The Dopamine Receptors

Second Edition

Edited by

Kim A. Neve

Humana Press

The Dopamine Receptors

The Receptors

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Preface

As sites of action for drugs used to treat schizophrenia and Parkinson's disease, dopamine receptors are among the most validated drug targets for neuropsychiatric disorders. Dopamine receptors are also drug targets or potential targets for other disorders such as substance abuse, depression, Tourette's syndrome, and attention deficit hyperactivity disorder. When chapters were being written for the first edition of "The Dopamine Receptors," published in 1997, researchers were still coming to grips with the discovery of novel dopamine receptor subtypes whose existence had not been predicted by pharmacological analysis of native tissue. Although we are still far from a complete understanding of the roles of each of the dopamine receptor subtypes, the decade since the publication of the first edition has seen the creation and characterization of mice deficient in each of the subtypes and the development of increasingly subtype-selective agonists and antagonists. Many of the chapters in this second edition rely heavily on new knowledge gained from these tools, but the use of knockout mice and subtype-selective drugs continues to be such a dominant theme in dopamine receptor research that these topics are also discussed in stand-alone chapters. The field of G protein-coupled receptors has advanced significantly since the publication of the first edition, with a model of GPCR signaling based on linear, compartmentalized pathways having been replaced by a more complex, richer model in which neurotransmitter effects are mediated by a signalplex composed of numerous signaling proteins, including multiple GPCRs, other types of receptors, such as ionotropic receptors, accessory and scaffolding proteins, and effectors. Again, although many chapter topics are affected by this more complex model, key aspects of the model are specifically addressed in new chapters on dopamine receptor-interacting proteins and on dopamine receptor oligomerization.

My goal has been to produce a book that will serve as a reference work on the dopamine receptors while also highlighting the areas of research that are most active today. To achieve this goal, I encouraged contributors to write chapters that set a broad area of research in its historical context and that look forward to new research opportunities. I hope that readers will agree with me that the authors have achieved that goal.

Portland, Oregon
March, 2009

Kim A. Neve

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Chapter 1
Historical Overview: Introduction to the Dopamine Receptors

Philip Seeman

Abstract A long-term search for the mechanism of action of antipsychotic drugs was motivated by a search for the cause of schizophrenia. The research between 1963 and 1975 led to the discovery of the antipsychotic receptor, now known as the dopamine D_2 receptor, the target for all antipsychotic medications. There are now five known dopamine receptors, all cloned. Although no appropriate animal model or brain biomarker exists for schizophrenia, it is known that the many factors and genes associated with schizophrenia invariably elevate the high-affinity state of the D_2 receptor or D_2^{High} by 100–900% in animals, resulting in dopamine supersensitivity. These factors include brain lesions; sensitization by amphetamine, phencyclidine, cocaine, or corticosterone; birth injury; social isolation; and more than 15 gene deletions in the pathways for the neurotransmission mediated by receptors for glutamate (NMDA), dopamine, GABA, acetylcholine, and norepinephrine. The elevation of D_2^{High} receptors may be the unifying mechanism for the various causes of schizophrenia.

Keywords Neuroleptic · Antipsychotic receptor · D_2^{High} receptor · Membrane stabilization · [^{3}H]haloperidol · Van Rossum hypothesis of schizophrenia · Dopamine supersensitivity · [^{3}H]domperidone

1.1 Introduction

The background to dopamine receptors is intimately associated with the history of antipsychotic drugs. The research in this field started with the development of antihistamines after the Second World War, with H. Laborit using these compounds to

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This chapter is dedicated to the memory of Hyman Niznik and Hubert H.M. Van Tol, pioneers in dopamine receptors.

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enhance analgesia [1]. In individuals receiving one of these series of medications, Laborit noticed a "euphoric quietude"; the patients were "calm and somnolent, with a relaxed and detached expression." Compound 4560 (now named chlorpromazine) was the most potent of the Rhone Poulenc compounds in the series.

Chlorpromazine was soon tested by many French physicians for various diseases. While Sigwald and Bouttier [2] were the first to use chlorpromazine as the only medication for a psychotic individual, they did not report their observations until 1953. The 1952 report by Delay et al. [3] showed that within 3 days [4, 5] chlorpromazine reduced hallucinations and stopped internal "voices" in eight patients, a significantly dramatic finding.

With the "neuroleptic" or antipsychotic action of chlorpromazine capturing the attention of the psychiatric community, the specific target of action for chlorpromazine became a research objective for basic scientists. The working assumption then, and still is the case now, was that the discovery of such a target might open the pathway to uncovering the biochemical cause of psychosis and possibly schizophrenia.

1.2 Membrane Stabilization by Antipsychotics

With the introduction of chlorpromazine to psychotic patients in state and provincial hospitals in North America in the late 1950s and early 1960s, the number of patients hospitalized with schizophrenia became markedly reduced. The basic science premise gradually emerged – if the target sites for antipsychotics could be found, then perhaps these sites were overactive in psychosis or schizophrenia. In the 1960s, however, no one agreed on what schizophrenia was. Inclusion criteria varied so much that it was impossible to decide which patients to study, let alone what to study. But everyone agreed that chlorpromazine and the many other new antipsychotic drugs, most of which were phenothiazines, alleviated the symptoms of schizophrenia, however defined.

But where in the nervous system does one start to look for an antipsychotic target? Moreover, were there many types of antipsychotic targets to identify?

With the advent of the electron microscope, the 1960s was an active decade of discovery of subcellular particles and cell membranes. In those days, therefore, it seemed reasonable to start by examining the actions of antipsychotics on cell membranes. In particular, did antipsychotics readily locate to cell surfaces and cell membranes and thereby alter membrane structure and function? Did antipsychotics target mitochondria, the structure of which was being rapidly revealed by electron microscopy?

In my own research in 1963, it was important to determine whether antipsychotics permeated cell membranes and whether the drugs were membrane active. I started with an artificial lipid film floating on water, and measured the film pressure with a 1 cm square of sand-blasted aluminum hanging into the bath (Wilhelmy method; [6]). Upon the addition of an antipsychotic to the water below the film, the

aluminum plate immediately rose, showing that the film pressure had been altered by the antipsychotic. This indicated that the antipsychotic molecules had entered into the single layer of lipid molecules floating on the water surface, expanding the intermolecular spaces between the lipid molecules. Therefore, could it be that cell membrane lipids were targets for antipsychotics?

To my surprise, however, when I omitted the lipid molecules, the addition of the antipsychotic still altered the surface pressure of the water surface. In other words, I had accidentally discovered that antipsychotics were surface active [7].

These surface-active potencies showed an excellent correlation with clinical antipsychotic potencies. However, I later realized that the antipsychotic concentrations were all in the micromolar range, a concentration subsequently found to be far in excess of that which was clinically effective in the plasma water or spinal fluid in patients taking antipsychotic medications.

Although all the antipsychotics were surface active and readily acted on artificial lipid films, it was essential to determine whether antipsychotics had similar membrane actions on human red blood cell membranes. In fact, this did occur, and it was found that low concentrations of antipsychotics readily expanded red blood cell membranes by ~0.1–1% and, in doing so, exerted an anti-hemolytic action by allowing the cells to become slightly larger and stabilized before hemolysis occurred [8–11].

This membrane stabilization by antipsychotics was also associated with electrical stabilization of the membrane. That is, it soon became clear that the antipsychotics were potent anesthetics, blocking nerve impulses at antipsychotic concentrations of between 20 nM and 1,000 nM (Fig. 1.1, top correlation line) [10, 12]. However, here too, these membrane-stabilizing concentrations were still in excess of those found clinically in the spinal fluid of treated patients (see following section). The driving criterion throughout this research was to find a target that was sensitive to the antipsychotic concentrations found in the spinal fluid of psychotic patients on maintenance doses of antipsychotic medications.

1.3 Therapeutic Concentrations of Antipsychotics

Although antipsychotics stabilize a variety of cellular and subcellular membranes [10], these antipsychotic concentrations are generally between 20 nM and 100 nM. The therapeutic molarities, however, were not known until the data on haloperidol were analyzed. In the case of haloperidol, for example, only 8% of haloperidol was free and not bound to plasma proteins [13]. Therefore, the active free concentration of haloperidol in the patient plasma water or in the spinal fluid would be between 1 nM and 2 nM [14, 15, 16]. Based on the standard pharmacological principle that the non-protonated form of tertiary amines readily permeates cell membranes [8], this concentration in the aqueous phase in the plasma is expected to be identical to the aqueous concentration of haloperidol in the spinal fluid.

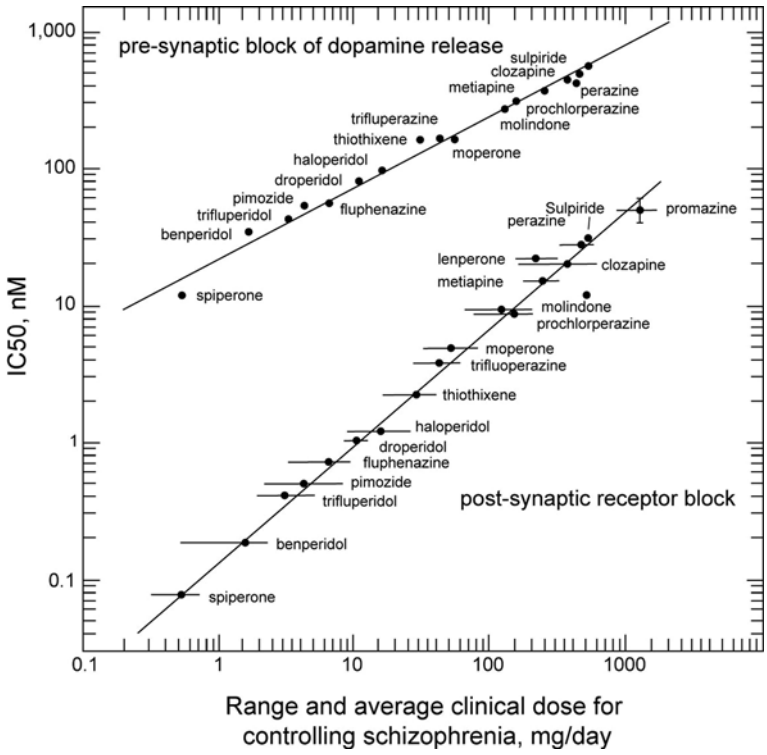

Fig. 1.1 All antipsychotic drugs inhibit the binding of [^{3}H]haloperidol to dopamine D_2 receptors (in calf striatal homogenate) in direct relation to the clinical antipsychotic potencies (*lower line*) [17,18,20]. The *upper line* indicates that antipsychotics also block the stimulated release of [^{3}H]dopamine (from rat striatal slices) at concentrations which correlate with their clinical potencies [12]; however, the antipsychotic concentrations required for this presynaptic action are much higher than those that inhibit [^{3}H]haloperidol binding to the D_2 receptors (*lower line*) or those which are found in the spinal fluid of patients being treated with antipsychotics [14] (re-drawn and adapted from [82] with permission)

1.4 Discovery of the Antipsychotic Dopamine Receptor

These latter calculations were critical for the discovery of the antipsychotic dopamine receptor [17, 18, 19]. That is, in order to detect or label a receptor with a dissociation constant of ∼1 nM for radioactive haloperidol, the specific activity of [^{3}H]haloperidol would have to be at least 10 Ci/mmol. However, the [^{3}H]haloperidol samples from Janssen Pharmaceutica (Belgium) kindly provided to the author's laboratory by Dr. J.J.P. Heykants in 1971 and by Dr. Jo Brugmans in 1972 had a specific activity of only 0.032–0.071 Ci/mmol, too low to detect specific binding for a site with an expected dissociation constant of ∼1 nM. Although New England Nuclear Corp. (Boston, MA) custom tritiated haloperidol for the author's laboratory, the specific activity was only ∼0.1 Ci/mmol.

Finally, after my extensive correspondence with Dr. Paul A.J. Janssen and Dr. J. Heykants, they asked I.R.E. Belgique (National Institut Voor Radio-Elementen, Fleurus, Belgium; Mr. M. Winand) to custom synthesize [^{3}H]haloperidol for the author's laboratory. I.R.E. Belgique soon thereafter provided us with relatively high specific activity [^{3}H]haloperidol (10.5 Ci/mmol) by June 1974.

This [^{3}H]haloperidol readily enabled us to detect the specific binding of [^{3}H]haloperidol to brain striatal tissue. Our laboratory submitted an abstract describing this to the Society for Neuroscience before the annual May 1975 deadline [17]. This report listed the following important IC_{50} values to inhibit the binding of [^{3}H]haloperidol: 2 nM for haloperidol, 20 nM for chlorpromazine, 3 nM for (+)butaclamol, and 10,000 nM for (–)butaclamol. The stereoselective action of butaclamol and the good correlation between the IC_{50} values and the clinical doses indicated that we had successfully identified the antipsychotic receptor. Moreover, of all the endogenous compounds tested, dopamine was the most potent in inhibiting the binding of [^{3}H]haloperidol, thus indicating that the antipsychotic receptor was a dopamine receptor.

The data of Seeman et al. [17] were confirmed by more extensive publications [18, 20, 21, 22], showing a clear correlation between the clinical potencies and the antipsychotic dissociation constants (Fig. 1.1, bottom correlation line).

At the CINP (Collegium Internationale Neuro-Psychopharmacologicum) meeting held in Paris in July 1975, during the evening courtyard reception at the City Hall of Paris, I rushed up to Dr. Paul Janssen and showed him the chart correlating the average clinical antipsychotic doses with the in vitro antipsychotic potencies. He laughed and said that averaging the clinical doses for each antipsychotic was like averaging all the religions of the world. Nevertheless, the correlation remains a cornerstone of the dopamine hypothesis of schizophrenia, still the major contender for an explanatory theory of schizophrenia causation.

1.5 Nomenclature of Dopamine Receptors

The receptor labeled by [^{3}H]haloperidol was later named the D2 receptor [23]. It is important to note that the data for the binding of [^{3}H]haloperidol identifying the antipsychotic receptor [17, 18] differed from the pattern of [^{3}H]dopamine binding described by Burt et al. [24] and Snyder et al. [25]. For example, the binding of [^{3}H]haloperidol was inhibited by ~10,000 nM dopamine, while that of [^{3}H]dopamine was inhibited by ~7 nM dopamine. For several years, this latter [^{3}H]dopamine binding site was termed the "D3 site" [26, 27], a term which is not to be confused with the discovery of the D_3 dopamine receptor [28]. As summarized in Table 1.1, there are now five different dopamine receptors that have been cloned.

At the same 1975 CINP meeting where I showed the correlation chart to Dr. Janssen, I happened to meet Dr. Sol Snyder in the lobby of the convention hotel and told him that I had custom prepared [^{3}H]haloperidol and that it was now available. The pattern of [^{3}H]haloperidol binding later published by Snyder et al.

[25] and by Burt et al. [24] agreed with my findings. The paper by Snyder et al. [25] kindly cited my paper of November, 1975, describing the [^{3}H]haloperidol-labeled antipsychotic receptor [18]. In addition, the publication of Burt et al. [24] kindly acknowledged the receipt of the drug samples of (+)- and (–)-butaclamol from our laboratory so that they could demonstrate stereoselective binding of [^{3}H]haloperidol.

Table 1.1 Key findings related to dopamine receptors

Year	Key findings related to dopamine receptors	Authors	References
1952	Analgesia and "euphoric quietude" with RP 4560	Laborit (Lacomme et al.)	[1]
1952–1953	Chlorpromazine (RP 4560) has effective antipsychotic action	Delay et al.; Sigwald and Bouttier	[2, 3]
1960	Very low amount of dopamine in Parkinson's diseased brain	Ehringer and Hornykiewicz	[29]
1963	Two antipsychotics increase normetanephrine and methoxytyramine	Carlsson and Lindqvist	[30]
1964	Three antipsychotics increase HVA and DOPAC; elimination delayed?	Andén et al.	[31]
1965	Dopamine can excite or inhibit neurons	Bloom et al.	[83]
1966	Dopamine hypothesis of schizophrenia outlined	Van Rossum	[33]
1971	Dopamine stimulates adenylate cyclase	Kebabian and Greengard	[38]
1971	Haloperidol measured in patient's plasma (see 1977 below)	Zingales et al.	[15]
1974	2.5 nM haloperidol blocks tritiated dopamine receptors	Seeman et al.	[19]
1974	Haloperidol blocks excitation in Helix	Struyker Boudier et al.	[84]
1975	Tritiated haloperidol labels dopamine receptors	Seeman et al.	[17, 18]
1975	Antipsychotic doses correlate with blockade of dopamine receptors	Seeman et al.	[18, 20]
1976	Sulpiride resolves two dopamine sites; no effect on adenylate cyclase	Roufogalis et al.	[42]
1976	Two dopamine receptors proposed: inhibitory and excitatory	Cools; Van Rossum	[35]

Table 1.1 (continued)

Year	Key findings related to dopamine receptors	Authors	References
1977	Dopamine stimulates adenylate cyclase in parathyroid	Brown et al.	[39]
1977	92% of plasma haloperidol bound, indicating 2 nM free in water	Forsman and Öhman	[13]
1978	Two dopamine receptors: coupled and uncoupled to adenylate cyclase	Spano et al.; Garau et al.	[36, 37]
1978	Presynaptic action of apomorphine reduces release of dopamine	Starke et al.	[53]
1978	Elevated D2 in postmortem schizophrenia brain	Lee et al.	[59]
1979	Names of D1 and D2 used	Kebabian and Calne	[23]
1979	Dopamine inhibits adenylate cyclase in ant pituitary	De Camilli et al.	[43]
1983	Identical antipsychotic Ki values at striatum and limbic D2 receptors	Seeman and Ulpian	[85]
1984	Kd values of D2 ligands depend on final tissue concentration	Seeman et al.	[56]
1984	D_2^{High} and D_{2Low} affinity states of D2 receptors	Wreggett and Seeman	[55]
1985	D_2^{High} is functional state of D2	McDonald et al.; George et al.	[51, 52]
1986	Elevated D2 measured in living schizophrenia patients	Wong et al.	[68]
1986	Labeling of D2 receptors in living humans by positron emission tomography	Farde et al.	[86]
1988	Antipsychotics occupy 60–80% of D2 in living schizophrenia patients	Farde et al.	[70]
1988–1989	Cloning of the rat D_{2Short} and D_{2Long} receptors	Bunzow et al.; Giros et al.	[46, 48]
1989	Cloning of the human D_{2Short} and D_{2Long} receptors	Grandy et al.	[47]
1989	90% of D2 receptors are in D_2^{High} state in brain slices	Richfield et al.	[54]
1989	Endogenous dopamine lowers radio-raclopride binding; relevant to PET	Seeman et al.	[81]
1990–1991	Dopamine D1 and D5 receptors cloned	Sunahara; Zhou et al.	[40,41,87]

Table 1.1 (continued)

Year	Key findings related to dopamine receptors	Authors	References
1990	Dopamine D3 receptor cloned	Sokoloff et al.	[28]
1991	Dopamine D4 receptor cloned	Van Tol et al.	[50]
1992	Block of D2 >80% by antipsychotics associated with Parkinsonism	Farde et al.	[69]
1992	Synaptic dopamine at rest is ∼2 nM, ∼100–200 nM during firing	Kawagoe et al.	[88]
1995	Drug Ki depends on fat solubility of ligand	Seeman and Van Tol	[57]
1996	Amphetamine-induced release of dopamine is higher in schizophrenia	Laruelle et al.	[80]
1998	D_{2Short} receptors located mostly in nigral neurones	Khan et al.	[89]
1999	Therapeutic doses of antipsychotics block 60–80% D2	Kapur et al.	[71]
1999	Isoleucine at position 154 in D2 causes myoclonus dystonia	Klein et al.	[90]
1999	Rapid release of clozapine and quetiapine from D2 receptors	Seeman et al.	[74]
2000	New $D_{2Longer}$ receptor	Seeman et al.	[49]
2003	Antipsychotics occupy more D2 in limbic areas than striatum	Bressan et al.	[75]
2005	Dopamine supersensitivity correlates with elevated D_2^{High} states	Seeman et al.	[91]
2005	Dopamine receptor contribution to action of PCP, LSD, and ketamine	Seeman et al.	[92]
2005	Higher D2 density in healthy identical twins of schizophrenia patients	Hirvonen et al.	[66]
2006	Markedly elevated D_2^{High} receptors in all animal models of psychosis	Seeman et al.	[93, 94]

1.6 Antipsychotic Accelerated Turnover of Dopamine

In 1960 Ehringer and Hornykiewicz [29] discovered that the content of dopamine was extremely low in the postmortem brains of patients who died with Parkinson's disease. This discovery immediately suggested that the well-known Parkinsonism

caused by antipsychotics was probably associated in some way with interference of dopamine neurotransmission by the antipsychotics. However, there were many possible molecular modes of interference, including presynaptic and postsynaptic mechanisms.

The finding of Ehringer and Hornykiewicz naturally stimulated brain research on dopamine. Carlsson and Lindqvist [30] soon reported that chlorpromazine and haloperidol increased the production of normetanephrine and methoxytyramine, metabolites of epinephrine and dopamine, respectively. To explain the increased production of these metabolites, these authors suggested that "the most likely [mechanism] appears to be that chlorpromazine and haloperidol block monoaminergic receptors in brain; as is well known, they block the effects of accumulated 5-hydroxytryptamine . . . ."

In other words, these authors proposed that antipsychotics blocked all three types of receptors for noradrenaline, dopamine, and serotonin, but they did not identify which receptor was selectively blocked or how to identify or test any of these receptors directly in vitro. The paper by Carlsson and Lindqvist [30] is often mistakenly cited as discovering the principle that antipsychotic drugs selectively block dopamine receptors. A year later, even the students of the Carlsson laboratory, Andén et al. [31], limited their speculation to proposing that "chlorpromazine and haloperidol delays the elimination of the (metabolites). . .," a hypothesis no longer held. Moreover, even after 7 years, although Andén et al. [32] reported that antipsychotics increased the turnover of both dopamine and noradrenaline, they could not show that the antipsychotics were selective in blocking dopamine; for example, chlorpromazine enhanced the turnover of noradrenaline and dopamine equally. Therefore, it remained for in vitro radioreceptor assays to detect the dopamine receptor directly and to demonstrate antipsychotic selectivity for the dopamine receptor.

In fact, when the antipsychotic dopamine receptor was discovered [18, 20], there was a peak surge in the rate of citations of the paper by Carlsson and Lindqvist [30], a peak stimulated by the actual discovery of the dopamine receptor method, as shown in Fig. 1.2. This figure also shows that there was approximately a 12-year interval between the onset of dopamine research and the research on dopamine receptors, indicating that the two fields were stimulated by separate developments.

1.7 The Dopamine Hypothesis of Schizophrenia, and Dopamine Receptors in the Human Brain

As already noted, the paper by Carlsson and Lindqvist [30] is often mistakenly cited as the origin of the dopamine hypothesis of schizophrenia. However, the dopamine hypothesis of schizophrenia was first outlined in 1967 by Van Rossum [33] (see [34]) as follows:

"The hypothesis that neuroleptic drugs may act by blocking dopamine receptors in the brain has been substantiated by preliminary experiments with a few

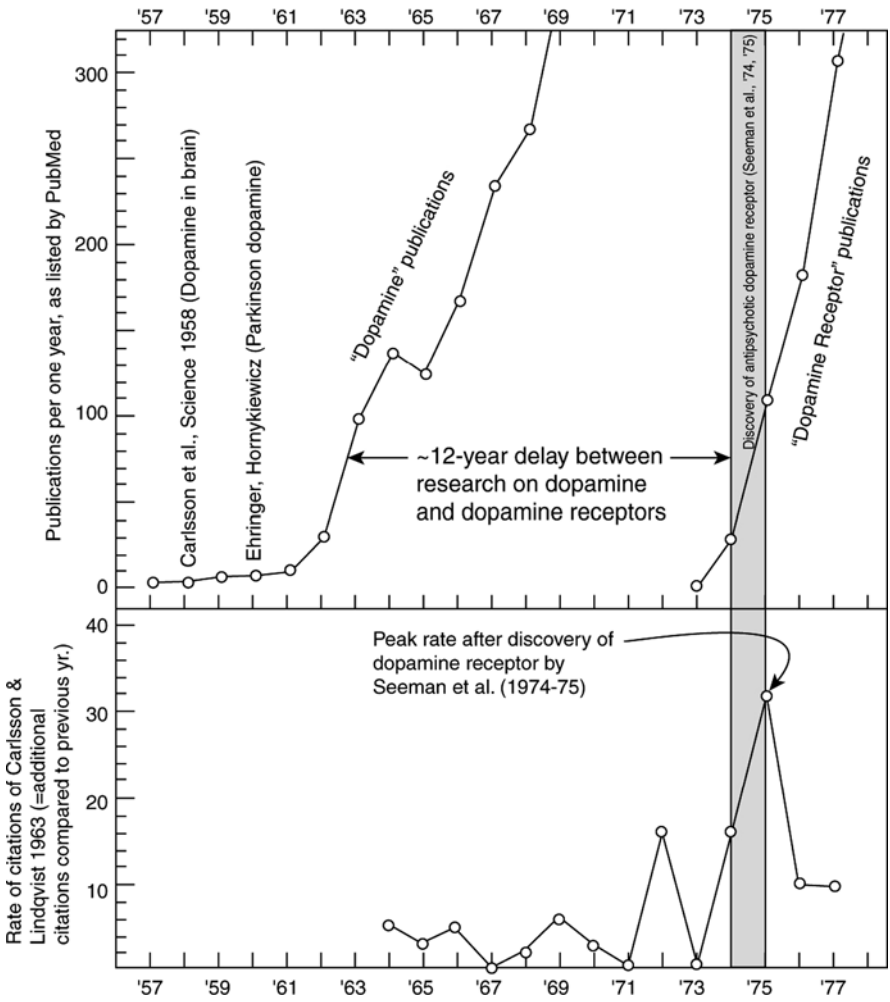

Fig. 1.2 *Top*: Annual number of publications on "dopamine" and on "dopamine receptors," as listed by PubMed online. Dopamine was found in brain tissue by Montagu [95] in Weil-Malherbe's laboratory [96, 97] and by Carlsson et al. [98]. There is a 12-year interval between the two sets of publications, suggesting that the two onsets of publications were stimulated by separate other publications. *Bottom*: Annual rate of citations (Web of Science, Thomson Scientific, Philadelphia, PA) of the article by Carlsson and Lindqvist [30], describing the increased production of normetanephrine and methoxytyramine by chlorpromazine or haloperidol. The citation rate of this 1963 article peaked in 1975 when the dopamine receptors were discovered [17, 18, 19] (from [82] with permission)

selective and potent neuroleptic drugs. There is an urgent need for a simple isolated tissue that selectively responds to dopamine so that less specific neuroleptic drugs can also be studied and the hypothesis further tested. . . . When the hypothesis of dopamine blockade by neuroleptic agents can be further substantiated it may have

fargoing consequences for the pathophysiology of schizophrenia. Over-stimulation of dopamine receptors could then be part of the etiology."

With the discovery of the antipsychotic dopamine receptor in vitro, it became possible to measure the densities and properties of these receptors directly not only in animal brain tissues but also in the postmortem human brain and, at a later time, in living humans by means of positron emission tomography. Many, but not all, of these findings directly or indirectly support the dopamine hypothesis of schizophrenia.

1.8 Key Advances Related to Dopamine Receptors

Many of the significant advances in dopamine receptors and the dopamine hypothesis of psychosis or schizophrenia are listed in Table 1.1. Between 1976 and 1979, it became clear that there were two main groups of dopamine receptors, D1 and D2 [23, 35, 36, 37]. The D1-like group of receptors were associated with dopamine-stimulated adenylate cyclase [38, 39], but were not selectively labeled by [^{3}H]haloperidol. The antipsychotic potencies at these D1 receptors did not correlate with clinical antipsychotic potency [26]. The D1-like receptors now consist of the cloned D_1 and D_5 receptors [40, 41].

The D2-like receptors did not stimulate adenylate cyclase and are now known to inhibit adenylate cyclase [42, 36, 37, 43, 44, 45]. The D2-like group now includes the cloned D_{2Short} [46, 47], D_{2Long} [48], $D_{2Longer}$ [49], D_3 [28], and D_4 dopamine receptors [50].

Moreover, each of these receptors has a state of high affinity and a state of low affinity for dopamine, with D_2^{High} being the functional state in the anterior pituitary [51, 52], in nigral dopamine terminals (presynaptic receptors [53]), and presumably in the nervous system itself. Although this latter point has not been unequivocably established, Richfield et al. [54] have found that 90% of the D_2 receptors in brain slices are in the D_2^{High} state. The D_2^{High} state can be quickly converted into the D_{2Low} state by guanine nucleotide [55].

The differences in findings on dopamine receptors between laboratories are explained by technically different methods and ligands. For example, the dissociation constant of a ligand at the D_2 receptor can vary enormously, depending on the final concentration of the tissue [56]. Moreover, fat-soluble ligands, such as [^{125}I]iodosulpride, [^{3}H]nemonapride, and [^{3}H]spiperone, invariably yield higher dissociation constants than less fat-soluble ligands (such as [^{3}H]raclopride) for competing drugs [21, 57]. This technical effect also occurs with positron emission tomography ligands [58].

Although the density of D_2 receptors in postmortem human schizophrenia tissues is elevated [26, 59, 60–62], some of this elevation may have resulted from the antipsychotic administered during the lifetime of the patient. An example of this elevation is shown in Fig. 1.3, where it may be seen that the postmortem tissues from half of the patients who died with schizophrenia revealed elevated densities of

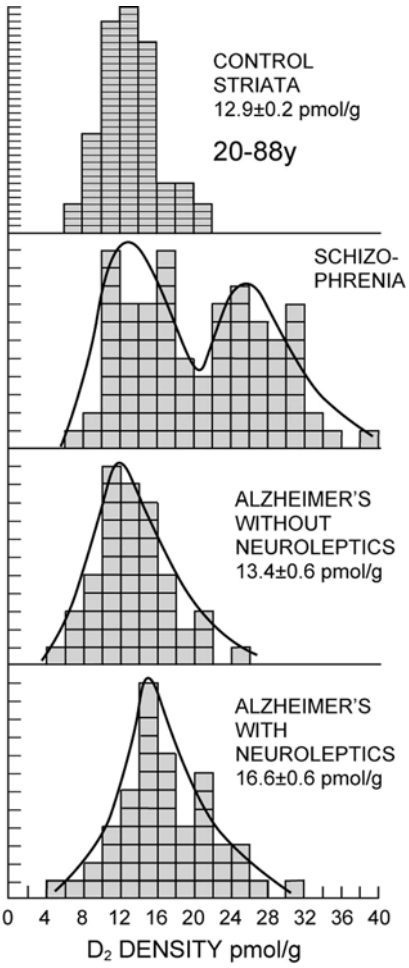

Fig. 1.3 Elevation of dopamine D_2 receptors in postmortem caudate–putamen tissues from patients who had died with schizophrenia. Each *box* indicates the D_2 density measured by saturation analysis with [^{3}H]spiperone (Scatchard method for Bmax; centrifugation method) [62]. The D_2 densities in the postmortem striata from schizophrenia patients exhibit a bimodal pattern, with half the values being two or three times the normal density. Most of the schizophrenia patients had been treated with antipsychotics during their lifetime. Although the Alzheimer patient tissues also revealed a small elevation of D_2 densities, the magnitude and pattern were different than that for schizophrenia (re-drawn and adapted from [82] with permission)

[^{3}H]spiperone-labeled D2-like receptors in the caudate–putamen tissue. The other half of the postmortem schizophrenia tissues were normal in D_2 density even though most of the patients were known to have also been treated with antipsychotics during their lifetime.

It is often surprising to encounter people who are resistant to advances in science. For example, I vividly recall one British psychiatrist standing up and shouting at me from the audience: "Post-mortem dopamine receptors? Do you actually expect me to believe that these dead receptors come to life and bind your radioactive material?" I answered that the same type of question was raised a century ago when people seriously questioned whether ferments could be isolated and still have activity, but that we can now buy crystallized enzymes for a few dollars and that these ferments are fully active. And, of course, thanks to many of the contributors to the present

book on "The Dopamine Receptors," one can now purchase frozen clones of the five different dopamine receptors.

1.9 Is D_2^{High} the Unifying Mechanism for Schizophrenia?

Throughout the years between 1963 and the present, the overall strategy has been to identify the main target of antipsychotic medications and then to determine whether these antipsychotic targets are overactive in schizophrenia or in animal models of psychosis. Has this strategy worked? The answer is yes. First, the primary target for antipsychotics, the dopamine D_2 receptor, has been identified, and, second, many avenues indicate that D_2^{High} (the high-affinity state of the D_2 receptor) may be the unifying mechanism for schizophrenia.

In particular, the following facts on dopamine receptors validate the 45-year search for a basic unifying mechanism for schizophrenia:

1. All antipsychotic drugs, including the newer dopamine partial agonists such as aripiprazole [22] or OSU 6162 [63], block dopamine D_2 receptors in direct relation to their clinical potency. Even the glutamate-type antipsychotic [64] has a significant dopamine partial agonist action on D_2 receptors [65].
2. The brain imaging by Hirvonen et al. [66] shows that the D_2 density is elevated in healthy identical co-twins of patients who have schizophrenia. This finding suggests that the elevation of D_2 receptors is necessary for psychosis. At the same time, however, the findings of Hirvonen et al. also illustrate that in addition to elevated D_2 receptors there is likely another factor precipitating the psychotic symptoms. This additional factor may well be that a certain proportion of D_2 receptors must convert into the high-affinity state.
   At the same time, the elevation of D_2 is becoming recognized as a valuable biomarker for prognosis and outcome in first-episode psychosis [67]. Earlier work had shown that the density of D_2 receptors labeled by [^{11}C]methylspiperone was elevated in drug-naive schizophrenia patients [68]. However, no such elevation of D_2 receptors was found in schizophrenia patients when [^{11}C]raclopride was used (Refs in [69]).
3. It has been consistently found that psychotic symptoms are alleviated when 65% to 75% of the brain D_2 receptors (as measured in the striatum) are occupied by antipsychotics [70, 69]. It is now considered unlikely that the blockade of serotonin-2 receptors assists in alleviating psychosis and affecting D_2 occupancy [71, 72, 73]. The antipsychotic occupancy of D_2 may or may not be higher in limbic regions [21, 74, 75, 76, 77].
4. In contrast to traditional antipsychotics such as chlorpromazine and haloperidol that can elicit Parkinsonism, clozapine and quetiapine do not produce Parkinsonism, consistent with the fact that clozapine and quetiapine dissociate rapidly from the D_2 receptor [21].
5. The psychotic symptoms in schizophrenia increase or intensify when the individual is challenged with psychostimulants at doses that have little effect in

control subjects. As reviewed by Lieberman et al. [78], 74–78% of patients with schizophrenia become worse with new or intensified psychotic symptoms after being given amphetamine or methylphenidate. Psychotic symptoms can also be elicited in this way in control subjects, but only in 0–26%.

6. In a meta-analysis of 27 studies (3,707 schizophrenia patients and 5,363 control subjects), Glatt and Jönsson [79] have found that the Ser311Cys polymorphism in the D_2 receptor was significantly associated with schizophrenia ($P = 0.002$–0.007), indicating that this polymorphism in D_2 may contribute a significant and reliable risk for the illness.
7. Amphetamine-induced release of endogenous dopamine in humans is a possible marker of psychosis [80], using the principle worked out in animals [81].
8. Although no appropriate animal model or brain biomarker exists for schizophrenia, it is known that the many factors and genes associated with schizophrenia invariably elevate dopamine D_2^{High} receptors by 100–900% in animals, resulting in dopamine supersensitivity. These factors include brain lesions; sensitization by amphetamine, phencyclidine, cocaine, or corticosterone; birth injury; social isolation; and more than 15 gene deletions in the pathways for the neurotransmission mediated by receptors for glutamate (NMDA), dopamine, GABA, acetylcholine, and norepinephrine. A list of these psychosis-precipitating factors is given in Table 1.2, along with the magnitude of the elevations that these factors elicit in the proportion of D_2^{High} receptors in the striata of mice or rats. The total density of D_2 generally does not change.

Table 1.2 Increase in D_2^{High} receptors in dopamine supersensitive animal models for psychosis

Percentage of increase in proportion of D_2^{High}	Treatment	References
	Sensitization by	
250%	Amphetamine	[93, 94]
180%	Phencyclidine	[91]
160%	Cocaine	[99]
125%	Caffeine	[100]
50%	Quinpirole	[94]
210%	Corticosterone	[91]
	Lesions of	
270%	Neonatal hippocampus	[91]
160%	Neonatal hippocampus	[94]
130%	Cholinergic lesion in cortex	[94]
100%	Entorhinal hippocampus	[101]
	Knockout of gene for	
200–900%	D4 receptor	[91]
60–340%	GRK6	[91, 94]
232%	Alpha-Adrenoceptor-1b	[102]
225%	$GABA_{B1}$	H. Mohler and P. Seeman (unpublished)
200%	Dopamine-beta-hydroxylase	[91, 94]

Table 1.2 (continued)

Percentage of increase in proportion of D_2^{High}	Treatment	References
160%	Trace amine-1 receptor	[103]
135%	RGS9-2	[91, 94]
133%	Nurr77	L.E. Trudeau, P. Seeman (unpublished)
129%	Postsynaptic density 95	J.-M. Beaulieu, P. Seeman (unpublished)
120%	Tyrosine hydroxylase (no dopamine)	[91]
90%	COMT	[91]
60–80%	Vesicular monoamine transporter	[104]
48%	RII beta (protein kinase A)	[91, 94]
39%	Dopamine transporter	[104]
	Other	
130–460%	Cesarian birth with anoxia (rat)	[91, 94]
228%	Rats socially isolated from birth	[105]
100%	Reserpine-treated rats	[91, 94]
	Animals not showing supersensitivity	
–7%	Dopamine D1 receptor knockout mice	[91, 94]
19%	Glycogen synthase kinase 3 knockout mice	[91, 94]
–75%	Adenosine A2A receptor knockout mice	[91, 94]
20%	mGluR5 knockout mice	[91, 94]

Abbreviations: COMT, catechol-O-methyl transferase; GABAB1, the B1 subtype of G protein-coupled receptors for GABA; GRK6, G protein-coupled receptor kinase 6; mGluR5, metabotropic glutamate receptor 5; Nurr77, orphan nuclear receptor 77; RII beta, the IIβ form of the regulatory subunit of cyclic AMP-dependent protein kinase; RGS9-2, regulator of G protein signaling 9-2

Because antipsychotic drugs directly block D_2 receptors, it is not surprising that antipsychotics also cause an increase in the proportion of D_2^{High} receptors. In fact, it has long been known that administration of antipsychotic drugs can induce dopamine supersensitivity and antipsychotic tolerance in animals. These effects are also found in humans and presumably are the basis for supersensitivity psychosis or rebound psychosis upon drug withdrawal. Although D_2^{High} receptors become elevated after long-term antipsychotics, these elevated D_2^{High} states readily reverse, unlike the essentially permanently elevated D_2^{High} states in the other animal models of psychosis mentioned above.

The strategy, the objective, and the questions on dopamine receptors still remain. What is the molecular pathway for antipsychotic action via the dopamine receptors? Are any of these steps specifically altered in schizophrenia? What is the intracellular biochemical mechanism of converting D_{2Low} into D_2^{High}?

At present, the most promising direction in this field is to examine the molecular basis of dopamine supersensitivity, because up to 70% of patients are supersensitive to either methylphenidate or amphetamine at doses that do not affect control humans. Moreover, as shown in Table 1.2, a wide variety of brain alterations (lesions, drug treatment, receptor knockouts) all lead to the final common target of elevated proportions of D_2 receptors in the D_2^{High} state. Therefore, the molecular control of the high-affinity state of D_2 is emerging as a central problem in this field. At present, there is uncertainty as to whether this high-affinity state of D_2 is controlled through Go or one of the Gi proteins, because this varies from cell to cell.

It is currently proposed that there are multiple pathways in the various types of psychosis that all converge to elevate the D_2^{High} state in specific brain regions and that this elevation elicits psychosis. This proposition is supported by the dopamine supersensitivity that is a common feature of schizophrenia and that also occurs in many types of genetically altered, drug-altered, and lesion-altered animals. Dopamine supersensitivity, in turn, correlates with D_2^{High} states. The finding that all antipsychotics, traditional and recent ones, act on D_2 receptors further supports the proposition.

Altogether, the dawn of the neurotransmitter era has proven to be an exciting chapter in neuropsychopharmacology. The art of psychiatry is becoming a science. It has been a privilege to participate in these developments. I thank my fellow students for making it possible.

References

1. Lacomme M, Laborit H, Le Lorier G, et al. [Obstetric analgesia potentiated by associated intravenous dolosal with RP 4560.] Bull Féd Soc Gynecol Obstet Lang Fr 1952;4:558–62.
2. Sigwald J, Bouttier D. 3-Chloro-10-(3'-dimethylaminopropyl)-phenothiazine hydrochloride in current neuro-psychiatry. Ann Méd Interne (Paris) 1953;54:150–82.
3. Delay J, Deniker P, Harl J-M. Traitement des états d'excitation et d'agitation par une méthode médicamenteuse dérivée de l'hibernithérapie. [Therapeutic method derived from hiberno-therapy in excitation and agitation states.] Ann. Méd-Psychol. (Paris) 1952;110:267–73.
4. Agid O, Kapur S, Arenovich T, et al. Delayed-onset hypothesis of antipsychotic action: a hypothesis tested and rejected. Arch Gen Psychiatry 2003;60:1228–35.
5. Kapur S, Arenovich T, Agid O, et al. Evidence for onset of antipsychotic effects within the first 24 hours of treatment. Am J Psychiatry 2005;162:939–46.
6. Wilhelmy L. Ann Physik Lpz 1863;119:177.
7. Seeman P, Bialy HS. The surface activity of tranquilizers. Biochem Pharmacol 1963;12:1181–91.
8. Seeman P. Erythrocyte membrane stabilization by local anesthetics and tranquilizers. Biochem Pharmacol 1966a;15;1753–66.
9. Seeman P. Membrane stabilization by drugs: tranquilizers, steroids and anesthetics. Int Rev Neurobiol 1966b;9:145–221.

10. Seeman, P. The membrane actions of anesthetics and tranquilizers. Pharmacol Rev 1972;24:583–655.
11. Seeman P, Weinstein J. Erythrocyte membrane stabilization by tranquilizers and antihistamines. Biochem Pharmacol 1966;15:1737–52
12. Seeman, P, Lee T. Antipsychotic drugs: Direct correlation between clinical potency and presynaptic action on dopamine neurones. Science 1975;188:1217–9.
13. Forsman A, Öhman R. Studies on serum protein binding of haloperidol. Curr Ther Res Clin Exp 1977;21:245–55.
14. Seeman, P. Dopamine receptor sequences. Therapeutic levels of neuroleptics occupy D_2, clozapine occupies D_4. Neuropsychopharmacology 1992;7:261–84.
15. Zingales IA. A gas chromatographic method for the determination of haloperidol in human plasma. J Chromatogr 1971;54:15–24.
16. Seeman, P. Therapeutic receptor-blocking concentrations of neuroleptics. Int Clin Psychopharmacol 1995;10 (Suppl 3):5–13.
17. Seeman P, Wong M, Tedesco J. Tranquilizer receptors in rat striatum. Soc Neurosci Abstr 1975;1:405.
18. Seeman P, Chau-Wong M, Tedesco J, et al. Brain receptors for antipsychotic drugs and dopamine: direct binding assays. Proc Natl Acad Sci USA 1975;72:4376–80.
19. Seeman P, Wong M, Lee T. Dopamine receptor-block and nigral fiber impulse-blockade by major tranquilizers. Fed Proc 1974;33:246.
20. Seeman P, Lee T, Chau-Wong M, et al. Antipsychotic drug doses and neuroleptic/dopamine receptors. Nature (Lond.) 1976;261:717–9.
21. Seeman, P. Atypical antipsychotics: mechanism of action. Can J Psychiat 2002;47:27–38.
22. Seeman P. Targeting the dopamine D2 receptor in schizophrenia. Expert Opin Ther Targets 2006;10:515–31.
23. Kebabian JW, Calne DB. Multiple receptors for dopamine. Nature 1979;277:93–6.
24. Burt DR, Creese I, Snyder SH. Properties of [^{3}H]haloperidol and [^{3}H]dopamine binding associated with dopamine receptors in calf brain membranes. Mol Pharmacol 1976;12: 800–12.
25. Snyder SH, Creese I, Burt DR. The brain's dopamine receptor: labeling with [^{3}H]dopamine and [^{3}H]haloperidol. Psychopharmacol Commun 1975;1:663–73.
26. Seeman, P. Brain dopamine receptors. Pharmacol Rev 1980;32:229–313.
27. Seeman, P. Nomenclature of central and peripheral dopaminergic sites and receptors. Biochem Pharmacol 1982;31:2563–8.
28. Sokoloff P, Giros B, Martres MP, et al. Molecular cloning and characterization of a novel dopamine receptor (D3) as a target for neuroleptics. Nature 1990;347:146–51.
29. Ehringer H, Hornykiewicz O. Distribution of noradrenaline and dopamine (3- hydroxytyramine) in the human brain and their behavior in diseases of the extrapyramidal system. Klin Wochenschr 1960;38:1236–9.
30. Carlsson A, Lindqvist M. Effect of chlorpromazine or haloperidol on formation of 3-methoxytyramine and normetanephrine in mouse brain. Acta Pharmacol Toxicol (Copenh) 1963;20:140–4.
31. Andén N-E, Roos B-E, Werdinius B, 1964. Effects of chlorpromazine, haloperidol and reserpine on the levels of phenolic acids in rabbit corpus striatum. Life Sci 1964;3:149–58.
32. Andén N-E, Butcher SG, Corrodi H, et al. Receptor activity and turnover of dopamine and noradrenaline after neuroleptics. Eur J Pharmacol 1970;11:303–14.
33. Van Rossum JM. The significance of dopamine-receptor blockade for the action of neuroleptic drugs. In: Brill H, Cole JO, Deniker P, Hippius H, Bradley PB, eds. Neuro-Psycho-Pharmacology, Proceedings of the Fifth International Congress of the Collegium Internationale Neuro-Psycho-pharmacologicum, March 1966, Excerpta Medica Foundation, Amsterdam, 1967;321–9.
34. Baumeister AA, Francis JL. Historical development of the dopamine hypothesis of schizophrenia. J History Neurosci 2002;11:265–77.

35. Cools AR, Van Rossum JM. Excitation-mediating and inhibition-mediating dopamine-receptors: a new concept towards a better understanding of electrophysiological, biochemical, pharmacological, functional and clinical data. Psychopharmacologia 1976;45: 243–54.
36. Spano PF, Govoni S, Trabucchi M. Studies on the pharmacological properties of dopamine receptors in various areas of the central nervous system. Adv Biochem Psychopharmacol 1978;19:155–65.
37. Garau L, Govoni S, Stefanini E, et al. Dopamine receptors: pharmacological and anatomical evidences indicate that two distinct dopamine receptor populations are present in rat striatum. Life Sci 1978;23:1745–50.
38. Kebabian JW, Greengard P. Dopamine-sensitive adenyl cyclase: possible role in synaptic transmission. Science 1971;174:1346–9.
39. Brown EM, Carroll RJ, Aurbach GD. Dopaminergic stimulation of cyclic AMP accumulation and parathyroid hormone release from dispersed bovine parathyroid cells. Proc Natl Acad Sci USA 1977;74:4210–3.
40. Sunahara RK, Niznik HB, Weiner DM, et al. Human dopamine D_1 receptor encoded by an intronless gene on chromosome 5. Nature 1990;347:80–3.
41. Sunahara RK, Guan H-C, O'Dowd B, et al. Cloning of the gene for a human dopamine D5 receptor with higher affinity for dopamine than D_1. Nature 1991;350:614–9.
42. Roufogalis BD, Thornton M, Wade DN. Specificity of the dopamine sensitive adenylate cyclase for antipsychotic antagonists. Life Sci 1976;19:927–34.
43. De Camilli P, Macconi D, Spada A. Dopamine inhibits adenylate cyclase in human prolactin-secreting pituitary adenomas. Nature 1979;278:252–4.
44. Meunier H, Labrie F. The dopamine receptor in the intermediate lobe of the rat pituitary gland is negatively coupled to adenylate cyclase. Life Sci 1982;30:963–8.
45. Onali P, Olianas MC, Gessa GL. Characterization of dopamine receptors mediating inhibition of adenylate cyclase activity in rat striatum. Mol Pharmacol 1985;28:138–45.
46. Bunzow JR, Van Tol HH, Grandy DK, et al. Cloning and expression of a rat D_2 dopamine receptor cDNA. Nature 1988;336:783–7.
47. Grandy DK, Marchionni MA, Makam H, et al. Cloning of the cDNA and gene for a human D_2 dopamine receptor. Proc Natl Acad Sci USA 1989;86:9762–976
48. Giros B, Sokoloff P, Martres MP, et al. Alternative splicing directs the expression of two D_2 dopamine receptor isoforms. Nature 1989;342:923–6.
49. Seeman P, Nam D, Ulpian C, et al. A new dopamine receptor, D2Longer, with a unique TG splice site, in human brain. Mol Brain Res 2000;76:132–41.
50. Van Tol HHM, Bunzow JR, Guan H-C, et al. Cloning of the gene for a human dopamine D_4 receptor with high affinity for the antipsychotic clozapine. Nature 1991;350:610–14.
51. McDonald WM, Sibley DR, Kilpatrick BF, et al. Dopaminergic inhibition of adenylate cyclase correlates with high affinity agonist binding to anterior pituitary D2 dopamine receptors. Mol Cell Endocrinol 1984;36:201–9.
52. George SR, Watanabe M, Di Paolo T, et al. The functional state of the dopamine receptor in the anterior pituitary is in the high affinity form. Endocrinology 1985;117:690–7.
53. Starke K, Reimann W, Zumstein A, et al. Effect of dopamine receptor agonists and antagonists on release of dopamine in the rabbit caudate nucleus in vitro. Naunyn Schmiedebergs Arch Pharmacol 1978;305:27–36.
54. Richfield EK, Young AB, Penney JB. Properties of D2 dopamine receptor autoradiography: high percentage of high-affinity agonist sites and increased nucleotide sensitivity in tissue sections. Brain Res 1986;383:121–8.
55. Wreggett KA, Seeman P. Agonist high- and low-affinity states of the D2 dopamine receptor in calf brain: partial conversion by guanine nucleotide. Mol Pharmacol 1984;25:10–17.
56. Seeman P, Ulpian C, Wreggett KA, et al. Dopamine receptor parameters detected by ^{3}H-spiperone depend on tissue concentration: analysis and examples. J Neurochem 1984;43:221–35.

57. Seeman P, Van Tol HHM. Deriving the therapeutic concentrations for clozapine and haloperidol: The apparent dissociation constant of a neuroleptic at the dopamine D2 or D4 receptor varies with the affinity of the competing radioligand. Eur J Pharmacol – Mol Pharmacol Section 1995;291:59–66.
58. Hagberg G, Gefvert O, Bergström M, et al. N-[^{11}C]methylspiperone PET, in contrast to [^{11}C]raclopride, fails to detect D_2 receptor occupancy by an atypical neuroleptic. Psychiatry Res 1998;82:147–60.
59. Lee T, Seeman P, Tourtellotte WW, et al. Binding of ^{3}H-neuroleptics and ^{3}H-apomorphine in schizophrenic brains. Nature 1978;274:897–900.
60. Lee T, Seeman P. Elevation of brain neuroleptic/dopamine receptors in schizophrenia. Am J Psychiat 1980;137:191–7.
61. Seeman, P. Dopamine receptors and the dopamine hypothesis of schizophrenia. Synapse 1987;1:133–52.
62. Seeman P, Ulpian C, Bergeron C, et al. Bimodal distribution of dopamine receptor densities in brains of schizophrenics. Science 1984;225:728–31.
63. Seeman P, Guan H-C. Dopamine partial agonist action of (-)OSU6162 is consistent with dopamine hyperactivity in psychosis. Eur J Pharmacol 2007;557:151–3.
64. Patil ST, Zhang L, Martenyi F, et al. Activation of mGlu2/3 receptors as a new approach to treat schizophrenia: a randomized Phase 2 clinical trial. Nat Med 2007;13:1102–10.
65. Seeman P, Caruso C, Lasaga M. Dopamine partial agonist actions of the glutamate receptor agonists LY 354,740 and LY 379,268. Synapse 2008;62:154–8.
66. Hirvonen J, van Erp TG, Huttunen J, et al. 2005. Increased caudate dopamine D_2 receptor availability as a genetic marker for schizophrenia. Arch Gen Psychiatry 2005;62:371–8.
67. Corripio I, Perez V, Catafau AM, Mena E, Carrio I, Alvarez E: Striatal D2 receptor binding as a marker of prognosis and outcome in untreated first-episode psychosis. Neuroimage 2006;29:662–6.
68. Wong DF, Wagner HN Jr, Tune LE, et al. Positron emission tomography reveals elevated D_2 dopamine receptors in drug-naive schizophrenics. Science 1986;234:1558–63.
69. Farde L, Nordstrom AL, Wiesel F-A, et al. Positron emission tomographic analysis of central D_1 and D_2 dopamine receptor occupancy in patients treated with classical neuroleptics and clozapine. Relation to extrapyramidal side effects. Arch Gen Psychiatry 1992;49:538–44.
70. Farde L, Wiesel F-A, Halldin C, et al. Central D_2-dopamine receptor occupancy in schizophrenic patients treated with antipsychotic drugs. Arch Gen Psychiatry 1988;45:71–6.
71. Kapur S, Zipursky RB, Remington G. Clinical and theoretical implications of 5-HT_2 and D_2 receptor occupancy of clozapine, risperidone, and olanzapine in schizophrenia. Am J Psychiatry 1999;156:286–93.
72. Nyberg S, Farde L. The relevance of serotonergic mechanisms in the treatment of schizophrenia has not been confirmed. J Psychopharmacol 1997;11:13–4.
73. Knable MB, Heinz A, Raedler T, et al. Extrapyramidal side effects with risperidone and haloperidol at comparable D_2 receptor occupancy levels. Psychiatry Res 1997;75:91–101.
74. Seeman P, Tallerico T. Rapid release of antipsychotic drugs from dopamine D2 receptors: an explanation for low receptor occupancy and early clinical relapse upon drug withdrawal of clozapine or quetiapine. Am J Psychiat 1999;156:876–84.
75. Bressan RA, Erlandsson K, Jones HM, et al. Is regionally selective D_2/D_3 dopamine occupancy sufficient for atypical antipsychotic effect? An in vivo quantitative [^{123}I]epidepride SPEC study of amisulpride-treated patients. Am J Psychiat 2003;160:1413–20.
76. Talvik M, Nordström AL, Nyberg S, et al. No support for regional selectivity in clozapine-treated patients: a PET study with [^{11}C]raclopride and [^{11}C]FLB 457. Am J Psychiat 2001;158:926–30.
77. Kapur S, Seeman P. Does fast dissociation from the dopamine D_2 receptor explain the action of atypical antipsychotics? - A new hypothesis. Am J Psychiat 2001;158:360–9.
78. Lieberman JA, Kane JM, Alvir J. Provocative tests with psychostimulant drugs in schizophrenia. Psychopharmacology 1987;91:415–33.

79. Glatt SJ, Jönsson EG. The Cys allele of the DRD2 Ser311Cys polymorphism has a dominant effect on risk for schizophrenia: evidence from fixed- and random-effects meta-analyses. Am J Med Genet B Neuropsychiatr Genet 2006;141:149–54.
80. Laruelle M, Abi-Dargham A, van Dyck CH, et al. Single photon emission computerized tomography imaging of amphetamine-induced dopamine release in drug-free schizophrenic subjects. Proc Natl Acad Sci USA 1996;93:9235–40.
81. Seeman P, Guan H-C, Niznik HB. Endogenous dopamine lowers the dopamine D_2 receptor density as measured by [^{3}H]raclopride: Implications for positron emission tomography of the human brain. Synapse 1989;3:96–7.
82. Seeman P. Dopamine and Dopamine Receptors. In: Ban TA, R Ucha Udabe R, eds. The Neurotransmitter Era in Neuropsychopharmacology. CINP, Scotland. Editorial Polemos. Buenos Aires, 2006:79–94.
83. Bloom FE, Costa E, Salmoiraghi GC. Anesthesia and the responsiveness of individual neurons of the caudate nucleus of the cat to acetylcholine, norepinephrine and dopamine administered by microelectrophoresis. J Pharmacol Exp Ther 1965;150:244–52.
84. Struyker Boudier HAJ, Gielen W, Cools AR, et al. Pharmacological analysis of dopamine-induced inhibition and excitation of neurones of the snail *Helix Aspersa.* Arch Int Pharmacodyn Ther 1974;209:324–31.
85. Seeman P, Ulpian, C. Neuroleptics have identical potencies in human brain limbic and putamen regions. Eur J Pharmacol 1983;94:45–148.
86. Farde L, Hall H, Ehrin E, et al. Quantitative analysis of D_2 dopamine receptor binding in the living human brain by PET. Science 1986;231:258–61.
87. Zhou QY, Grandy DK, Thambi L, et al. Cloning and expression of human and rat D1 dopamine receptors. Nature 1990;347:76–80.
88. Kawagoe KT, Garris PA, Wiedemann DJ, et al. Regulation of transient dopamine concentration gradients in the microenvironment surrounding nerve terminals in the rat striatum. Neuroscience 1992;51:55–64.
89. Khan ZU, Mrzljak L, Gutierrez A, et al. Prominence of the dopamine D_2 short isoform in dopaminergic pathways. Proc Natl Acad Sci USA 1998;95:7731–6.
90. Klein C, Brin MF, Kramer P, et al. Association of a missense change in the D_2 dopamine receptor with myoclonus dystonia. Proc Natl Acad Sci USA 1999;96:5173–6.
91. Seeman P, Weinshenker D, Quirion R, et al. Dopamine supersensitivity correlates with D_2^{High} states, implying many paths to psychosis. Proc Nat Acad Sci USA 2005;102: 3513–8.
92. Seeman P, Ko F, Tallerico T. Dopamine receptor contribution to the action of PCP. LSD, and ketamine psychotomimetics. Mol Psychiatry 2005;877–83.
93. Seeman P, Tallerico T, Ko F, et al. Amphetamine-sensitized animals show a marked increase in dopamine D_2^{High} receptors occupied by endogenous dopamine, even in the absence of acute challenges. Synapse 2002;46:235–9.
94. Seeman P, Schwarz J, Chen JF, et al. Psychosis pathways converge via D_2^{High} dopamine receptors. Synapse 2006;60:319–46.
95. Montagu KA. Catechol compounds in rat tissues and in brains of different animals. Nature 1957;180:244–5.
96. Weil-Malherbe H, Bone AD. Intracellular distribution of catecholamines in the brain. Nature 1957;180:1050–1.
97. Weil-Malherbe H, Bone AD. Effect of reserpine on the intracellular distribution of catecholamines in the brain stem of the rabbit. Nature 1958;181:1474–5.
98. Carlsson A, Lindqvist M, Magnusson T, et al. 1958. On the presence of 3-hydroxytyramine in brain. Science 1958;127:471.
99. Briand LA, Flagel SB, Seeman P, et al. Cocaine self-administration produces a persistent increase in dopamine D_2^{High} receptors. Eur Neuropsychopharmacol 2008;18:551–6.
100. Simola N, Morelli M, Seeman P. Increase of dopamine D_2^{High} receptor density in the striatum of rats sensitized to caffeine motor effects. Synapse 2008;62:394–7.

101. Sumiyoshi T, Seeman P, Uehara T, et al. Increased proportion of high-affinity dopamine D2 receptors in rats with excitotoxic damage of the entorhinal cortex, an animal model of schizophrenia. Mol Brain Res 2005;140:116–9.
102. Tassin J-P, Torrens Y, Salomon L, et al. Elevated dopamine D_2^{High} receptors in alpha-1b adrenoceptor knockout supersensitive mice. Synapse 2007;61:569–72.
103. Wolinsky TD, Swanson CJ, Smith KE, et al. The trace Amine 1 receptor knockout mouse: an animal model with relevance to schizophrenia. Genes Brain Behav 2007;6:628–39.
104. Seeman P, Hall FS, Uhl G. Increased dopamine D_2High receptors in knockouts of the dopamine transporter and the vesicular monoamine transporter may contribute to spontaneous hyperactivity and dopamine supersensitivity. Synapse 2007;61:573–6.
105. King MV, Seeman P, Marsden C, et al. Increased dopamine D_2High receptors in rats reared in social isolation. Synapse 2009;63:476–83.

Chapter 2
Gene and Promoter Structures of the Dopamine Receptors

Ursula M. D'Souza

Abstract The dopamine receptors have been classified into two groups, the D_1-like and D_2-like dopamine receptors, respectively, based on molecular biology and pharmacological studies. The D_1-like dopamine receptors comprise the D_1 and D_5 dopamine receptors and the D_2-like dopamine receptors include the D_2, D_3 and D_4 dopamine receptors. The gene structures of these two classes of receptors are dissimilar with respect to the organization of their coding and regulatory regions. First, the D_2-like dopamine receptor genes have revealed the presence of coding exons separated by introns whereas the D_1-like dopamine receptor genes consist of a single exon and thus are intronless. Second, examination of the 5′-regulatory regions reveals the presence of non-coding exon(s) several kilobases upstream from their coding exons in the D_2 and D_3 dopamine receptor genes, while regulatory regions of the D_1-like dopamine receptor genes have only one non-coding exon that is separated by a small intron from the coding exon. However, in general, characterization of the 5′-flanking regions of the dopamine receptor genes demonstrates that they lack TATA boxes or CCAAT boxes, are GC rich and have several consensus binding sites for the transcription factor Sp1. The regulatory region of the D_2 dopamine receptor gene is similar to that in the D_3 dopamine receptor gene as they both contain an initiator-like element suggesting transcription initiation from this position and are under strong negative regulation in mammalian cell cultures. Furthermore, amongst the dopamine receptor genes, the 5′-flanking regions of the D_3 and D_5 dopamine receptors have much lower GC content than those in the D_1, D_2 and D_4 dopamine receptor genes. Nevertheless, overall, the promoter regions of all the dopamine receptor genes are regulated in a cell-specific manner, including the additional promoter of the D_1 dopamine receptor gene located within intron 1. There are several studies that have identified transcription factors (DNA binding proteins) that regulate the dopamine receptor genes,

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with more experimental data generated for D_1 and D_2 compared to the D_3, D_4 and D_5 genes. Therefore, all the evidence suggests that the genes encoding the dopamine receptor subtypes have diverse transcriptional regulation mechanisms that result in cell-specific expression patterns that are coupled with different molecular functions.

Keywords Dopamine receptors · Promoters · Transcriptional regulation · Gene structure

2.1 Dopamine Receptors

Dopamine is a catecholamine neurotransmitter that mediates several important physiological functions in both the central and peripheral nervous system. In the brain, it plays a major role in the control of motor function, reward, emotional expression, neuroendocrine release and behavioural homeostasis. Dopamine induces cellular and biochemical effects by interacting with its cell surface receptors [1, 2]. These receptors belong to the superfamily of G protein-coupled receptors having seven transmembrane domains and were first classified in the 1970s based primarily on pharmacological and biochemical studies, which included the rank order of receptor agonist and antagonist affinities [3], and the ability of dopamine to stimulate cAMP formation, by the activation of adenylyl cyclase in the central nervous system [4]. Later on in the 1980s four distinct dopamine receptor subtypes termed D_1, D_2, D_3 and D_4 were proposed on the basis of radioligand binding studies [5, 6]. However, this terminology was soon abandoned when the proposed D_3 and D_4 dopamine receptors were realized to be high-affinity states of the D_1 and D_2 dopamine receptors, respectively. In the 1990s, modification of this dopamine receptor classification was necessary after the development of molecular biology experimental techniques such as PCR cloning which revealed a much larger number of dopamine receptors than originally postulated (see [7] for a review). The current nomenclature for dopamine receptors is based on their structure, pharmacological specificity and effector responses. Consequently, the studies have divided the dopamine receptors into two groups called the D_1-like and the D_2-like dopamine receptors. The D_1-like dopamine receptor group is composed of the D_1 and D_5 dopamine receptors, sometimes also referred to as D_{1A} and D_{1B} dopamine receptors, respectively. The D_2-like dopamine receptors are the D_2, D_3 and D_4 dopamine receptors which include the two D_2 dopamine receptor isoforms, the different isoforms of D_3 and the polymorphic forms of the D_4 dopamine receptors. Molecular cloning of the dopamine receptor genes paved the way for the characterization of their 5′-flanking and promoter regions to understand their transcriptional control. This information further enabled the identification of key polymorphisms (single nucleotide and tandem repeats) within

these regulatory regions which have been found to be associated with several neuropsychiatric and behavioural disorders. Interestingly, these genetic variants within the regulatory regions of the dopamine receptor genes have been found to have functional effects at a molecular and cellular level (reviewed in [8–11]). Thus this provides plausible molecular mechanisms underlying the aetiology of psychiatric phenotypes.

This chapter will describe the gene and promoter structures of the dopamine receptors under subheadings of the D_1-like and D_2-like dopamine receptor genes. The section on the D_1-like dopamine receptor genes will be divided into D_1 and D_5 dopamine receptor genes. Similarly, the subdivision on the D_2-like dopamine receptor genes will be separated into the D_2, D_3 and D_4 dopamine receptor genes. Every section for each gene will be further divided into two parts: one focusing on the topic of gene structure and organization and the other concentrating on the regulatory and promoter regions. All this information has been summarized in Table 2.1 and should be referred for the overall description of each of the dopamine receptor subtypes. However, specific details can be obtained from original references that have been cited in each section.

The themes of transcriptional gene regulation and gene expression have been described concisely in several reports [12–17]. More recently, several other levels of gene regulation are currently being studied and include RNA interference which is important in gene silencing [18] and non-coding RNA (ncRNA) that comprise a hidden layer of internal signals that control various levels of gene expression [19, 20]. These non-coding RNAs include rRNA and tRNA involved in mRNA translation, small nuclear RNA (snRNA) implicated in splicing, small nucleolar RNA (snoRNA) involved in the modification of rRNA and microRNA (miRNA) which function as repressors at the level of post-transcriptional control. Furthermore, since DNA is packaged into a nucleoprotein complex known as chromatin, it is becoming important to understand this structure together with histone modification and cytosine methylation in gene regulation [21].

In general the first stage in characterizing the 5′-flanking and promoter region(s) of a gene involves isolation of a genomic clone that harbours the transcription initiation site and contains the upstream sequence of the gene. This is followed by the determination of the exon/intron organization to identify any untranslated region and then measurement of the transcriptional activity of the upstream regulatory sequences [12]. These strategies involve the generation of serial 5′-deletion plasmid constructs fused with a reporter gene such as luciferase or chloramphenicol acetyltransferase (CAT). These constructs are transiently transfected into mammalian cell lines to determine transcriptional activity of the mutant fragments in vitro or they can be used in vivo to generate transgenic mice. Furthermore, electrophoretic mobility assays and yeast one-hybrid studies have also focused on identifying which DNA binding proteins (transcription factors) interact within the regulatory domains of the genes coding for the dopamine receptors. The findings generated from the methods described above are described and discussed below.

Table 2.1 Description of gene organization and transcriptional regulation of the dopamine receptor genes

	Gene organization [coding and 5′-regulatory region]	Features in promoter region	Upstream regulation	Regulation by transcription factors
D2-like dopamine receptors				
D_2 dopamine receptor	One non-coding exon separated from seven coding exons by large intron	Two promoter regions, initiator-like element, no CCAAT or TATA boxes, 80% GC	Two silencer regions in mammalian cell lines	AP1, retinoids Sp1/Sp3, Zif68, DRRF, nuclear factor-κB
D_3 dopamine receptor	Two non-coding exons separated from six coding exons by large intron	One promoter region, initiator-like element, no CCAAT or TATA boxes, 52% GC	Two silencer regions in neuroblastoma and hepatoblastoma cell lines	DRRF
D_4 dopamine receptor	Four or five coding exons (depends on species) separated by introns	One promoter region, no CCAAT or TATA boxes, CpG island, over 50% GC	Two silencer regions in neuroblastoma and retinoblastoma cell lines	Sp1
D1-like dopamine receptors				
D_1 dopamine receptor	One non-coding exon separated from the coding exon by small intron	Two promoter regions, no CCAAT or TATA boxes, 80% GC	Two activator regions and one silencer region in neuroblastoma cell lines	Sp1, POU, Brn4, Meis2, TGIF, ZIC, Sp3, DRRF
D_5 dopamine receptor	One non-coding exon separated from the coding exon by small intron	One promoter region, no CCAAT or TATA boxes, not GC rich	One activator and one silencer region in neuroblastoma cell lines	Not known to date

2.2 D_2-Like Dopamine Receptor Genes

2.2.1 D_2 Dopamine Receptor Genes

2.2.1.1 Gene Structure and Organization

The cloning of the rat D_2 dopamine receptor was a major breakthrough in the neuroscience field [22]. The cloning strategy that was employed utilized the coding region of the hamster β_2-adrenergic receptor [23]. This genomic DNA sequence was used to probe a rat genomic library for the presence of homologous fragments in Southern blot analysis. Under low-stringency hybridization conditions several clones were found, and one clone (clone RGB-2) was further characterized. This clone consisted of a 0.8 kb EcoRI–PstI fragment that revealed a high degree of similarity to the nucleotide sequence of the putative transmembrane domain of the hamster β_2-adrenergic receptor. The 0.8 kb EcoRI–PstI fragment of RGB-2 was then used to probe a rat brain cDNA library. A full-length cDNA of 2,455 bases was isolated that encoded a protein of 415 amino acids. A hydrophobicity plot of this amino acid sequence indicated that it belonged to the family of G protein-coupled receptors, as it consisted of seven putative transmembrane domains [24]. Subsequently, the human pituitary cDNA ($hPITD_2$) was cloned using rat brain D_2 dopamine receptor cDNA as a hybridization probe [25]. The human and rat nucleotide sequences were found to be 90% identical and they indicated 96% homology at the amino acid level. When $hPITD_2$ cDNA was expressed in mouse Ltk^- cells, the protein showed a pharmacological profile which was essentially identical to that obtained with the cloned rat D_2 dopamine receptor [22]. However, the human pituitary D_2 dopamine receptor encoded a protein of 444 amino acids, 29 amino acids longer than the rat D_2 dopamine receptor. DNA sequence analysis showed that the coding sequence had seven exons interrupted by six introns and that the additional amino acid sequence was encoded by a single exon (exon 5) of 87 base pairs, which was present in the putative third cytoplasmic loop of the receptor. These D_2 dopamine receptors of different sizes from the two species were referred to as the D_{2L} (long) and D_{2S} (short) forms and it was postulated that they were produced by alternative splicing of mRNA [25]. The human D_2 dopamine receptor gene was found to localize to chromosome 11q23-24 [26].

The structure and organization of the rat D_2 dopamine receptor gene was subsequently further delineated when demonstrated that the gene contains eight exons and spans at least 50 kb [27]. This research group identified seven coding exons (numbered as exons 2–8), including the alternatively expressed exon (exon 6), clustered in approximately 13 kb of the genome which revealed a similar structure to the human D_2 dopamine receptor gene. Furthermore, they also identified a non-coding exon termed as exon 1, thus generating a different exon numbering system to that previously used for the human D_2 dopamine receptor gene [25]. Additionally, the same research group consequently analysed the structure of the human D_2 dopamine receptor [28]. Like the rat D_2 dopamine receptor gene, the human D_2 dopamine receptor gene was found to contain at least eight exons and spans at least 52 kb. The

coding exons 2–8 are clustered within 14 kb and the non-coding exon 1 is separated from exon 2 by at least 38 kb. Similarly, the mouse D_2 dopamine receptor gene was found to span at least 30 kb with the coding exons 2–8 clustered in ~11 kb and the non-expressed exon 1 located at least 18 kb away from exon 2 revealing an analogous organization to the rat and human D_2 dopamine receptor genes [29]. Each intron/exon boundary was also sequenced in the mouse D_2 dopamine receptor gene and compared with the rat and human species [29]. The position of all boundaries was conserved in all three species except for intron 4 which contains a variant donor splice site (a GC dinucleotide instead of the canonical GT) in the mouse and rat but not in the human D_2 dopamine receptor gene [27].

Other studies also demonstrated that alternative splicing produces the expression of two rat D_2 dopamine receptor isoforms [27, 30–32]. Furthermore, similar investigations were performed on both the rat and human D_2 dopamine receptor isoforms [31], on the rat and bovine D_2 dopamine receptor isoforms [33–36] and for the mouse D_2 dopamine receptor isoforms [29]. In the literature the long form of the D_2 dopamine receptor was referred to as the D_{2L}, $D_{2(long)}$, D_{2A}, $D_{2(444)}$ or $D_{2\text{-}in}$, whereas the short form was termed D_{2S}, $D_{2(short)}$, D_{2B}, $D_{2(415)}$ or $D_{2\text{-}o}$.

2.2.1.2 Promoter Structure and Transcriptional Regulation

A short fragment of 500 bp from the translational start site of the rat D_2 dopamine receptor gene was initially sequenced [27]. No transcriptional elements such as CCAAT or TATA boxes were found but the region was 78% GC rich and consisted of several Sp1-like binding sites. Subsequently, the analysis of the promoter region of the rat D_2 dopamine receptor gene was comprehensively determined [37]. This analysis included cloning of exon 1, identification of its 5′-end, determination of the transcription start sites and the ability of D_2 promoter deletion mutants to transcribe the reporter gene chloramphenicol acetyltransferase (CAT) in various cell lines. The rat D_2 dopamine receptor gene spans at least 50 kb with coding exons 2–7 clustered in approximately 13 kb of genome, revealing that intron 1 is very long and over 20 kb [27]. A 21-mer oligonucleotide probe consisting of exon 1 sequences [27] was used to screen a rat genomic library [37]. A 1.3 kb region including all of exon 1, its 5′-flanking region and part of intron 1 was sequenced. S1 nuclease analysis indicated three consecutive nucleotides as the main transcription start sites and several weaker sites also noted upstream from the 3′-end of exon 1. The results also reveal no exon further upstream to the non-coding exon 1 in the D_2 dopamine receptor gene. The +1 was designated as the adenine that corresponds to one of the strong S1 signals and to one of the 5′-cDNA ends generated by RACE (rapid amplification of cDNA ends). The promoter region of the D_2 dopamine receptor gene was found to lack TATA and CCAAT boxes and is rich in GC content (reaching 80% in some portions) with several putative binding sites for the transcription factor Sp1. An initiator-like sequence was sited between nucleotides –6 and +11, suggesting transcription initiation from this position. Transient expression assays using 5′-deletion mutant constructs controlling transcription of the CAT gene were determined in murine neuroblastoma cells (NB41A3) that endogenously express the

D_2 dopamine receptor gene. Strongest transcriptional activity was found between nucleotides –75 and –30 and silencing activity was present between nucleotides –217 and –76. DNase I footprinting studies using nuclear extract from NB41A3 cells suggested Sp1 binding to its consensus sequence at nucleotide –48 but inhibition of Sp1 binding at nucleotide –86 by the extract. The D_2 promoter showed no transcription activity of the heterologous CAT gene in rat glioma C6, mouse embryonal NIH 3T3 and human hepatoblastoma Hep G2 cells, indicating that it is regulated in a tissue-specific manner.

Subsequently, another study demonstrated the transcription of the rat D_2 dopamine receptor gene from two promoter regions [38]. Using single-stranded ligation to single-stranded cDNA (SLIC), the gene was found to contain two transcription start sites: the major one located about 320 bp upstream from the 3′-end of the first exon and a minor site 70 bp further upstream. Transient expression assays with fusion constructs consisting of fragments of the rat D_2 promoter region and the luciferase reporter gene confirmed the presence of two independent TATA-lacking promoter regions. Both promoters independently induced transcription of the luciferase gene in C6 glioma cells, fibroblasts and GH3 and MMQ rat pituitary cell lines, although only the MMQ cells express the D_2 dopamine receptor. The transcriptional activity was enhanced in the presence of both promoters and modified by the upstream sequences. These data differ from that derived by [37] and were suggested to be due to the use of different reporter gene assays with varying sensitivities and/or the utilization of different cell lines [38].

The negative modulator of the rat D_2 dopamine receptor gene was further analysed [39]. In this study, a small deletion series within the negative modulator fused with the CAT reporter gene was used to transfect the D_2-expressing cells, NB41A3. The results identified two *cis*-acting functional DNA sequences. The first is a 41 bp segment between nucleotides –116 and –76 (D_2Neg-B) and the second is a 26 bp segment between nucleotides –160 and –135 (D_2Neg-A). D_2Neg-B decreased transcription from the D_2 promoter by 45%, whereas D_2Neg-A in the presence of the downstream negative modulator reduced transcription down to the level of a promoterless vector. Furthermore, DNase I footprinting, gel mobility shift and competitive cotransfection experiments suggested that D_2Neg-A functions without *trans*-acting factors, while D_2Neg-B interacts with nuclear factors at its Sp1 binding sequences. Gel supershift assays with anti-Sp1 antibody and UV cross-linking experiments revealed that a novel 130 kDa factor as well as Sp1 interacts with D_2Neg-B in NB41A3 cells. The novel protein that recognizes Sp1 binding sequences in the D_2 gene negative modulator was also found to be present in rat striatum nuclear extract.

In the case of the human D_2 dopamine receptor gene only a small fragment of the 5′-flanking region was isolated and sequenced which enabled screening of genetic variants [40]. A significant polymorphism in the D_2 promoter is the –141C Ins/Del (insertion/deletion), where one or two cytosines are found as part of a putative binding site for the transcription factor Sp1. Constructs consisting of the –141C Del allele cloned into a plasmid with the luciferase reporter gene demonstrated lower transcriptional activity in human retinoblastoma Y-79 cells (that express D_2) and

human kidney 293 cells (D_2 non-expressing) compared to the –141C Ins allele [40]. Interestingly, it was additionally demonstrated in case–control studies that the –141C Del allele was significantly lower in schizophrenic patients than in control subjects in Japanese and Swedish populations [40, 41].

The promoter region of the D_2 dopamine receptor has been found to be regulated by other transcription factors including retinoids [42, 43], AP1 [44], Sp1/Sp3 [45] and Zif268 in the rat [46], by nuclear factor-κB in human [47] and by dopamine receptor regulating factor (DRRF) in mouse [48]. The latter report showed that DRRF is a zinc finger transcription factor that binds to GC and GT boxes in the D_2 dopamine receptor promoters and effectively displaces Sp1 and Sp3 from these sequences. Highest levels of DRRF mRNA were found in mouse brain in areas including olfactory bulb and tubercle, nucleus accumbens, striatum, hippocampus, amygdala and frontal cortex. Interestingly, these brain regions also express abundant levels of dopamine receptors, indicating the importance of DRRF in regulating dopaminergic neurotransmission. In the D_2-expressing NB41A3 cells, DRRF potently inhibited transcription from the D_2 promoter, whereas it was found to activate the D_2 promoter in NS20Y and TE671 cells. In vivo experiments show that DRRF mRNA is significantly altered in striatum and nucleus accumbens brain regions in mice treated with acute and chronic doses of cocaine and haloperidol. Furthermore, in situ hybridization studies in mice have shown that DRRF mRNA is expressed uniquely during development with high levels observed at E12, E14 and E16 in various tissues [49]. DRRF expression during development is also found in particular brain regions such as the neopallial cortex, olfactory lobe and corpus striatum. This pattern of DRRF distribution during embryogenesis overlaps with that found in the adult brain and with the expression profile of dopamine receptors both in adult and during development. Additionally, the promoter region of murine DRRF was characterized, revealing tissue-specific activity, suggesting that it shares structural and functional similarities with the dopamine receptor genes that it regulates [50]. More recently, it has been found that DRRF auto-regulates its own promoter by competing with Sp1 and that both AP1 and AP2 modulate its expression [51]. Additionally a small segment of the rat D_2 promoter has been found to be regulated by corticosterone and oestrogen in NB41A3 cells in vitro [52, 53].

Moreover, DNA methylation has been demonstrated within the promoter region of the human D_2 dopamine receptor gene, suggesting that this transcription regulatory mechanism plays a role in controlling human D_2 dopamine receptor gene expression [54]. Lately, the 5′-regulatory region of the human D_2 dopamine receptor gene has been found to have methylated cytosines mainly in three clusters [55].

2.2.2 D_3 Dopamine Receptor Genes

2.2.2.1 Gene Structure and Organization

The molecular cloning of the rat D_3 dopamine receptor gene was performed using reverse transcription polymerase chain reaction (RT-PCR) [56]. Genomic and cDNA

libraries were screened using a probe derived from the D_2 dopamine receptor sequence published earlier [22]. The positive clone that was obtained coded for a protein of 446 amino acid residues, and hydrophobicity analysis of the clone indicated seven putative transmembrane regions characteristic of G protein-coupled receptors. The D_3 dopamine receptor gene contained introns similar to the D_2 dopamine receptor gene, and 75% homology existed between the rat D_{2S} and D_3 dopamine receptor genes within the transmembrane regions. Consistent with this high sequence homology, the pharmacological properties of the D_3 receptor were similar to but distinct from those of the D_2 dopamine receptor. The rat D_3 dopamine receptor gene contains six coding exons separated by five introns. In humans, this gene is located on chromosome 3, band 3q13.3 [57], with a coding region consisting of six exons over 53 kb and an open reading frame of only 400 amino acids. This difference of 46 amino acid residues between the rat and human D_3 dopamine receptors is located within the third cytoplasmic loop of the protein [58].

The polymerase chain reaction amplification of mRNA from rat brain revealed the existence of two shorter isoforms of the D_3 dopamine receptor in addition to the D_3 dopamine receptor itself [59]. The isoforms were suggested to result from different processes of alternative splicing. One form was produced by splicing of an exon whose absence deletes the third transmembrane domain, resulting in a protein (termed D_3(TM3-del)) having no dopaminergic ligand binding activity. The second isoform resulted from splicing at a receptor site that coded for half the second extracellular loop and part of the fifth transmembrane domain (referred to as D_3(O2-del)). Other research groups have also demonstrated splice variants of the D_3 dopamine receptor in the rat and human brain [60–62], but none of the truncated proteins encoded by these variants has dopamine receptor activity. However, the alternatively spliced short isoform of the mouse D_3 dopamine receptor that lacks 63 nucleotides in the third cytoplasmic loop was found to bind dopaminergic ligands [63]. This alternative splicing reflects the presence of a sixth intron found in the mouse D_3 receptor gene [64, 65]**.** The functional and physiological role of the truncated forms of the D_3 dopamine receptors is not known, but it has been suggested that they could be formed for controlling the amount of active D_3 dopamine receptors [59]. Defects in the regulation of alternative splicing of the receptor could result in formation of inactive D_3 dopamine receptors and may be associated with psychiatric disorders.

2.2.2.2 Promoter Structure and Transcriptional Regulation

The D_3 dopamine receptor gene has been implicated in neuropsychiatric disorders and found to be regulated following antipsychotic drug treatment [66, 67]. To begin with only a short segment of the 5′-untranslated region of the D_3 dopamine receptor gene was described in the mouse [65] and human [62]. However, a comprehensive investigation of the gene's transcriptional control was elucidated when the 5′-flanking region was characterized by isolating the 5′-end of its cDNA as well as 4.6 kb of genomic sequence [68]. Analysis of this region revealed the presence of two new (untranslated) exons of 196 bp and 120 bp, designated exon 1 and exon

2, that are separated by an 855-bp intron located several kilobases (at least 4 kb) upstream of the previously published coding exons. This evidence shows that the rat D_3 dopamine receptor gene is organized into eight exons and is comparable to the structure of the rat D_2 dopamine receptor gene. The rat D_2 dopamine receptor gene has a single non-coding exon located at least 35 kb upstream from its first of seven coding exons [27, 37, 38]. However, sequence comparison between the 5′-UTR and 5′-flanking regions of the rat D_2 and D_3 dopamine receptor genes shows substantial homology. On the other hand, the D_{1A} dopamine receptor gene has been found to have a different organization with a non-coding exon separated from a single coding exon by a small intron [69, 70], which is 116 bp in humans (see Section 2.3). There is no sequence homology between the 5′-flanking region of the D_{1A} and the rat D_3 dopamine receptor genes.

The transcription initiation site of the rat D_3 dopamine receptor gene determined by primer extension analysis and repeated rounds of 5′-RACE (rapid amplification of cDNA ends) was found to consist of a pyrimidine-rich consensus "initiator" sequence, similar to the rat D_2 dopamine receptor gene [37, 68]. The promoter region of the rat D_3 dopamine receptor gene did not reveal any TATA and CCAAT boxes but unlike that of D_1 and D_2 dopamine receptor genes has only 52% GC content. These results demonstrate that the rat D_3 dopamine gene has similarities with the rat D_2 dopamine receptor gene as both are transcribed from a TATA-less promoter that has an initiator element. Functional studies of rat D_3 promoter deletion mutants fused to the CAT reporter gene were carried out in a human medulloblastoma cell line (TE671 cells) [68]. These cells endogenously express the D_3 dopamine receptor [71]. Strongest transcriptional activity was determined within 36 nucleotides upstream of the transcriptional start site and a potent silencer identified between bases –37 and –86 which extends to –537 as transcriptional activity is noticeably and gradually reduced with the addition of sequences between –36 and –537 until complete inhibition. There was a small recovery of reporter gene activity with the addition of nucleotides –538 to –782, suggesting the presence of a potential activator. Additionally, another weaker silencer is located between nucleotides –783 and –1,046. These data suggest that the rat D_3 dopamine receptor gene is under intense negative regulation and similar to that observed with the rat D_2 dopamine receptor gene. Interestingly, none of the D_3 deletion mutant constructs showed any transcriptional activity in COS-7 (African green monkey kidney) or C6 rat glioma cells, which are not known to express the D_3 dopamine receptor gene endogenously. However, the shortest construct having 36 nucleotides from the transcriptional start site also showed significant transcriptional activity in OK (opossum kidney) and HepG2 (human hepatoblastoma) cells even though the evidence suggests that these cells do not express the D_3 dopamine receptor mRNA. However, unlike the potent silencing effect of the longer upstream regulatory regions of the D_3 gene in TE671 cells, these fragments showed only weak inhibition in OK cells or strong activation in HepG2 cells. Thus although the core promoter of the rat D_3 dopamine receptor gene is active in these three different cell types, its regulation by the upstream elements varies in D_3- and non-D_3-expressing cells. The presence of specific transcription factors in the different cell lines could help explain the complex differential

regulation of the rat D_3 dopamine receptor gene. Interestingly, the transcription factor dopamine receptor regulating factor (DRRF) was found to activate the regulatory region of the rat D_3 dopamine receptor gene in TE671 cells [48].

Subsequently, a 9 kb genomic fragment of the human D_3 dopamine receptor gene was isolated upstream from the translational start site [72]. Studies using 5′-RACE identified three additional exons, and transcriptional activity was found in two putative 500 bp 5′-regions derived from brain tissue and lymphoblast cells following transfection in human cell lines. However, interpretations of these findings were ambiguous as the transcriptional start site was not accurately determined and no series of deletion constructs tested for transcriptional activity. The main focus of this report appeared to be identification of single nucleotide polymorphisms in the 5′-region of the gene, but no association was found with schizophrenia.

2.2.3 D_4 Dopamine Receptor Genes

2.2.3.1 Gene Structure and Organization

The human D_4 dopamine receptor was cloned and characterized after screening various cell lines for other D_2-like dopamine receptors [73]. The cloning strategy employed in the discovery of this receptor utilized the D_2 dopamine receptor sequence that encoded for the sixth and seventh putative transmembrane regions [22]. This fragment of the rat D_2 dopamine receptor served as a probe for screening genomic and cDNA libraries under low- and high-stringency conditions. The genomic intron–exon organization of the human D_4 dopamine receptor gene indicated the presence of five coding exons for a protein of 387 amino acids. Hydrophobicity analysis of the protein sequence indicated seven putative transmembrane regions, which suggested that it belonged to the family of G protein-coupled receptors. The pharmacological characteristics of the D_4 dopamine receptor resembled that of the D_2 and D_3 dopamine receptors. However, the "atypical neuroleptic" drug clozapine had higher affinity for D_4 than for D_2 and D_3 dopamine receptors.

The rat analogue of the human D_4 dopamine receptor gene was cloned, also revealing high affinity for clozapine [74]. This rat gene was found to have only four coding exons which encoded a protein of 368 amino acids, suggesting an additional splice site in the human D_4 dopamine receptor gene. However, despite the differences in gene structure the rat gene shares a high homology of 73% and 77% with the human D_4 gene at the amino acid and nucleic acid level, respectively. Interestingly, the amino acid homology of the gene between the two species increased to 89–96% when only the transmembrane regions were considered. The majority of the differences observed amongst the rat and human D_4 dopamine receptor genes occurred within the third cytoplasmic loop, which have only 50% amino acid identity. In the human gene, this region contains an unusual splice site within intron 3 (a donor/acceptor site of TC/CT is present instead of the GT/AG). Additionally, the rat D_4 dopamine receptor mRNA was detected in the cardiovascular system in addition to the brain, suggesting that this receptor is an important

dopamine receptor in the peripheral nervous system [74]. Similar to the rat D_4 gene, the murine D_4 dopamine receptor gene was found to have four coding exons that span over 30 kb [75]. The gene encodes a 387 amino acid protein displaying 80% and 95% homology with the human and rat D_4 dopamine receptors, respectively, at the amino acid level. Likewise, at the nucleotide level the mouse D_4 dopamine receptor gene revealed 79% and 93% homology with the human and rat D_4 dopamine receptor genes, respectively. The most conserved regions were seen within the transmembrane domains that are thought to form the ligand binding site.

Three polymorphic forms of the D_4 dopamine receptor in humans were further discovered [76]. These were the three most common variants, having 2-, 4- or 7-fold imperfect repeats of a 48 bp sequence in the putative third cytoplasmic loop of the receptor, located in exon 3 of the gene. This was the first example of polymorphic variation observed in catecholamine receptors. Although the different forms of the receptor showed slightly different pharmacological profiles with drugs spiperone and clozapine, they all coupled to G proteins [76]. Similarly, the same group later found that the polymorphic repeat sequences conferred only small differences in pharmacological binding properties [77, 78] and also in functional properties to inhibit cyclic adenosine monophosphate [79]. Therefore, it was concluded from the evidence that there was no direct relationship between length of the polymorphism and changes in these activities. The D_4 dopamine receptor variants were also capable of coupling to several G protein ($G_{i\ \alpha}$) subtypes, but no evidence of any quantitative difference in G protein coupling related to repeat length was observed [80]. However, transcriptional differences were observed when the repeat variants were cloned downstream from the luciferase gene in expression vectors and tested in a somatomammotrophic (GH4C1) cell line [81]. Constructs having 7 repeat sequences significantly suppressed expression of the reporter gene compared to those consisting of the 2 and 4 repeats, which was suggested to be via mechanisms involving RNA stability or translational efficiency. More recently, dopamine was found to be more potent at D_4 receptors having 2 and 7 repeats than those with 4 repeats, suggesting that the actions of dopamine and therapeutic drugs on D_4 dopamine receptors may vary amongst individuals depending on the variants they have [82].

At least 19 different repeat unit sequences, used in 25 different haplotypes that code for 18 different unique receptor variants, were identified in the human D_4 dopamine receptor gene [83]. More recently, an extra 35 different alleles have been detected in the population having single nucleotide polymorphisms [84]. The 7-repeat allele was initially reported to be associated with the personality of novelty seeking [85, 86] and then more lately with attention deficit hyperactivity disorder (ADHD) [87–90].

2.2.3.2 Promoter Structure and Transcriptional Regulation

The 5′-flanking region of the human D_4 dopamine receptor gene was isolated and sequenced, revealing a transcription initiation region located between –501 and –436 bp relative to the first nucleotide of the translational codon [91]. A CpG island

spanned the region from –900 to +500 bp (which is over 50% GC rich) but no TATA or CCAAT boxes were present in the 5′-flanking region. However, the region was found to have consensus binding sites for transcription factors including Sp1. These properties are similar in the 5′-regulatory sequences of the D_1, D_2, D_3 and D_5 dopamine receptor genes. Functional analysis of deletion constructs fused to the CAT reporter gene and transiently transfected into IMR32 (neuroblastoma) and Y-79 (retinoblastoma) cells demonstrated promoter activity in the region around –591 and –123 bp and the presence of a negative modulator between –770 and –679 bp. However, no transcriptional activity was observed in human epithelial (HeLa) cells, suggesting cell-specific regulation of the human D_4 dopamine receptor gene.

The 5′-flanking region of the human D_4 dopamine receptor contains several polymorphisms [92]. One of these is a –521 C/T polymorphism that showed weak association to schizophrenia. Functional studies demonstrated that this promoter polymorphism had reduced transcriptional activity compared to the C allele in human neuroblastoma cells (Y-79) [93]. However, more lately the –521 C/T polymorphism showed no significant differences in transcriptional activity in three human neuroblastoma (SK-N-F1, IMR32 and Y-79) cell lines [94]. The discrepancy in results between the two studies was suggested to be due to the use of different reporter vectors and variation in the length of the cloned fragment that harboured the polymorphism. Interestingly, the transcriptional regulation of the human D_4 dopamine receptor gene was also further analysed in SK-N-F1, Y-79, IMR32 and HeLa cells [94]. The highest transcriptional activity was observed between nucleotides –668 and –389 from the translational start site and a putative silencer region was located from –1,571 to –800 bp. These results together with the previous findings on the regulatory region of the D_4 dopamine receptor gene [91] suggest that the gene may possess two negative modulators, one of which was not functional in the cell systems utilized in the study by [94].

A tandem duplication of 120 bp located 1.2 kb upstream from the initiation codon and approximately 850 bp upstream from the transcription start site was identified in the human D_4 dopamine receptor gene [95]. This polymorphism has been found to be associated with ADHD [96–98]. Transient transfection in human neuroblastoma cells and other human cell lines with coupled luciferase reporter gene assays demonstrated that the duplication had lower transcriptional activity in human neuroblastoma cells (SK-N-MC, SH-SY5Y), human embryonic kidney cells (HEK293) and HeLa cells compared to the non-duplicated form [99]. These data also suggested that the D_4 dopamine receptor gene may have an alternative promoter in an intron region, as the tandem duplication revealed promoter activity. This is yet to be confirmed, but this speculation is in agreement with a previous report that suggested that the promoter region characterized by [91] could be in an intron region due to the discrepancy in size of mRNA from previous studies [100]. They also suggested that a large intron could separate the coding exons of the D_4 dopamine receptors from potential untranslated exons similar to that observed for the other D_2-like receptors [100], and the interpretation of findings from the functional studies of the tandem duplication were discussed in light of these considerations [99].

A recent study has verified these functional data, also showing that the duplicated allele but this case in constructs comprising the major part of the 5′-regulatory region showed lower transcriptional activity in human cell lines Y-79, SK-N-F1 and HeLa compared to the non-duplicated form [101]. They also identified a 4-repeat allele of this polymorphism which showed dose-dependent functional effects with the lowest transcriptional activity in the cell lines tested.

Capillary electrophoretic mobility shift assays were used to compare the binding capacity of the transcription factor Sp1 to the polymorphic 120 bp sequence in the human D_4 dopamine receptor gene [102]. The data suggest enhanced binding capacity of the transcription factor to the duplicated form using HeLa nuclear extracts. However, these data have not been verified independently using other cell culture systems and/or other techniques such as standard electrophoretic mobility shift assays (EMSA).

2.3 D_1-Like Dopamine Receptor Genes

2.3.1 Gene Structure and Organization of D_1-Like Dopamine Receptors

The human and rat D_1 dopamine receptors were cloned by several groups [103–106]. This was achieved using a cloning strategy based on the nucleotide sequence of the D_2 dopamine receptor gene [22]. The cloned D_1 dopamine receptor encodes a protein of 446 amino acids and the gene consists of one coding exon and thus was found to be intronless. Hydrophobicity analysis of the amino acid sequence of the receptor indicated that it belonged to the family of G protein-coupled receptors, as it consists of the characteristic seven stretches of hydrophobic amino acid residues. The structural features of this receptor include a short third cytoplasmic loop and a long carboxyl-terminal tail, in contrast to the D_2 dopamine receptor with a long third cytoplasmic loop and short carboxyl-terminal tail. Furthermore, the cloned D_1 dopamine receptor when transfected into a cell line was able to stimulate adenylyl cyclase in response to dopamine. This clearly indicated that cellular physiological responses mediated at the D_1 dopamine receptor were different from those mediated at the D_2 dopamine receptor. A second member of the D_1 dopamine receptor subfamily was then cloned and referred to as the D_5 dopamine receptor as it had affinity for dopamine and lower expression in the brain compared to the D_1 dopamine receptor [107]. After the cloning of the D_5 dopamine receptor, the cloning of the rat D_1 dopamine receptor subtype was reported [108]. They referred to this receptor as the D_{1B} dopamine receptor, and the previously cloned D_1 dopamine receptors were termed D_{1A} dopamine receptor [103–106]. The cloned rat D_{1B} dopamine receptor encoded a protein of 475 amino acids and belonged to the family of proteins that couple to G proteins. The distribution of the receptor was found to be similar to that of the D_5 dopamine receptor and it was suggested that the D_{1B} dopamine receptor is the rat ortholog of the D_5 dopamine receptor, and therefore the terms D_{1B} and D_5

were used interchangeably to describe the same receptor [109]. The current standard nomenclature for dopamine receptors refers to these subtypes as D_1 and D_5 (http://www.iuphar-db.org/GPCR/ChapterMenuForward?chapterID=1282).

Interesting developments arose when three genes related to the D_1 dopamine receptor were identified in the human genome [110–112]. The authors demonstrated that one of the genes lacked introns and was found to function as human D_5 dopamine receptor. However, the other two genes were pseudogenes. The transcript of one of these pseudogenes is expressed in several brain regions and produces a protein of 154 amino acids [113]. The human D_1 dopamine receptor is located on chromosome 5 at q35.1 [114] and the functional human D_5 dopamine receptor gene is localized to chromosome 4p16.1 [115]. The first and second pseudogenes of the D_5 dopamine receptor genes demonstrated chromosome localization to 2p11.1-p11.2 and 1q21.1, respectively [115].

2.3.2 Promoter Region of the D_1 Dopamine Receptor Gene

The 5′-flanking region of the human D_1 dopamine receptor gene was characterized by sequencing a 2.3 kb genomic fragment from –2,571 to –236 bp relative to the adenosine of the first methionine codon [69]. S1 nuclease mapping and reverse transcription PCR revealed the presence of a small intron of 116 bp (–599 to –484) and an exon of about 440 bases in the 5′-non-coding region upstream from the coding exon in the human D_1 dopamine receptor gene which was previously thought to be intronless [103–105]. The rat D_1 dopamine receptor gene was also found to have a small intron in its 5′-untranslated region [70] and additional information on its 5′-flanking region has been described at the end of the section. The human D_1 dopamine receptor gene has multiple transcription initiation sites located between –1,061 and –1,040. The promoter region lacks a TATA box and a CCAAT box, is rich in G+C content (up to 80% in some regions) and has several putative binding sites for the general transcription factor Sp1 [69]. Thus the promoter region of D_1 dopamine receptor was similar to that in the D_2 gene having similar features. However, it also has consensus sequences for a putative cAMP response element and binding sites for the transcription factors AP1 and AP2. Transient expression assays suggested the presence of a positive modulator between nucleotides –1,340 and –1,102 and a negative modulator between –1,730 and –1,341 in the murine neuroblastoma cell line (NS20Y), with no or very low transcriptional activity in NB41A3, C6 and HepG2 cells which do not express the D_1 dopamine receptor gene, suggesting regulation in a tissue-specific manner. It was later demonstrated that the transcriptional activity of the intron within the human D_1 dopamine receptor gene is higher than the upstream promoter by 12-fold in SK-N-MC cells and by 5.5-fold in NS20Y cells in an orientation-dependent manner [116]. Studies in SK-N-MC cells additionally revealed that longer D_1 mRNAs including exon 1 are degraded 1.8 times faster than the shorter D_1 transcripts. Thus all this evidence indicates that the human D_1 dopamine receptor gene is transcribed in neural cells from a second strong promoter that is located in the intron and generates shorter transcripts

deficient in exon 1. However, only this short D_1 dopamine receptor transcript was present in human and rat kidneys [117]. These data demonstrate that the upstream promoter is active only in neural cells, whereas the intron promoter is active in both neuronal and renal cells. However, the activator region (nucleotides –1,154 to –1,136) that enhances transcriptional activity of the upstream promoter in SK-N-MC and NS20Y could not activate this promoter in OK cells [117]. Furthermore, gel shift assays using nuclear extracts from either OK cells or rat kidney tissue showed no protein binding to the activator region. The results suggest that differential expression of long and short D_1 dopamine receptor transcripts is due to tissue-specific expression of the activator protein binding to the activator region promoting transcription from the upstream promoter. It was suggested that absence of this protein would result in a non-functional D_1 upstream promoter in the kidney.

The activator region of the human D_1 dopamine receptor gene was further analysed and found to localize to two regions (–1,154 to –1,137 and –1,197 to –1,154) which increased promoter activity [118]. In addition, both the –1,197 to –1,154 region and the core promoter located downstream to –1,102 resulted in cell-specific D_1 dopamine receptor gene expression. Additionally, DNase I footprinting and gel shift assays revealed DNA protein interactions mainly in the region between –1,197 and –1,116. Furthermore, functional significance of nuclear factors interacting with the activator regions of the human D_1 dopamine receptor gene was determined in the neuroblastoma cell line SK-N-MC using competitive cotransfections with different fragments of this region. Novel transcription factors interacted with the D_1-Act-1 (–1,197 to –1,152) sequence even though it does not have consensus binding sites for known transcription factors. DNA binding proteins interact with D_1-Act-2 (–1,154 to –1,116) sequence as a complex that includes Sp1 or Sp1-like protein as well as a novel factor. However, even though recombinant AP2 binds to some of its consensus sequences in the D_1 dopamine receptor gene, it was suggested that it is unlikely that AP2 has a significant role in positively modulating the basal expression of the gene in NS20Y cells as the AP2 consensus sequences in the D_1 dopamine activator could not bind to nuclear extracts of these cells [118].

Supplementary transcriptional regulation studies of the human D_1 dopamine receptor gene by cAMP revealed location of two cAMP responsive regions in exon 1, both of which interacted with nuclear proteins in D_1-expressing cells SK-N-MC [119]**.** The segment of D_1 gene between these two regions strongly interacted with nuclear proteins following forskolin/IBMX which directly increases cAMP levels. A different study demonstrated that 6.4 kb upstream region on the human D_1 dopamine receptor gene is sufficient to confer tissue-specific expression in specific D_1-expressing brain regions of transgenic mice in vivo and in neuroblastoma cells in vitro [120].

The regulatory regions of the rat and mouse D_1 dopamine receptor genes have additionally been studied. The promoter region of the rat D_1 dopamine receptor gene was initially characterized locating the transcription start site to 864 bp upstream from the translational start site [70]. Like the human gene, the 5′-flanking region of the rat gene does not have any TATA or CCAAT boxes but is G + C rich, with binding sites for the transcription factors Sp1, Ap1 and Ap2 and with potential

cAMP and glucocorticoid response element sequences. Transfection studies using fusion constructs with the CAT reporter gene demonstrated that the rat D_1 promoter is active in D_1-expressing neuroblastoma NS20Y cells but inactive in glioma C6 and human embryonic kidney (HEK293) cells that do not express the D_1 dopamine receptor. A fragment of 735 bp of the 5′-flanking region of the gene produced this cell-specific promoter activity which was responsive to cAMP, suggesting an autoregulation mechanism by which stimulation of the gene exerts a positive feedback on its expression.

Second, the 5′-flanking region of the murine D_1 dopamine receptor gene was investigated to determine the utility of its promoters in brain tissue-specific expression of transgenes [121]. The presence of two functional promoters was confirmed similar to that found for the rat and human D_1 dopamine receptor genes [116, 117]. Transient expression analyses using CAT fusion constructs revealed that the murine D_1 upstream promoter fused with the activator region of the human D_1 gene has strong transcriptional activity in NS20Y cells that express D_1 but not in renal (OK), glial (C6) and hepatic (HepG2) cells. This suggests that the hybrid construct harbours neural cell-specific elements and this could be tested for the neuronal-specific expression of transgenes in vivo [121].

Most of the evidence for the interaction of DNA binding proteins with the regulatory region of the D_1 dopamine receptor has been generated from studies with the human gene. The transcription factors that have been found to regulate the human D_1 dopamine receptor gene include POU factors such as Brn-4 [122, 123] and also Meis2 (myeloid ecotropic viral integration site 1) and TGIF (5′-TG-3′-interacting factor) which belong to the family of three-amino acid extension loop (TALE) homeobox proteins [124]. Furthermore, the zinc finger protein ZIC2 and factor Sp3 were found to repress Sp1-induced activation of the human D_1 dopamine receptor gene, an effect that involved the activator region in the regulatory fragment of the gene [125]. The hormone oestrogen has additionally been found to upregulate transcription of the human D_1 dopamine receptor in neuroblastoma cell lines [126]. The zinc finger-type transcription factor DRRF has been found to repress the promoter region of the D_1 dopamine receptor in NS20Y cells and activate the D_1 dopamine receptor promoter in TE671 cells [48]. This protein binds to the GC and GT boxes in the D_1 dopamine receptor promoter and effectively displaces Sp1 and Sp3 from these sequences. Thus DRRF regulates dopamine receptor subtypes and has opposing effects depending on cellular context.

2.3.3 Promoter Region of the D_5 Dopamine Receptor Gene

There is some evidence on the transcriptional regulation of the D_5 dopamine receptor following characterization of the 5′-flanking and promoter regions of the human gene [127]. Comparison of genomic and cDNA sequences revealed the presence of two exons separated by a small intron. The 5′-flanking region showed no TATA and CCAAT boxes but contained several putative binding sites for the transcription factors Sp1 and Ap1, thus revealing a similar structure to the other dopamine receptor

genes. However, the D_5 regulatory region is not GC rich and has a major transcription initiation site determined 2,125 bp upstream from the translational start site, in contrast to [12, 127]. Constructs consisting of the luciferase gene and up to 500 bp of the 5′-transcription initiation site showed transcriptional activity in SK-N-SH cells but not COS-7, CHO (Chinese hamster ovary), NB41A3 and SK-N-MC cells. Deletion analysis indicated that the regulatory region of the D_5 dopamine receptor gene has a positive modulator at 119–182 bp and a negative modulator 251–500 bases upstream from the transcriptional start site in the neuroblastoma cell line SK-N-MC cells. This information resulted in an understanding of the regulatory control mechanisms of the human D_5 dopamine receptor genes. In addition, even though a polymorphic dinucleotide (TC) repeat in the promoter region of the human gene was identified and demonstrated to have transcriptional activity in human neuroblastoma cells (SK-N-SH), there were no significant differences in the functional effect of these genetic variants [128]. To date there are no reports on the 5′-flanking regions of the D_5 dopamine receptor gene from other species.

References

1. Strange PG. New insights into dopamine receptors in the central nervous system. Neurochem Int 1993; 22(3):223–36.
2. Zeng C, Zhang M, Asico LD, Eisner GM, Jose PA. The dopaminergic system in hypertension. Clin Sci (Lond) 2007; 112(12):583–97.
3. Seeman P, Guan HC. Dopamine D1 and D2 receptors are sensitive to the cationic form of apomorphine. Mol Pharmacol 1987; 32(6):760–3.
4. Kebabian JW, Petzold GL, Greengard P. Dopamine-sensitive adenylate cyclase in caudate nucleus of rat brain, and its similarity to the "dopamine receptor". Proc Natl Acad Sci USA 1972; 69(8):2145–9.
5. Seeman P. Brain dopamine receptors. Pharmacol Rev 1980; 32(3):229–313.
6. Sokoloff P, Martres MP, Schwartz JC. Three classes of dopamine receptor (D-2, D-3, D-4) identified by binding studies with 3H-apomorphine and 3H-domperidone. Naunyn Schmiedebergs Arch Pharmacol 1980; 315(2):89–102.
7. Andersen PH, Gingrich JA, Bates MD et al. Dopamine receptor subtypes: beyond the D1/D2 classification. Trends Pharmacol Sci 1990; 11(6):231–6.
8. Wong AH, Buckle CE, Van Tol HH. Polymorphisms in dopamine receptors: what do they tell us? Eur J Pharmacol 2000; 410(2–3):183–203.
9. McAllister TW, Summerall L. Genetic polymorphisms in the expression and treatment of neuropsychiatric disorders. Curr Psychiatry Rep 2003; 5(5):400–09.
10. Ueno S. Genetic polymorphisms of serotonin and dopamine transporters in mental disorders. J Med Invest 2003; 50(1–2):25–31.
11. D'Souza UM, Craig IW. Functional polymorphisms in dopamine and serotonin pathway genes. Hum Mutat 2006; 27(1):1–13.
12. Mouradian MM, Minowa MT, Minowa T. Dopamine receptor genes: promoters and transcriptional activation. In Dopamine receptors and transporters. Marcel Dekker Niznik HB, eds. New York: Marcel Dekker, 205–35. 1994.
13. Latchman D. Gene regulation. In: A eukaryotic perspective 3rd ed. Cheltenham: Stanley Thornes, Ltd. 1998.
14. Carey M, Smale ST. Transcriptional regulation in eukaryotes: concepts, strategies and techniques. Cold Spring Harbor, New York: Cold Spring Harbor Laboratory Press. 2000.

15. Fessele S, Maier H, Zischek C, Nelson PJ, Werner T. Regulatory context is a crucial part of gene function. Trends Genet 2002; 18(2):60–63.
16. D'Souza UM, Kel A, Sluyter F. From transcriptional regulation to aggressive behavior. Behav Genet 2003; 33(5):549–62.
17. Dunn RK, Kingston RE. Gene regulation in the postgenomic era: technology takes the wheel. Mol Cell 2007; 28(5):708–14.
18. Kim DH, Rossi JJ. Strategies for silencing human disease using RNA interference. Nat Rev Genet 2007; 8(3):173–84.
19. Mattick JS, Makunin IV. Non-coding RNA. Hum Mol Genet 2006; 15 Spec No 1:R17–29.
20. Chen K, Rajewsky N. The evolution of gene regulation by transcription factors and microRNAs. Nat Rev Genet 2007; 8(2):93–103.
21. Rando OJ. Chromatin structure in the genomics era. Trends Genet 2007; 23(2):67–73.
22. Bunzow JR, Van Tol HH, Grandy DK et al. Cloning and expression of a rat D2 dopamine receptor cDNA. Nature 1988; 336(6201):783–7.
23. Dixon RA, Kobilka BK, Strader DJ et al. Cloning of the gene and cDNA for mammalian beta-adrenergic receptor and homology with rhodopsin. Nature 1986; 321(6065):75–9.
24. Dohlman HG, Bouvier M, Benovic JL, Caron MG, Lefkowitz RJ. The multiple membrane spanning topography of the beta 2-adrenergic receptor. Localization of the sites of binding, glycosylation, and regulatory phosphorylation by limited proteolysis. J Biol Chem 1987; 262(29):14282–88.
25. Grandy DK, Marchionni MA, Makam H et al. Cloning of the cDNA and gene for a human D2 dopamine receptor. Proc Natl Acad Sci USA 1989; 86(24):9762–6.
26. Grandy DK, Litt M, Allen L et al. The human dopamine D2 receptor gene is located on chromosome 11 at q22-q23 and identifies a TaqI RFLP. Am J Hum Genet 1989; 45(5): 778–85.
27. O'Malley KL, Mack KJ, Gandelman KY, Todd RD. Organization and expression of the rat D2A receptor gene: identification of alternative transcripts and a variant donor splice site. Biochemistry 1990; 29(6):1367–71.
28. Gandelman KY, Harmon S, Todd RD, O'Malley KL. Analysis of the structure and expression of the human dopamine D2A receptor gene. J Neurochem 1991; 56(3):1024–9.
29. Mack KJ, Todd RD, O'Malley KL. The mouse dopamine D2A receptor gene: sequence homology with the rat and human genes and expression of alternative transcripts. J Neurochem 1991; 57(3):795–801.
30. Giros B, Sokoloff P, Martres MP, Riou JF, Emorine LJ, Schwartz JC. Alternative splicing directs the expression of two D2 dopamine receptor isoforms. Nature 1989; 342(6252): 923–6.
31. Dal Toso R, Sommer B, Ewert M et al. The dopamine D2 receptor: two molecular forms generated by alternative splicing. EMBO J 1989; 8(13):4025–34.
32. Monsma FJ, Jr., McVittie LD, Gerfen CR, Mahan LC, Sibley DR. Multiple D2 dopamine receptors produced by alternative RNA splicing. Nature 1989; 342(6252):926–9.
33. Chio CL, Hess GF, Graham RS, Huff RM. A second molecular form of D2 dopamine receptor in rat and bovine caudate nucleus. Nature 1990; 343(6255):266–9.
34. O'Dowd BF, Nguyen T, Tirpak A et al. Cloning of two additional catecholamine receptors from rat brain. FEBS Lett 1990; 262(1):8–12.
35. Rao DD, McKelvy J, Kebabian J, MacKenzie RG. Two forms of the rat D2 dopamine receptor as revealed by the polymerase chain reaction. FEBS Lett 1990; 263(1):18–22.
36. Miller JC, Wang Y, Filer D. Identification by sequence analysis of a second rat brain cDNA encoding the dopamine (D2) receptor. Biochem Biophys Res Commun 1990; 166(1): 109–12.
37. Minowa T, Minowa MT, Mouradian MM. Analysis of the promoter region of the rat D2 dopamine receptor gene. Biochemistry 1992; 31(36):8389–96.
38. Valdenaire O, Vernier P, Maus M, Dumas Milne Edwards JB, Mallet J. Transcription of the rat dopamine-D2-receptor gene from two promoters. Eur J Biochem 1994; 220(2):577–84.

39. Minowa T, Minowa MT, Mouradian MM. Negative modulator of the rat D2 dopamine receptor gene. J Biol Chem 1994; 269(15):11656–62.
40. Arinami T, Gao M, Hamaguchi H, Toru M. A functional polymorphism in the promoter region of the dopamine D2 receptor gene is associated with schizophrenia. Hum Mol Genet 1997; 6(4):577–82.
41. Jonsson EG, Nothen MM, Neidt H et al. Association between a promoter polymorphism in the dopamine D2 receptor gene and schizophrenia. Schizophr Res 1999; 40(1):31–6.
42. Samad TA, Krezel W, Chambon P, Borrelli E. Regulation of dopaminergic pathways by retinoids: activation of the D2 receptor promoter by members of the retinoic acid receptor-retinoid X receptor family. Proc Natl Acad Sci U S A 1997; 94(26):14349–54.
43. Valdenaire O, Maus-Moatti M, Vincent JD, Mallet J, Vernier P. Retinoic acid regulates the developmental expression of dopamine D2 receptor in rat striatal primary cultures. J Neurochem 1998; 71(3):929–36.
44. Wang J, Miller JC, Friedhoff AJ. Differential regulation of D2 receptor gene expression by transcription factor AP-1 in cultured cells. J Neurosci Res 1997; 50(1):23–31.
45. Yajima S, Lee SH, Minowa T, Mouradian MM. Sp family transcription factors regulate expression of rat D2 dopamine receptor gene. DNA Cell Biol 1998; 17(5):471–9.
46. Takeuchi Y, Miyamoto E, Fukunaga K. Activation of the rat dopamine D2 receptor promoter by mitogen-activated protein kinase and Ca2+/calmodulin-dependent protein kinase II pathways. J Neurochem 2002; 83(4):784–96.
47. Bontempi S, Fiorentini C, Busi C, Guerra N, Spano P, Missale C. Identification and characterization of two nuclear factor-kappaB sites in the regulatory region of the dopamine D2 receptor. Endocrinology 2007; 148(5):2563–70.
48. Hwang CK, D'Souza UM, Eisch AJ et al. Dopamine receptor regulating factor, DRRF: a zinc finger transcription factor. Proc Natl Acad Sci U S A 2001; 98(13):7558–63.
49. D'Souza UM, Lammers CH, Hwang CK, Yajima S, Mouradian MM. Developmental expression of the zinc finger transcription factor DRRF (dopamine receptor regulating factor). Mech Dev 2002; 110(1–2):197–201.
50. Lee SH, Kim YM, Yajima S et al. Genomic organization and promoter characterization of the murine dopamine receptor regulating factor (DRRF) gene. Gene 2003; 304:193–9.
51. Lee SH, Jang MK, Lee OH et al. Transcriptional auto-regulation of the dopamine receptor regulating factor (DRRF) gene. Mol Cell Endocrinol 2008; 289(1–2):23–28.
52. Lammers CH, D'Souza UM, Qin ZH, Lee SH, Yajima S, Mouradian MM. Regulation of striatal dopamine receptors by corticosterone: an in vivo and in vitro study. Brain Res Mol Brain Res 1999; 69(2):281–5.
53. Lammers CH, D'Souza U, Qin ZH, Lee SH, Yajima S, Mouradian MM. Regulation of striatal dopamine receptors by estrogen. Synapse 1999; 34(3):222–7.
54. Popendikyte V, Laurinavicius A, Paterson AD, Macciardi F, Kennedy JL, Petronis A. DNA methylation at the putative promoter region of the human dopamine D2 receptor gene. Neuroreport 1999; 10(6):1249–55.
55. Zhang AP, Yu J, Liu JX et al. The DNA methylation profile within the 5′-regulatory region of DRD2 in discordant sib pairs with schizophrenia. Schizophr Res 2007; 90(1–3): 97–103.
56. Sokoloff P, Giros B, Martres MP, Bouthenet ML, Schwartz JC. Molecular cloning and characterization of a novel dopamine receptor (D3) as a target for neuroleptics. Nature 1990; 347(6289):146–51.
57. Le Coniat M, Sokoloff P, Hillion J et al. Chromosomal localization of the human D3 dopamine receptor gene. Hum Genet 1991; 87(5):618–20.
58. Giros B, Martres MP, Sokoloff P, Schwartz JC. [Gene cloning of human dopaminergic D3 receptor and identification of its chromosome]. C R Acad Sci III 1990; 311(13):501–8.
59. Giros B, Martres MP, Pilon C, Sokoloff P, Schwartz JC. Shorter variants of the D3 dopamine receptor produced through various patterns of alternative splicing. Biochem Biophys Res Commun 1991; 176(3):1584–92.

60. Snyder LA, Roberts JL, Sealfon SC. Alternative transcripts of the rat and human dopamine D3 receptor. Biochem Biophys Res Commun 1991; 180(2):1031–5.
61. Nagai Y, Ueno S, Saeki Y, Soga F, Yanagihara T. Expression of the D3 dopamine receptor gene and a novel variant transcript generated by alternative splicing in human peripheral blood lymphocytes. Biochem Biophys Res Commun 1993; 194(1):368–74.
62. Griffon N, Crocq MA, Pilon C et al. Dopamine D3 receptor gene: organization, transcript variants, and polymorphism associated with schizophrenia. Am J Med Genet 1996; 67(1):63–70.
63. Fishburn CS, Belleli D, David C, Carmon S, Fuchs S. A novel short isoform of the D3 dopamine receptor generated by alternative splicing in the third cytoplasmic loop. J Biol Chem 1993; 268(8):5872–78.
64. Fu D, Skryabin BV, Brosius J, Robakis NK. Molecular cloning and characterization of the mouse dopamine D3 receptor gene: an additional intron and an mRNA variant. DNA Cell Biol 1995; 14(6):485–92.
65. Park BH, Fishburn CS, Carmon S, Accili D, Fuchs S. Structural organization of the murine D3 dopamine receptor gene. J Neurochem 1995; 64(2):482–6.
66. Wang W, Hahn KH, Bishop JF, Gao DQ, Jose PA, Mouradian MM. Up-regulation of D3 dopamine receptor mRNA by neuroleptics. Synapse 1996; 23(3):232–5.
67. D'Souza U, McGuffin P, Buckland PR. Antipsychotic regulation of dopamine D1, D2 and D3 receptor mRNA. Neuropharmacology 1997; 36(11–12):1689–96.
68. D'Souza UM, Wang W, Gao DQ et al. Characterization of the 5′ flanking region of the rat D(3) dopamine receptor gene. J Neurochem 2001; 76(6):1736–44.
69. Minowa MT, Minowa T, Monsma FJ, Jr., Sibley DR, Mouradian MM. Characterization of the 5′ flanking region of the human D1A dopamine receptor gene. Proc Natl Acad Sci U S A 1992; 89(7):3045–9.
70. Zhou QY, Li C, Civelli O. Characterization of gene organization and promoter region of the rat dopamine D1 receptor gene. J Neurochem 1992; 59(5):1875–83.
71. Levavi-Sivan B, Park BH, Fuchs S, Fishburn CS. Human D3 dopamine receptor in the medulloblastoma TE671 cell line: cross-talk between D1 and D3 receptors. FEBS Lett 1998; 439(1–2):138–42.
72. Anney RJ, Rees MI, Bryan E et al. Characterisation, mutation detection, and association analysis of alternative promoters and 5′ UTRs of the human dopamine D3 receptor gene in schizophrenia. Mol Psychiatry 2002; 7(5):493–502.
73. Van Tol HH, Bunzow JR, Guan HC et al. Cloning of the gene for a human dopamine D4 receptor with high affinity for the antipsychotic clozapine. Nature 1991; 350(6319):610–4.
74. O'Malley KL, Harmon S, Tang L, Todd RD. The rat dopamine D4 receptor: sequence, gene structure, and demonstration of expression in the cardiovascular system. New Biol 1992; 4(2):137–46.
75. Fishburn CS, Carmon S, Fuchs S. Molecular cloning and characterisation of the gene encoding the murine D4 dopamine receptor. FEBS Lett 1995; 361(2–3):215–9.
76. Van Tol HH, Wu CM, Guan HC et al. Multiple dopamine D4 receptor variants in the human population. Nature 1992; 358(6382):149–52.
77. Asghari V, Schoots O, van Kats S et al. Dopamine D4 receptor repeat: analysis of different native and mutant forms of the human and rat genes. Mol Pharmacol 1994; 46(2):364–73.
78. Jovanovic V, Guan HC, Van Tol HH. Comparative pharmacological and functional analysis of the human dopamine D4.2 and D4.10 receptor variants. Pharmacogenetics 1999; 9(5):561–8.
79. Asghari V, Sanyal S, Buchwaldt S, Paterson A, Jovanovic V, Van Tol HH. Modulation of intracellular cyclic AMP levels by different human dopamine D4 receptor variants. J Neurochem 1995; 65(3):1157–65.
80. Kazmi MA, Snyder LA, Cypess AM, Graber SG, Sakmar TP. Selective reconstitution of human D4 dopamine receptor variants with Gi alpha subtypes. Biochemistry 2000; 39(13):3734–44.

81. Schoots O, Van Tol HH. The human dopamine D4 receptor repeat sequences modulate expression. Pharmacogenomics J 2003; 3(6):343–8.
82. Wedemeyer C, Goutman JD, Avale ME, Franchini LF, Rubinstein M, Calvo DJ. Functional activation by central monoamines of human dopamine D(4) receptor polymorphic variants coupled to GIRK channels in Xenopus oocytes. Eur J Pharmacol 2007; 562(3):165–73.
83. Lichter JB, Barr CL, Kennedy JL, Van Tol HH, Kidd KK, Livak KJ. A hypervariable segment in the human dopamine receptor D4 (DRD4) gene. Hum Mol Genet 1993; 2(6):767–73.
84. Grady DL, Chi HC, Ding YC et al. High prevalence of rare dopamine receptor D4 alleles in children diagnosed with attention-deficit hyperactivity disorder. Mol Psychiatry 2003; 8(5):536–45.
85. Benjamin J, Li L, Patterson C, Greenberg BD, Murphy DL, Hamer DH. Population and familial association between the D4 dopamine receptor gene and measures of Novelty Seeking. Nat Genet 1996; 12(1):81–4.
86. Ebstein RP, Novick O, Umansky R et al. Dopamine D4 receptor (D4DR) exon III polymorphism associated with the human personality trait of Novelty Seeking. Nat Genet 1996; 12(1):78–80.
87. Faraone SV, Biederman J, Weiffenbach B et al. Dopamine D4 gene 7-repeat allele and attention deficit hyperactivity disorder. Am J Psychiatry 1999; 156(5):768–70.
88. Mill J, Curran S, Kent L et al. Attention deficit hyperactivity disorder (ADHD) and the dopamine D4 receptor gene: evidence of association but no linkage in a UK sample. Mol Psychiatry 2001; 6(4):440–4.
89. Langley K, Marshall L, van den BM et al. Association of the dopamine D4 receptor gene 7-repeat allele with neuropsychological test performance of children with ADHD. Am J Psychiatry 2004; 161(1):133–8.
90. Li D, Sham PC, Owen MJ, He L. Meta-analysis shows significant association between dopamine system genes and attention deficit hyperactivity disorder (ADHD). Hum Mol Genet 2006; 15(14):2276–84.
91. Kamakura S, Iwaki A, Matsumoto M, Fukumaki Y. Cloning and characterization of the 5′-flanking region of the human dopamine D4 receptor gene. Biochem Biophys Res Commun 1997; 235(2):321–6.
92. Paterson AD, Sunohara GA, Kennedy JL. Dopamine D4 receptor gene: novelty or nonsense? Neuropsychopharmacology 1999; 21(1):3–16.
93. Okuyama Y, Ishiguro H, Toru M, Arinami T. A genetic polymorphism in the promoter region of DRD4 associated with expression and schizophrenia. Biochem Biophys Res Commun 1999; 258(2):292–5.
94. Kereszturi E, Kiraly O, Barta C, Molnar N, Sasvari-Szekely M, Csapo Z. No direct effect of the -521 C/T polymorphism in the human dopamine D4 receptor gene promoter on transcriptional activity. BMC Mol Biol 2006; 7:18.
95. Seaman MI, Fisher JB, Chang F, Kidd KK. Tandem duplication polymorphism upstream of the dopamine D4 receptor gene (DRD4). Am J Med Genet 1999; 88(6):705–9.
96. McCracken JT, Smalley SL, McGough JJ et al. Evidence for linkage of a tandem duplication polymorphism upstream of the dopamine D4 receptor gene (DRD4) with attention deficit hyperactivity disorder (ADHD). Mol Psychiatry 2000; 5(5):531–6.
97. Mill J, Fisher N, Curran S, Richards S, Taylor E, Asherson P. Polymorphisms in the dopamine D4 receptor gene and attention-deficit hyperactivity disorder. Neuroreport 2003; 14(11):1463–6.
98. Kustanovich V, Ishii J, Crawford L et al. Transmission disequilibrium testing of dopamine-related candidate gene polymorphisms in ADHD: confirmation of association of ADHD with DRD4 and DRD5. Mol Psychiatry 2004; 9(7):711–7.
99. D'Souza UM, Russ C, Tahir E et al. Functional effects of a tandem duplication polymorphism in the 5′flanking region of the DRD4 gene. Biol Psychiatry 2004; 56(9):691–7.
100. Todd RD, O'Malley KL. The dopamine receptor DRD4 gene: are duplications distracting? Trends Pharmacol Sci 2001; 22(2):55–6.

101. Kereszturi E, Kiraly O, Csapo Z et al. Association between the 120-bp duplication of the dopamine D4 receptor gene and attention deficit hyperactivity disorder: genetic and molecular analyses. Am J Med Genet B Neuropsychiatr Genet 2007; 144(2):231–6.
102. Ronai Z, Guttman A, Keszler G, Sasvari-Szekely M. Capillary electrophoresis study on DNA-protein complex formation in the polymorphic 5′ upstream region of the dopamine D4 receptor (DRD4) gene. Curr Med Chem 2004; 11(8):1023–9.
103. Dearry A, Gingrich JA, Falardeau P, Fremeau RT, Jr., Bates MD, Caron MG. Molecular cloning and expression of the gene for a human D1 dopamine receptor. Nature 1990; 347(6288):72–6.
104. Zhou QY, Grandy DK, Thambi L et al. Cloning and expression of human and rat D1 dopamine receptors. Nature 1990; 347(6288):76–80.
105. Sunahara RK, Niznik HB, Weiner DM et al. Human dopamine D1 receptor encoded by an intronless gene on chromosome 5. Nature 1990; 347(6288):80–3.
106. Monsma FJ, Jr., Mahan LC, McVittie LD, Gerfen CR, Sibley DR. Molecular cloning and expression of a D1 dopamine receptor linked to adenylyl cyclase activation. Proc Natl Acad Sci U S A 1990; 87(17):6723–7.
107. Sunahara RK, Guan HC, O'Dowd BF et al. Cloning of the gene for a human dopamine D5 receptor with higher affinity for dopamine than D1. Nature 1991; 350(6319):614–9.
108. Tiberi M, Jarvie KR, Silvia C et al. Cloning, molecular characterization, and chromosomal assignment of a gene encoding a second D1 dopamine receptor subtype: differential expression pattern in rat brain compared with the D1A receptor. Proc Natl Acad Sci U S A 1991; 88(17):7491–5.
109. Schwartz JC. Multiple dopamine receptors: functional implications. Clin Neuropharmacol 1992; 15(Suppl 1 Pt A):1A-2A.
110. Grandy DK, Zhang YA, Bouvier C et al. Multiple human D5 dopamine receptor genes: a functional receptor and two pseudogenes. Proc Natl Acad Sci U S A 1991; 88(20):9175–9.
111. Weinshank RL, Adham N, Macchi M, Olsen MA, Branchek TA, Hartig PR. Molecular cloning and characterization of a high affinity dopamine receptor (D1 beta) and its pseudogene. J Biol Chem 1991; 266(33):22427–35.
112. Nguyen T, Bard J, Jin H et al. Human dopamine D5 receptor pseudogenes. Gene 1991; 109(2):211–8.
113. Nguyen T, Sunahara R, Marchese A, Van Tol HH, Seeman P, O'Dowd BF. Transcription of a human dopamine D5 pseudogene. Biochem Biophys Res Commun 1991; 181(1):16–21.
114. Grandy DK, Zhou QY, Allen L et al. A human D1 dopamine receptor gene is located on chromosome 5 at q35.1 and identifies an EcoRI RFLP. Am J Hum Genet 1990; 47(5): 828–34.
115. Grandy DK, Allen LJ, Zhang Y, Magenis RE, Civelli O. Chromosomal localization of three human D5 dopamine receptor genes. Genomics 1992; 13(4):968–73.
116. Lee SH, Minowa MT, Mouradian MM. Two distinct promoters drive transcription of the human D1A dopamine receptor gene. J Biol Chem 1996; 271(41):25292–9.
117. Lee SH, Wang W, Yajima S, Jose PA, Mouradian MM. Tissue-specific promoter usage in the D1A dopamine receptor gene in brain and kidney. DNA Cell Biol 1997; 16(11): 1267–75.
118. Minowa MT, Minowa T, Mouradian MM. Activator region analysis of the human D1A dopamine receptor gene. J Biol Chem 1993; 268(31):23544–51.
119. Minowa MT, Lee SH, Mouradian MM. Autoregulation of the human D1A dopamine receptor gene by cAMP. DNA Cell Biol 1996; 15(9):759–67.
120. Severynse DM, Colapietro AM, Box TL, Caron MG. The human D1A dopamine receptor gene promoter directs expression of a reporter gene to the central nervous system in transgenic mice. Brain Res Mol Brain Res 1995; 30(2):336–46.
121. Lee SH, Yajima S, Mouradian MM. Neural cell line-specific regulatory DNA cassettes harboring the murine D1A dopamine receptor promoter. Neurosci Res 1999; 34(4): 225–34.

122. Okazawa H, Imafuku I, Minowa MT, Kanazawa I, Hamada H, Mouradian MM. Regulation of striatal D1A dopamine receptor gene transcription by Brn-4. Proc Natl Acad Sci U S A 1996; 93(21):11933–8.
123. Imafuku I, Kamei M, Kanazawa I, Mouradian MM, Okazawa H. POU transcription factors differentially regulate the D1A dopamine receptor gene in cultured cells. Biochem Biophys Res Commun 1996; 222(3):736–41.
124. Yang Y, Hwang CK, D'Souza UM, Lee SH, Junn E, Mouradian MM. Three-amino acid extension loop homeodomain proteins Meis2 and TGIF differentially regulate transcription. J Biol Chem 2000; 275(27):20734–41.
125. Yang Y, Hwang CK, Junn E, Lee G, Mouradian MM. ZIC2 and Sp3 repress Sp1-induced activation of the human D1A dopamine receptor gene. J Biol Chem 2000; 275(49):38863–9.
126. Lee SH, Mouradian MM. Up-regulation of D1A dopamine receptor gene transcription by estrogen. Mol Cell Endocrinol 1999; 156(1–2):151–7.
127. Beischlag TV, Marchese A, Meador-Woodruff JH et al. The human dopamine D5 receptor gene: cloning and characterization of the 5′-flanking and promoter region. Biochemistry 1995; 34(17):5960–70.
128. Beischlag TV, Nam D, Ulpian C, Seeman P, Niznik HB. A polymorphic dinucleotide repeat in the human dopamine D5 receptor gene promoter. Neurosci Lett 1996; 205(3):173–6.

Chapter 3
Structural Basis of Dopamine Receptor Activation

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Abstract G protein-coupled receptors (GPCRs) are seven transmembrane (TM) proteins representing the largest and most universally expressed cell surface receptors and are present in almost all species and in a wide variety of cells. Here we will focus our attention on the catecholamine-binding GPCRs and in particular on the dopamine receptors. The catecholamine-binding GPCRs form a group of rhodopsin-like GPCRs composed of adrenoceptors, which are endogenously activated by epinephrine and norepinephrine, and dopamine receptors. We review the different "molecular switches" involved in GPCR activation and we emphasize the importance of extracellular loop 2 (ECL2) in ligand binding. A better understanding of the functional role of ECL2 can be achieved after the release of the crystal structures of B2AR and rhodopsin, which are consistent with dopamine D2 receptor substituted cysteine accessibility method (SCAM) experimental data. Even though reconstituted GPCR monomers appear sufficient to activate a G protein, in the native setting their dimerization/oligomerization may modulate activation through changes at the dimerization interface or a larger-scale reorientation of the protomers. Therefore, the structural aspects of oligomerization and their importance for receptor activation and signaling are also addressed.

Keywords Catecholamine-binding GPCRs · Dopamine receptors · Binding site · ECL2 · GPCR oligomerization · GPCR–G Protein interaction · Activation · Structural rearrangements

3.1 Introduction

G protein-coupled receptors (GPCRs) are seven transmembrane (TM) proteins representing the largest and most universally expressed cell surface receptors. GPCRs

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are present in almost all species and in a wide variety of cells [1–8]. They play important roles in a broad array of cellular functions and in disease and represent the targets for a large fraction of existing drugs [9–12]. GPCRs are classified into three major classes based on the size of the N termini, on sequence homology, the identity of conserved residues within the seven TM domains that participate in ligand binding, mode of action, and pharmacology [13, 14]. The largest family is Class A (more than 90%), which comprises rhodopsin as well as receptors for biogenic amines, peptides, and odorants. Class B receptors are a much smaller group and include receptors for large peptides such as secretin, cytokines, thrombin, and glucagon. Class C receptors (comprised of approximately 12 members) include the γ-aminobutyric acid B receptor ($GABA_B$), eight metabotropic glutamate receptors, the Ca^{2+} sensing receptor, as well as some pheromone and taste receptors [15]. GPCRs, upon ligand binding, induce dissociation of G proteins into their G_α and $G_{\beta\gamma}$ components and ultimately modulate the activity of enzyme or ion channel effectors [5, 16–19].

Structurally, GPCRs are made up of seven TM segments connected by three intracellular and three extracellular loops (ICL, ECL), and Class A receptors share important functionally conserved sites identified as structural motifs that act as functional microdomains, such as the D(E)RY motif in TM3 and NPXXY in TM7 [8, 20–28]. The first GPCR structure, bovine rhodopsin, was solved in 2000 [29], and there was much anticipation that many other GPCR structures would be rapidly forthcoming. Although a number of different rhodopsin structures were solved, 7 years passed without any other GPCR structures, in support of the unique biochemical properties of rhodopsin, including its high abundance and its unusual stability, retaining function under conditions that denature other GPCRs, due to the covalently bound 11-*cis*-retinal, which maintains the receptor in an inactive conformation [30–32].

At the end of 2007, two new crystal structures of the human β2 adrenergic receptor (B2AR) were solved, including the wild-type receptor bound to an antibody fragment and an engineered receptor with T4 lysozyme inserted into the third intracellular loop [31–33]. Although the B2AR crystallographic structures are quite similar to rhodopsin with a root mean square deviation of 1.6 Å, there are some interesting differences, which impact on considerations of the structure of the dopamine receptor family, for which a structure is not yet available. Very recently two new structures of the β1 adrenergic receptor [34] and the adenosine A2 receptor [35] have been solved, and while there are interesting differences, the overall structures are again quite similar.

Here we will focus our attention on the catecholamine-binding GPCRs and in particular on the dopamine receptors. The catecholamine-binding GPCRs form a group of rhodopsin-like GPCRs composed of adrenoceptors, which are endogenously activated by epinephrine and norepinephrine, and dopamine receptors [36]. For the adrenoceptors, there are three main classes based on their pharmacological properties, amino acid sequences, and signaling mechanisms. These adrenoceptor classes were subsequently divided in humans into three subtypes each: α_1 (α_{1A}, α_{1B}, α_{1D}), α_2 (α_{2A}, α_{2B}, α_{2C}), and β (β_1, β_2, β_3). These receptors respond to the

neurotransmitters/hormones, norepinephrine and epinephrine, which play key roles in regulation of cardiovascular function, energy metabolism, and blood pressure [15]. In contrast to adrenoceptors, dopamine receptors in human are divided into two classes: D_1-like receptors (D_{1A} or D_1 and D_{1B} or D_5) and D_2-like receptors (D_2, D_3, and D_4). While sharing some common properties, each receptor displays unique properties including affinity for dopamine, specificity for G protein coupling and signaling, and specific neuronal distributions [37]. Furthermore, in the case of the D_2 receptor (D2R) subfamily, there are two isoforms: the long isoform D_{2L} and the short isoform D_{2S}, generated by alternative splicing of an 87-bp exon. This splicing event leads to an additional 29 amino acids in the ICL3 of the isoform D_{2L} [38].

3.2 Transmembrane Segments and Activation

TM segment interactions are a key determinant in the assembly and stability of the native structure of membrane proteins [39–41]. As the sequence conservation within the membrane-spanning regions is high, it is thought that class A GPCRs share a similar architecture [16, 17, 41, 42], which has been supported to date by the four different receptors for which we have crystal structures. The catecholamine-binding GPCRs share within their TM regions 20–26% sequence identity with rhodopsin [36]. For example, the sequence identity between the TM domains of rhodopsin and B2AR is 21%, between rhodopsin and D2R is 25%, and between D2R and B2AR is 38% [36].

Some of the most important features of the TM domains are the kinks and bends generated by prolines and glycines, respectively [23, 43–46]. Serines, threonines, and cysteines can also bend the α-helices that constitute the TM domains [24, 47]. In rhodopsin, TM1 possesses a proline-induced kink that bends it inward, toward the helix bundle. It was proposed that other GPCRs, which do not have this proline in TM1, including the D2R, might be packed somewhat differently with TM1 more distant from the bundle [8, 24, 48]. Consistent with such an orientation of TM1, the extracellular segment of TM1 of D2R did not seem to contribute to the binding site based on substituted cysteine accessibility method (SCAM) studies [8, 49]. The B2AR 3D structure validated this hypothesis because its TM1 is comparatively straight [32]. Moreover, although the TM segments in rhodopsin and B2AR have similar orientations, there are some differences: the angles between TM1, TM3, and TM6 and the membrane are different from their counterparts in rhodopsin, TM4 is translated away from the center of the receptor, and TM5 is translated closer to the center of the receptor [32].

It is hypothesized that GPCRs exist as an ensemble of various conformational states that are in a dynamic equilibrium, and that agonist binding and subsequent activation occur through a series of conformational intermediates [50, 51]. Ligands have the ability to stabilize or possibly induce specific conformations [52]. Mutations that disrupt stabilizing non-covalent interactions favor more active receptor conformations by increasing the movement of the TM segments relative to

each other [52]. Although there are different ligand-binding modes in the different GPCR classes, activation processes are thought to result from similar conformational changes involving the TM domain [53–59]. In particular, rotation and outward movement of TM6 likely open a crevice allowing for interaction with the C terminus of the G protein α-subunit and triggering GPCR activation [59–61].

Many GPCRs show a considerable amount of basal, agonist-independent activity, reflecting GPCR structural flexibility and the existence of conformational ensembles [52]. The study of constitutively active GPCRs has contributed to our understanding of the activation mechanism [57, 62–65]. Mutation of certain residues in GPCRs significantly increases their constitutive activation [66, 67] by breaking crucial intramolecular interactions between amino acid residues that normally constrain the receptor to its inactive state [21, 24, 62]. Many residues that produce constitutive activation when mutated are linked through packing interactions with residues that are essential for receptor activation by side chain rearrangement on adjacent TMs and/or by larger-scale TM movements [62, 68–70]. Some of the most well-known constitutively active mutants (CAMs) are those that disrupt the highly conserved (D/E)R(Y/W) amino acid sequence present in 72% of class A GPCRs. By contrast, an "ionic lock" is crucially involved in maintaining the inactive state of the receptor [21, 24, 71–73]. This is exemplified in a network of hydrogen bonding and charge interactions between Glu134$^{3.49}$ and Arg135$^{3.50}$ at the cytoplasmic end of TM3 and Glu247$^{6.30}$ and Thr251$^{6.34}$ at the cytoplasmic end of TM6 of rhodopsin (Ballesteros general number in the superscript [7]) [23, 24, 74]. In the B2AR structures, the "ionic lock" is broken, which may account for the residual basal activity of the B2AR bound to the inverse agonist carazolol [31, 33, 51]. Recent computational studies suggest that the ionic lock dynamically forms and unforms in association with conformational change in ICL2 [75].

Besides the "ionic lock," there are other "molecular switches" involving non-covalent intramolecular interactions that must be altered to achieve an active state. The "rotamer toggle switch" involves Phe290$^{6.52}$, which is accessible in the binding site crevice and serves as a "sensor," a change in the bend of TM6 at the highly conserved residue Pro288$^{6.50}$, and a change in the rotamer of Trp265$^{6.48}$ upon activation of rhodopsin and related family members [46]. Although carazolol does not directly interact with the "toggle switch" in the B2AR TM6, it seems to modulate the rotameric state of Trp286 indirectly by interacting with Phe289$^{6.51}$ and Phe290$^{6.52}$ [31–33, 76, 77]. These kinds of molecular switches can be studied experimentally and computationally [6, 25, 71, 72].

3.3 The Binding Site

SCAM studies, experimental approaches such as studies of chimeric receptors and point mutants, as well as molecular modeling allowed for the identification of amino acids that line the putative binding pocket of the D2R [48, 78–96] (Fig. 3.1). The findings for D2R are in agreement with results for other catecholamine-binding receptors [36]. The binding crevice has two polar regions common to all these

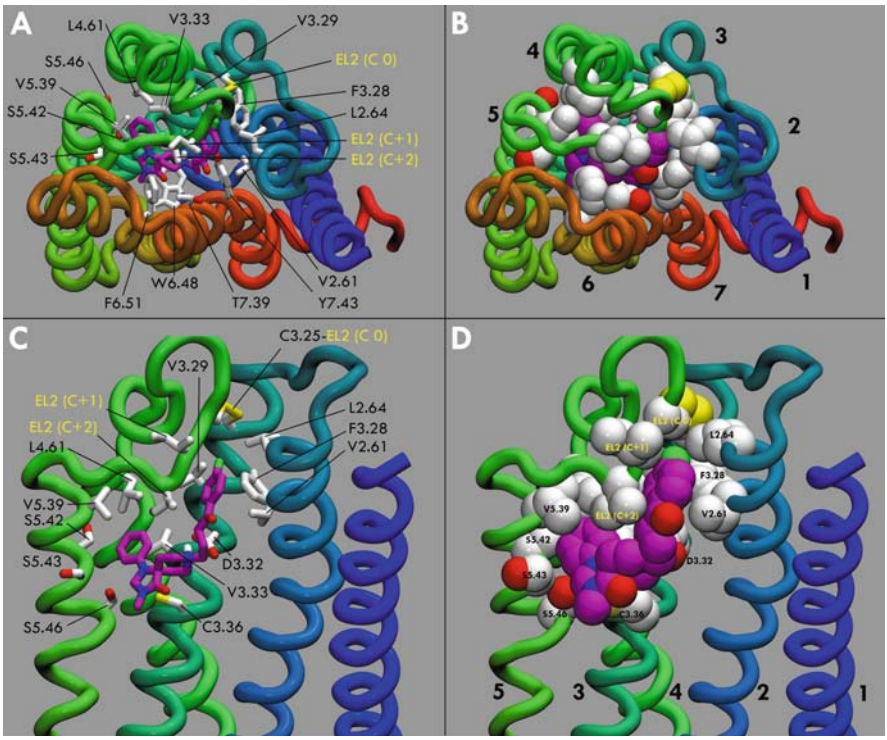

Fig. 3.1 *N*-Methylspiperone (MSP) docked into the binding site crevice of the dopamine D_2 receptor (D2R). Panel (**A**): From an extracellular perspective, TMs 1–7 are colored from *blue* to *red*. MSP (carbon *magenta*, stick) is nestled in the binding site and capped by EL2. D2R side chains within 4 Å of MSP are shown (carbon *white*, stick) and labeled according to the Ballesteros and Weinstein indexing system. Residues in EL2, $Ile183^{EL2(C+1)}$, and especially $Ile184^{EL2(C+2)}$ (labeled in *yellow*), provide substantial hydrophobic contacts to the ligand. Other residues within 4 Å of MSP are $Val91^{2.61}$, $Leu94^{2.64}$, $Phe110^{3.28}$, $Val111^{3.29}$, $Asp114^{3.32}$, $Val115^{3.33}$, $Cys118^{3.36}$, $Leu171^{4.61}$, $Cys182^{EL2(C\,0)}$, $Val190^{5.39}$, $Ser193^{5.42}$, $Ser194^{5.43}$, $Ser197^{5.46}$, $Trp357^{6.48}$, $Phe360^{6.51}$, $Thr383^{7.39}$, and $Tyr387^{7.43}$ [D2R (*short*) UNIPROT sequence P14416-2]. Panel (**B**): To emphasize the hydrophobic packing of the EL2 and crevice residues with bound MSP, the contact side chains from **A** are rendered as van der Waals spheres. TMs are numbered. Panel (**C**): Peering into the crevice from a side view (TMs 6–7 are removed) reveals some key interactions between MSP and the D2R binding site crevice and EL2. Key interactions include a hydrogen bond-reinforced ionic interaction between $Asp114^{3.32}$ and the ligand's amine moiety, deep occupancy of the MSP's phenyl-imidazolidinone group in the primary binding cleft centered between TMs 3 and 5–6, and contacts between the fluorophenyl group with residues in EL2 and TMs 2,3, and 7 (not shown). Missing from this view are the aromatic contacts from TM6 to the ligand's piperazine and phenyl-imidazolidinone moieties and potential hydrogen bonding between $Thr383^{7.39}$ and $Tyr387^{7.43}$ and the butyrophenonyl keto group. Panel (**D**): Same view as Panel **C** with side chains rendered as van der Waals spheres. EL2 contact residues are labeled in *yellow*. TMs are numbered

receptors: $Asp^{3.32}$, which forms ionic interactions with the protonated amine of biogenic amines, and $Ser^{5.42}$, $Ser^{5.46}$, and $Ser^{5.43}$ of TM5, which interact by hydrogen bonding with the *meta*-OH and *para*-OH of the catecholamine. The β-hydroxyl group of (nor)epinephrine, which is not found in dopamine, interacts with $Asn^{6.55}$. $Phe^{5.47}$, $Trp^{6.48}$, $Phe^{6.51}$, and $Phe^{6.52}$ are also expected to interact with the aromatic ring of the ligands [36]. In an exhaustive computational study, Xhaard et al. [36] demonstrated that the docked ligand tends to be in an extended conformation because $Asp^{3.32}$ and TM5 residues are distant from each other, at the opposite ends of the binding pocket.

Prior to the determination of the crystal structure of rhodopsin, Simpson et al. [91] used data from SCAM studies to guide an exploration of the structural basis of the pharmacological specificity of D2R and D4R. Combined substitution of four to six of the residues that faced the binding site crevice in the D2R but were not conserved in the D4R switched the affinity of the receptors for several chemically distinct D4-selective antagonists by three orders of magnitude in both directions (D2- to D4-like and D4- to D2-like). The mutated residues were in TM2, TM3, and TM7 and were predicted to form a divergent cluster that differentiated D2R and D4R binding, which has been supported as well by subsequent studies [94, 95, 97]. Ortore et al. [98] have docked different ligands to both D2R and D4R and have proposed that another difference between the two receptors seems to be due to the extracellular loop 2 (ECL2) region (see below), which is differentially situated in the receptor models, although it should be noted that loop modeling is a complex and developing science [99, 100]. Ligand binding to many members of the GPCR family is regulated allosterically by cations. For example, Na^+ is important for the D2R, and Zn^{2+} was shown to interact with D1, D2, and D4 receptors [101].

3.4 Extracellular Loop 2

It is widely accepted that the extracellular loops (especially ECL2) are of great importance for accommodating high molecular weight GPCR ligands (peptides and proteins). In the rhodopsin structure ECL2 forms a lid-like structure over retinal, but the precise role of ECL2 in binding other lower molecular weight, drug-like ligands is less clear [102]. In more than 800 GPCRs encoded in the human genome, the average size of ECL2 is 27 residues, with a deviation of 13 residues [103]. For nearly all rhodopsin-like GPCRs, the disulfide bond between $Cys^{3.25}$ (Cys-107 in D2R) and the conserved Cys in E2 (Cys_e2, Cys-182 in D2R) connects ECL2 with the extracellular end of TM3, and this disulfide bond (SS-E2) is crucial to the structural integrity and function of many GPCRs. This disulfide bridge is found in more than 90% of GPCRs [14, 103]. The removal of SS-E2 by mutagenesis severely disrupts ligand binding to muscarinic acetylcholine receptors [104, 105] and destabilizes the high-affinity state of the B2AR [106]. Moreover, antagonist protected the B2AR from the effects of reduction by dithiothreitol [107]. Thus, SS-E2 is protected by a conformational change or steric block within the binding site.

In rhodopsin, ECL2 forms a twisted, buried β-hairpin structure that folds deeply into the TM domain with one strand contacting retinal and forming interactions with other extracellular loops [102]. It forms a lid-like structure that shields the retinal in a hydrophobic pocket [51]. The orientation of ECL2 is maintained by the SS-E2 described above [29, 108].

Several reports have implicated ECL2 in ligand specificity in aminergic and other small molecule ligand GPCRs. Zhao et al. [109] found that substitution of three consecutive residues in ECL2 interconverted the ligand specificity for particular antagonists between that of α_{1B}AR and α_{1A}AR. Substitution of a single residue in ECL2 interconverted the pharmacological specificities of canine 5-HT_{1D} and human 5-HT_{1D} receptor [110]. Similarly, substitution of ECL2 and TM5 changed the subtype specificity of the 5-HT_{1D} receptor to that of the 5-HT_{1B} receptor and vice versa [111]. Thus, although it has been argued that the presence of ECL2 within the TMD may be a feature unique to rhodopsin [112, 113], it has also been proposed that ECL2 contributes directly to forming the binding site of aminergic and certain other small molecule ligand GPCRs [114]. To address this issue, SCAM studies were carried out in the short ECL2 of D2R [115]. The reaction of five of these mutants with sulfhydryl reagents inhibited antagonist binding, and bound antagonist protected two, I184C and N186C, the second and fourth residues after the highly conserved Cys_e2 (C+2, C+4). The pattern of accessibility in ECL2 was consistent with a structure similar to that of bovine rhodopsin, in which E2b, the part of ECL2 C-terminal to the conserved disulfide bond, is deeper in the binding site crevice than is E2a, the N-terminal part of ECL2, and E2b was inferred to contribute directly to the binding site in the D2R and probably in other aminergic GPCRs as well (Fig. 3.1).

More recently, the effects of ECL2 mutations on agonist and antagonist binding have been studied in the V1a vasopressin receptor (Class A) ECL2 by a systematic alanine-scanning mutagenesis technique that identified four aromatic amino acids, located in the middle of the ECL2 near the conserved disulfide bond and conserved throughout this subfamily of peptide GPCRs, as important for agonist binding and receptor activation [116]. $Trp206^{(C+1)}$ and $Phe209^{(C+4)}$ were hypothesized to be important for ligand binding and $Tyr218^{(C+13)}$ and $Phe189^{(C-16)}$ appear to be important for orientation/stability of ECL2 over the binding pocket [116]. Furthermore, Klco et al. showed that disruption of ECL2 of the complement C5a receptor (C5aR) by random mutagenesis generated many receptors able to activate G proteins even in the absence of ligands [14]. The authors postulated that ECL2 acts as a negative regulator of C5aR activation possibly by making multiple contacts with the TM domain to stabilize the inactive state.

Through their studies of the serotonin 5-$HT_{4(a)}$ receptor Baneres et al. have suggested the existence of different arrangements of ECL2 depending whether the bound ligand was an agonist (partial or full) or an inverse agonist [117]. In contrast, antagonist binding was inferred not to induce any structural changes of ECL2. Therefore, as in the case of D2R, ECL2 appears to participate in the binding site and rearranges upon activation. Despite the constraint provided by the conserved disulfide bond between ECL2 and the top of TM3, Avlani et al. showed that the

flexibility in ECL2 of the muscarinic acetylcholine M_2 receptor (M_2 mAChR) and its capacity to achieve an open conformation is necessary for the binding of both allosteric and orthosteric ligands [4]. They postulated ECL2 as a gatekeeper with respect to entrance into the orthosteric binding site crevice.

Other studies stress ECL2's importance for ligand binding as in the M_3 muscarinic acetylcholine receptor (M3R) and the thyroid-stimulating hormone receptor (TSHR). ECL2 of the M3R was subjected to random mutagenesis. In contrast to the model proposed by Klco et al. [14], the results of this study suggested that specific ECL2 residues stabilize the active state of the M3R, and are required for efficient agonist-induced M3R activation [108]. The authors also proposed a mechanism in which conformational flexibility in the ECL2 loop is required for efficient receptor activation [108]. Kleinau et al. suggested an activation mechanism in which TM6 glides along ECL2 according to the diverse receptor activation states. Disruption of this critical interface by introduction of mutations in the TSHR alters its basal activity [118].

The crystal structure of B2AR provided the first non-rhodopsin ECL2 structure with which to address these hypothesized functional roles. The conformation of B2AR ECL2 and its orientation to the TMD are significantly different from that in rhodopsin, rendering the binding site crevice of B2AR directly exposed to the water phase [31–33]. Strikingly, however, the ligand-binding residue positions in E2b of rhodopsin and B2AR are remarkably consistent, if counted from CysE2, namely the C+2 and C+4 positions, even though rhodopsin has eight extra residues between CysE2 and the start of TM5 at position 5.36. This is in remarkable agreement with the SCAM studies in D2R [115], in which protection by antagonist suggests that the same two positions, Ile184 and Asn186, face the binding site crevice. In this study several residues in D2R were also found to be accessible to MTS reagent but not protected by ligand, and these were proposed to line the ligand entry pathway. This is most obvious in the C+1 position, because E2b can easily be aligned between D2R and B2AR, whereas E2a varies significantly. In the B2AR structure, C+1 is in the vestibule through which the extracellular milieu gains access to the bound carazolol [31–33]. Given the consistency between the D2R SCAM experimental data for ECL2 with both the rhodopsin and B2AR structures, it is likely that C+2 and C+4 play an important role in ligand binding in the other catecholamine receptors as well, and play an important role in ligand specificity (see Fig. 3.1).

3.5 GPCR Oligomerization

Class C GPCRs, including the metabotropic glutamate receptors and γ-aminobutyric acid type B ($GABA_B$) receptors, have been shown to form homo- and heterodimers in the plasma membrane, with important consequences for trafficking of receptors to the cell surface and for ligand-induced activation and G protein coupling [119]. Class C GPCRs have unique characteristics with dimerization potential: an N-terminal Venus flytrap (VTF) module with structural and functional homology to bacterial periplasmic proteins [120], and cysteine-rich domains (CRDs) [121].

Although the formation of dimers for Class C GPCRs is clear, there is still some controversy regarding the existence of dimers in Class A receptors [122, 123]. Nonetheless, there is increasing agreement that Class A GPCRs can interact to form homo- or heterodimers/oligomers [40, 124–149]. Evidence for dopamine receptor homo- and heteromerization is reviewed extensively in Chapter 10; here we focus on structural aspects of oligomerization and the relationship of oligomerization to receptor activation and signaling.

For rhodopsin, dimers and higher-order oligomers have been visualized in disc membranes by atomic force microscopy [150], and an oligomeric arrangement has been inferred in its native environment [12, 151]. Oligomerization also has been inferred from ligand-binding studies [132, 152–155]. Guo et al. recently demonstrated using biophysical and biochemical approaches that the D2R forms higher-order oligomers in living cells at physiological levels of expression [147].

3.5.1 GPCR Oligomerization and Signaling

What is physiologically most relevant is understanding the role of the dimeric or oligomeric organization of GPCRs in signaling [156, 157]. Indeed, one of the great challenges in GPCR biology today is strengthening the weak mechanistic link between the physical interactions of receptors in the membrane and signaling cross talk of presumed heterodimers or hetero-oligomers. There is a great deal of evidence from many laboratories that many GPCRs interact as heterodimers (reviewed in [158, 159]). As indicated above, a number of findings support the existence of higher-order homo-oligomers as well [150, 155, 160, 161]. This raises the possibility that GPCR heteromers may interact not as heterodimers per se but rather as higher-order hetero-oligomers composed of homodimer subunits.

A large number of studies have demonstrated signaling cross talk between coexpressed GPCRs [162]. In almost all cases, however, the mechanistic link between heteromerization and signaling is tenuous. Although activation of two coexpressed receptors may be essential, signaling cross talk could nonetheless take place downstream of parallel homomeric receptor-mediated G protein activation and in such a case would not be a direct result of heteromeric signaling. Such a downstream cross talk mechanism, while often ignored, is very difficult to rule out. One example of this complexity is a recent fascinating study of a putative D1–D2R heterodimer that has been carried out both in heterologous cells [163] and in the brain [164]. These receptors appear to be coexpressed in some neurons in vivo [164]. In heterologous cells they have been inferred to physically interact based on fluorescence resonance energy transfer (FRET) [165, 166] as well as co-internalization [167, 168] and co-retention of mutants [169]. Activating both D1 and D2Rs leads to Gq-mediated signaling [163, 164], whereas D_1 signaling is normally Gs/olf mediated and D_2 signaling is normally Go/i mediated. These findings are intriguing and open exciting avenues of drug design targeted selectively to specific heteromers [170]. However, the plot appears thicker, as D1R-mediated Gq signaling has been observed in the brain [171, 172] where in some studies it has been shown to be insensitive to D2R

blockade [173], suggesting a role for other cellular factors in the coupling of D1R to the Gq pathway. Evidence for a priming effect for D1R-mediated Gq signaling is an example of such a potential mechanism [174, 175].

D2R has also been reported to interact with the dopamine D_3 receptor (D3R), and coexpression of D_2 and D_3 receptors has been reported to modulate the function of both receptors [176, 177]. More recently the D2R has been shown to modulate and to physically associate with the dopamine transporter as well [178, 179].

In addition to its reported interactions with receptors from the dopamine subfamily, there is a substantial literature on heteromerization of D2R with multiple other Class A receptors. There is evidence for direct physical interaction between D2R and the SST5 somatostatin receptor [180], D2R and adenosine A2A receptor [181, 182], and D2R and CB1 cannabinoid receptor [183]. In each of these cases, changes in signaling were observed upon receptor coexpression, with either altered D2R pharmacology by the partner protomer and/or an alteration in the properties of the partner in response to drugs acting at the D2R. In the case of the D2R–CB1 heteromer, dual-agonist mediated activation of Gs was reported, although neither receptor alone is able to activate this Gα subunit [183]. These results are intriguing and suggest the possibility of an untapped level of pharmacological diversity for new compound development, as well as a host of potential roles for in vivo signaling specificity for these putative heteromers. However, in none of these studies is it possible to rule out downstream signaling cross talk and thus to establish incontrovertibly that direct signaling by the D2R heteromer is responsible for the cross talk.

Such a mechanistic interrogation of heteromeric signaling in Class A GPCRs has been difficult. Our mechanistic understanding of the functional role of GPCR dimerization is more advanced in the Class C receptors, due in part to the availability of a clever adaptation of the endoplasmic reticulum (ER) retention signal from the $GABA_B$ receptor to enable controlled cell surface expression and signaling by defined metabotropic glutamate receptor (mGluR) heterodimers [184]. These studies have shown evidence for asymmetric activation of the heterodimer [185, 186]. Furthermore, one agonist can activate the dimer, but two agonists are required for full activation [187]. In addition, within the same Class C, T1R3 taste receptors are known to form functional heterodimers with either T1R1 or T1R2 in order to respond to a large panel of ligands and to trigger umami and sweet taste sensations, respectively (reviewed in [188]).

Unfortunately, related approaches with ER retention signals have been unsuccessful in Class A receptors, and it has not been possible to differentiate clearly the role of each subunit in homomeric and heteromeric signaling with coexpressed receptors. However, multiple lines of study do suggest interaction between Class A receptors in a heteromeric functional unit. Thus, for example, ligand-binding dissociation kinetics have recently been linked to the GPCR dimerization process (reviewed in [189]). In chemokine receptor heteromers, a CCR2-selective drug accelerates the dissociation of a CCR5- or CXCR4-selective drug when the receptors are coexpressed in heterologous cells and in native lymphocytes [190–192]. Moreover, although it remains to be proven conclusively, it seems reasonable to

infer that bivalent drugs engaging two different receptors, i.e., heteromer-selective compounds, might act simultaneously on two protomers in a heteromer and thereby directly activate downstream heteromer-specific signaling machinery [193–195] raising the possibility of their selective therapeutic potential [196]. Although there is evidence of G protein signaling by coexpressed nonfunctional receptor chimeras, this was proposed to occur by transmembrane domain swapping [197], which is unlikely to be universal [198]. Curiously, coexpression of two loss of function glycoprotein hormone receptors (receptors with either agonist binding or the ability to activate G proteins compromised) [199–201] led to function, but among Class A receptors such rescue seems to be limited to glycoprotein hormone receptors, which have very large extracellular N-terminal binding sites. This is similar to the transactivation seen in the Class C $GABA_B$ receptor, in which agonist binding to one protomer signals to G protein through the second protomer [184].

Another major question facing the field is the relationship between findings in heterologous cells and in ex vivo or in vivo cell systems. Most studies have focused on heterologous cells, but new approaches are being developed, including heteromer-specific antibodies (L. Devi, personal communication) as well as transgenic approaches with modified receptors.

Recent studies of purified B2AR and rhodopsin reconstituted into nanodiscs [202, 203] or in detergent solution [204] have demonstrated clearly that these receptors as monomers *can* activate G proteins. If, however, these receptors are indeed organized as dimers (or higher-order units) in native membranes, these elegant biophysical studies beg the physiologically relevant question. That is, if the receptors are capable of functioning as monomers but are closely associated as dimers or oligomers in the membrane, then what functional role does the second protomer play in drug binding and G protein activation? For example, in the $GABA_BB$ receptor the GB2 subunit is necessary for high-affinity binding of agonist to GB1 [205]. Studies in the D2R indicate that conformational change at the TM4 dimer interface is part of the receptor activation mechanism [145], although we cannot as yet establish whether this is achieved by changes in one or both protomers. Similarly, in the LBT4 Baneres and colleagues have shown evidence receptor for conformational changes in protomer B upon agonist binding to protomer A [185], again consistent with a role for the dimer interface in activation.

3.5.2 GPCR Oligomers – Structural Considerations

Dimer interface has been the subject of various studies over the years because of its crucial value in elucidating the structural mechanism(s) for cross talk between receptors within an oligomeric arrangement [145]. Guo et al. have shown that in the D2R TM4 forms a symmetrical dimer interface and that a conformational change at this interface is part of the receptor activation mechanism [145, 206]. Based on atomic force microscopy (AFM) maps of rhodopsin, Liang et al. proposed an oligomeric model in which TM4, TM5, and ECL2 form a dimeric interface, whereas contacts between TM1, TM2, and the cytoplasmic loop connecting TM5 and TM6

facilitate the formation of oligomers [151, 160, 207, 208]. TM1 and TM4 were postulated to be the most common interfaces of oligomerization by a correlated mutation analysis-based method [143, 144, 209].

It is unclear if other dimer orientations are also permissible [119]. For example, besides TM4 and TM5 of rhodopsin [160], other TMs have been implicated in dimer interfaces [210]: TM6 of the β_2-adrenergic, cholecystokinin, and leukotriene B_4 receptors [211, 212], TM5 and TM6 of the adrenergic–muscarinic chimera [213–216], TM1 and TM4 of the D2R [145, 147, 206], TM1 and TM7 of the α-adrenergic receptor [217], TM1 and TM4 of the chemokine receptor [218], TM4, TM1, and TM5/6 in the β1-adrenoceptor [130], TM1, TM2, and TM4 in the complement C5a [219], and TM5 in the adenosine A2A receptor [220].

Bouvier et al. showed that a peptide derived from the TM6 of the B2AR inhibits dimerization of these receptors and proposed a helix–helix interaction involving a conserved GxxxG motif on TM6 [221]. Although TM1 and TM4 can form simultaneous symmetric interfaces in an oligomeric structure [147], TM6 cannot form a symmetrical interface in this oligomer, although it might contribute to an asymmetrical interface.

3.5.3 Oligomer Rearrangements upon Activation

Even though GPCR monomers appear sufficient to activate a G protein [122, 202, 203, 222], their dimerization may modulate this activation through changes at the dimerization interface or a larger-scale reorientation of the two subunits [145, 147, 162, 223, 224]. Cross-linking in the D2R homodimer suggested a conformational rearrangement at the TM4 dimer interface upon receptor activation, passing from a conformation consistent with the 1N3M pdb file to an alternative TM4 interface [145, 147]. Consequently, the D2R inactive state is consistent with the AFM model while the active state is consistent with a squid rhodopsin electron cryomicroscopy (ECM) model [145, 147]. Consistent with this proposal, cross-links of the TM4 interface activated D2R, even in the absence of agonist [145, 147]. This idea was substantiated by recent studies. Brock et al. showed that an agonist-induced rearrangement may indeed occur in the activation of the dimeric metabotropic glutamate receptors [225]. Similarly, a possible dimeric rearrangement was also observed in the mGluR1α receptor. Upon ligand binding, although the distance between ICL1 and ICL2 in each protomer is unchanged, the distance between the ICL1s becomes larger, whereas that between ICL2s becomes smaller [226]. Damian et al. have also shown that in the leukotriene B_4 (LTB_4) receptor, conformational changes take place in one of the protomers upon activation of the other [185].

These observations suggest that in addition to the activation-related conformational changes within a GPCR protomer after activation (mainly a conformational change in TM6 and an associated opening of a binding cleft for G protein between TM6 and TM3) [227], it seems that a rearrangement of the interface of the two protomers is also vital for activation [145, 147]. Mechanisms that might account for this conformational rearrangement include a rigid body clockwise rotation of

contacting TM4s upon activation, protomer displacement involving a large movement and reorganization, or partner change among protomer partners [145, 147], although Niv et al. found using computational methods that rigid body rotation of interacting TM4s is an unlikely mechanism [228].

3.5.4 GPCR Oligomerization and GPCR–G Protein Interactions

MGlu receptor heteromers have been inferred to activate with individual protomers in an asymmetrical relationship [186, 229]. For the BLT1 receptor, the active form of the receptor dimer also is nonsymmetric with only one subunit reaching the fully active state [230]. A single agonist per dimer appears to be sufficient for activation of heterodimeric receptors such as $GABA_B$ [205, 231] and T1R receptors [232]. Similar findings have been reported for the mGlu receptor [187], but other findings in mGlu receptors suggest that activation by agonist binding to both protomers produces greater activation [187]. G protein-specific interactions have been proposed to account for such asymmetric behavior [185, 233, 234]. Jastrzebska et al. speculated that activation of a GPCR dimer could be achieved by a single protomer and that the combination of interactions including the regions of specific trimeric G proteins and two protomers facilitates more efficient coupling [235].

In the classic view, supported by innumerable mutagenesis studies of GPCRs, a monomeric GPCR interacts through ICL2, ICL3, and/or proximal carboxyl-terminal regions with a single heterotrimeric G protein. Structural studies of the receptor–G protein interface have led to the identification of several points of contact between the G protein and the receptor on both α- and β/γ-subunits [236]. When the first crystal structure of a heterotrimeric G protein was solved, it was argued that the surface area of a GPCR monomer was too small to account for the simultaneous interaction with both α- and β/γ-subunits of a G protein [29, 60, 236–238]. A single G protein molecule might instead interact with a GPCR dimer [19, 29, 160, 236, 239–241]. Consistent with this, Baneres and Parello have shown that activated leukotriene B_4 (LTB_4) receptor BLT1 dimer and $G_{\alpha 12\beta 1\gamma 2}$ form an assembly containing one G protein heterotrimer and one receptor dimer (242). If the signaling unit is a GPCR dimer complexed with a heterotrimeric G protein, then both *cis*- and *trans*-activation between two protomers may occur [145].

In recent years, increasing attention has been placed on developing an improved understanding of the interaction between G proteins and the D2R. Senogles et al. have demonstrated that random point mutations in the ICL3 of $D2R_s$ modify G_i protein coupling specificity. Specifically, ICL3 mutations R233G and A234T alter the predicted helical character of ICL3 and disrupt the $D2R_s$/G protein interface [243]. Moreover, a receptor-mimetic peptide derived from the N terminus of D2R ICL3 (D_2N) directly activates G_i/G_o proteins [244, 245]. The crystallographic structure of D_2N with $G\alpha_{i1}$ has further elucidated D_2N/G protein interactions, suggesting that the α4/β6 region of Gα (residues Q304/E308 and T321) is connected to a short basic cluster of D_2N and ^{11}RRRK^{14} (corresponding to ^{216}RRRK^{219} in human D2R) [246].

3.5.5 Consequences of GPCR Oligomerization

One of the most fundamental aspects of oligomerization is its importance for GPCR pharmacology. Ligand binding to GPCRs may result in changes in the binding characteristics of additional ligands targeting the same GPCR, creating a cooperative effect on the binding of another GPCR through an allosteric mechanism [247–249]. Stabilization of a particular conformation of the dimer by a bifunctional agonist might lead to an increase of specificity and efficacy of the signaling [250]. A number of different functionalities and pharmacologic characteristics have been reported and attributed to the generation of GPCR heterodimer/oligomer complexes. Nevertheless, it is crucial to keep in mind that these effects can not only be attributed to direct protein–protein interactions but also to indirect effects produced via downstream signaling and feedback control [251].

The capacity of a GPCR to alter the binding affinity of its binding partner may ultimately be applied clinically in future drug development. More than 50% of all drugs with annual worldwide sales of more than $50 billion regulate the function and activity of many GPCRs in attempts to treat various diseases and disorders [131, 252]. As previously mentioned, GPCR dimerization is important prior to plasma membrane delivery, and incorrect folding may interfere with dimerization and can lead to alteration in cell surface delivery and function [253–255]. In designing potential drugs that may take advantage of our growing knowledge of GPCR structure and function, taking into account oligomerization and heterodimer formation may be critical. Moreover, since receptor heterodimers can generate distinct signals from their corresponding homodimers, understanding the structural basis for higher-order receptor structure may offer a means to improve tissue selectivity and improve drug therapeutic function [251].

References

1. Soyer O, Dimmic MW, Neubig RR, Goldstein RA. Using evolutionary methods to study G-protein coupled receptors. Pac Symp Biocomput 2002;7:625–36.
2. Soyer OS, Dimmic MW, Neubig RR, Goldstein RA. Dimerization in aminergic G-protein-coupled receptors: application of a hidden-site class model of evolution. Biochemistry 2003;42:14522–31.
3. Bockaert J, Pin JP. Molecular tinkering of G protein-coupled receptors: an evolutionary success. EMBO J 1999;18:1723–9.
4. Avlani VA, Gregory KJ, Morton CJ, Parker MW, Sexton PM, Christopoulos A. Critical role for the second extracellular loop in the binding of both orthosteric and allosteric G protein-coupled receptor ligands. J Biol Chem 2007.
5. Weinstein H. Protein interactions in GPCR signaling: a very moving story. Abstr Pap Am Chem Soc 2006;231:102.
6. Niv MY, Skrabanek L, Filizola M, Weinstein H. Modeling activated states of GPCRs: the rhodopsin template. J Comput Aided Mol Des 2006;20:437–48.
7. Ballesteros J, Weinstein H. Integrated methods for the construction of three-dimensional models of structure-function relations in G protein-coupled receptors. Methods Neurosci 1995;25:366.

8. Ballesteros JA, Shi L, Javitch JA. Structural mimicry in G-protein-coupled receptors: implications of the high-resolution structure of rhodopsin for structure-function analysis of rhodopsin-like receptors. Mol Pharmacol 2001;60:1.
9. Thompson, Menard, Pombal, Grillner. Forebrain dopamine depletion impairs motor behavior in lamprey. Eur J Neurosci 2008;27:1452–60.
10. Chan WY, McKinzie DL, Bose S, et al. Allosteric modulation of the muscarinic M-4 receptor as an approach to treating schizophrenia. Proc Natl Acad Sci U S A 2008;105:10978–83.
11. Thompson MD, Burnham WM, Cole DE. The G protein-coupled receptors: pharmacogenetics and disease. Crit Rev Clin Lab Sci 2005;42:311–92.
12. Palczewski K, Hofmann KP, Baehr W. Rhodopsin – advances and perspectives. Vision Res 2006;46:4425–6.
13. Bouchard C, Ribeiro P, Dube F, Anctil M. A new G protein-coupled receptor from a primitive metazoan shows homology with vertebrate aminergic receptors and displays constitutive activity in mammalian cells. J Neurochem 2003;86:1149–61.
14. Klco JM, Wiegand CB, Narzinski K, Baranski TJ. Essential role for the second extracellular loop in C5a receptor activation. Nat Struct Mol Biol 2005;12:320–6.
15. Minneman K. Heterodimerization and surface localization of G protein coupled receptors. Special Issue: In Memory of Art Hancock 2007;73:1043–50.
16. Lefkowitz RJ. The superfamily of heptahelical receptors. Nat Cell Biol 2000;2:E133–36.
17. Lefkowitz RJ. Historical review: a brief history and personal retrospective of seven-transmembrane receptors. Trends Pharmacol Sci 2004;25:413–22.
18. Lefkowitz RJ. Seven transmembrane receptors: a brief personal retrospective. Biochim Biophys Acta 2007;1768:748–55.
19. Maggio R., Innamorati G., Parenti M. G protein-coupled receptor oligomerization provides the framework for signal discrimination. J Neurochem 2007;103:1741–52.
20. Han DS, Wang SX, Weinstein H. Active state-like conformational elements in the beta(2)-AR and a photoactivated intermediate of rhodopsin identified by dynamic properties of GPCRs. Biochemistry 2008;47:7317–21.
21. Huang P, Li J, Chen CG, Visiers I, Weinstein H, Liu-Chen LY. Functional role of a conserved motif in TM6 of the rat mu opioid receptor: constitutively active and inactive receptors result from substitutions of Thr6.34(279) with Lys and Asp. Biochemistry 2001;40:13501–9.
22. Prioleau C, Visiers I, Ebersole BJ, Weinstein H, Sealfon SC. Conserved helix 7 tyrosine acts as a multistate conformational switch in the 5HT2C receptor – Identification of a novel "locked-on" phenotype and double revertant mutations. J Biol Chem 2002;277:36577–84.
23. Visiers I, Ballesteros JA, Weinstein H. Three-dimensional representations of G protein-coupled receptor structures and mechanisms. G Protein Pathways, Pt a, Receptors 2002;343:329–71.
24. Ballesteros JA, Jensen AD, Liapakis G, et al. Activation of the beta(2)-adrenergic receptor involves disruption of an ionic lock between the cytoplasmic ends of transmembrane segments 3 and 6. J Biol Chem 2001;276:29171–7.
25. Konvicka K, Guarnieri F, Ballesteros JA, Weinstein H. Proposed structure for the NPxxY sequence motif in transmembrane segment 7 of G-protein coupled receptors. Biophys J 1997;72:A74.
26. Konvicka K, Guarnieri F, Ballesteros JA, Weinstein H. A proposed structure for transmembrane segment 7 of G protein-coupled receptors incorporating an Asn-Pro/Asp-Pro motif. Biophys J 1998;75:601–11.
27. Bhattacharya M, Babwah AV, Ferguson SS. Small GTP-binding protein-coupled receptors. Biochem Soc Trans 2004;32:1040–4.
28. Bhattacharya S, Hall S, Vaidehi N. Agonist-induced conformational changes in Bovine rhodopsin: insight into activation of G-protein-coupled receptors. J Mol Biol 2008;382:539–55.
29. Palczewski K, Kumasaka T, Hori T, et al. Crystal structure of rhodopsin: A G protein-coupled receptor. Science 2000;289:739–45.

30. Day PW, Rasmussen SGF, Parnot C, et al. A monoclonal antibody for G protein-coupled receptor crystallography. Nature Methods 2007;4:927–9.
31. Rasmussen SGF, Choi HJ, Rosenbaum DM, et al. Crystal structure of the human beta(2) adrenergic G-protein-coupled receptor. Nature 2007;450:383–U4.
32. Cherezov V, Rosenbaum DM, Hanson MA, et al. High-resolution crystal structure of an engineered human beta(2)-adrenergic G protein-coupled receptor. Science 2007;318: 1258–65.
33. Rosenbaum DM, Cherezov V, Hanson MA, et al. GPCR engineering yields high-resolution structural insights into beta(2)-adrenergic receptor function. Science 2007;318: 1266–73.
34. Warne T, Serrano-Vega MJ, Baker JG, et al. Structure of a beta(1)-adrenergic G-protein-coupled receptor. Nature 2008;454:486–491.
35. Jaakola VP, Griffith MT, Hanson MA, et al. The 2.6 angstrom crystal structure of a human A(2A) adenosine receptor bound to an antagonist. Science 2008;322:1211–7.
36. Xhaard H, Rantanen VV, Nyronen T, Johnson MS. Molecular evolution of adrenoceptors and dopamine receptors: Implications for the binding of catecholamines. J Med Chem 2006;49:1706–19.
37. Missale C, Nash R, Robinson S, Jaber M, Caron M. Dopamine receptors: from structure to function. Physiol Rev 1998;78:189–225.
38. Pivonello R, Ferone D, de Herder WW, et al. Dopamine receptor expression and function in corticotroph ectopic tumors. J Clin Endocrinol Metab 2007;92:65–9.
39. Thevenin D, Lazarova T. Stable interactions between the transmembrane domains of the adenosine A2A receptor. Protein Sci 2008;17:1188–99.
40. Javitch JA. The ants go marching two by two: oligomeric structure of G-protein-coupled receptors. Mol Pharmacol 2004;66:1077–82.
41. Ballesteros J, Palczewski K. G protein-coupled receptor drug discovery: implications from the crystal structure of rhodopsin. Curr Opin Drug Discov Devel 2001;4:561–74.
42. Lefkowitz RJ. Seven transmembrane receptors: something old, something new. Acta Physiol 2007;190:9–19.
43. Sansom MSP, Weinstein H. Hinges, swivels and switches: the role of prolines in signalling via transmembrane alpha-helices. Trends Pharmacol Sci 2000;21:445–51.
44. Visiers I, Ballesteros J, Weinstein H. A spatially ordered sequence of intramolecular rearrangements observed from simulations of agonist-related activation of 5HT(2C) receptors. Biophys J 2000;78:394Pos.
45. Ceruso MA, Weinstein H. Structural mimicry of proline kinks: tertiary packing interactions support local structural distortions. J Mol Biol 2002;318:1237–49.
46. Shi L, Liapakis G, Xu R, Guarnieri F, Ballesteros JA, Javitch JA. Beta2 adrenergic receptor activation. Modulation of the proline kink in transmembrane 6 by a rotamer toggle switch. J Biol Chem 2002;277:40989–96.
47. Ballesteros JA, Deupi X, Olivella M, Haaksma EEJ, Pardo L. Serine and threonine residues bend alpha-helices in the chi(1) = g(–) conformation. Biophys J 2000;79:2754–60.
48. Shi L, Simpson MM, Ballesteros JA, Javitch JA. The first transmembrane segment of the dopamine D2 receptor: accessibility in the binding-site crevice and position in the transmembrane bundle. Biochemistry 2001;40:12339–48.
49. Liapakis G, Simpson MM, Javitch JA. The substituted-cysteine accessibility method (SCAM) to elucidate membrane protein structure. Curr Protoc Neurosci 2001;Chapter 4:Unit 4.15.
50. Hubbell WL, Altenbach C, Hubbell CM, Khorana HG, Douglas CR. Rhodopsin structure, dynamics, and activation: a perspective from crystallography, site-directed spin labeling, sulfhydryl reactivity, and disulfide cross-linking. Adv Protein Chem 2003;63: 243–90.
51. Shukla AK, Sun J-P, Lefkowitz RJ. Crystallizing thinking about the {beta}2-adrenergic receptor. Mol Pharmacol 2008;73:1333–8.

52. Kobilka BK, Deupi X. Conformational complexity of G-protein-coupled receptors. Trends Pharmacol Sci 2007;28:397–406.
53. Schwartz TU, Schmidt D, Brohawn SG, Blobel G. Homodimerization of the G protein SRbeta in the nucleotide-free state involves proline cis/trans isomerization in the switch II region. Proc Natl Acad Sci U S A 2006;103:6823–8.
54. Schwartz TW, Frimurer TM, Holst B, Rosenkilde MM, Elling CE. Molecular mechanism of 7TM receptor activation – a global toggle switch model. Annu Rev Pharmacol Toxicol 2006;46:481–519.
55. Sheikh SP, Vilardarga JP, Baranski TJ, et al. Similar structures and shared switch mechanisms of the beta2-adrenoceptor and the parathyroid hormone receptor. Zn(II) bridges between helices III and VI block activation. The J Biol Chem 1999;274: 17033–41.
56. Binet V, Duthey BA, Lecaillon J, et al. Common structural requirements for heptahelical domain function in class A and class C GPCRS. J Biol Chem 2007.
57. Park PS, Lodowski DT, Palczewski K. Activation of G protein-coupled receptors: beyond two-state models and tertiary conformational changes. Ann Rev Pharmacol Toxicol 2008;48:107–41.
58. Xu W, Li J, Chen CG, et al. Comparison of the amino acid residues in the sixth transmembrane domains accessible in the binding-site crevices of mu, delta, and kappa opioid receptors. Biochemistry 2001;40:8018–29.
59. Javitch JA, Fu DY, Liapakis G, Chen JY. Constitutive activation of the beta(2) adrenergic receptor alters the orientation of its sixth membrane-spanning segment. J Biol Chem 1997;272:18546–9.
60. Bourne H. How receptors talk to trimeric G proteins. Curr Opin Cell Biol 1997;9: 134–42.
61. Elling CE, Frimurer TM, Gerlach LO, Jorgensen R, Holst B, Schwartz TW. Metal ion site engineering indicates a global toggle switch model for seven-transmembrane receptor activation. J Biol Chem 2006;281:17337–46.
62. Gether U, Ballesteros JA, Seifert R, SandersBush E, Weinstein H, Kobilka BK. Structural instability of a constitutively active G protein-coupled receptor – agonist-independent activation due to conformational flexibility. J Biol Chem 1997;272: 2587–90.
63. Li J, Huang P, Chen CG, de Riel JK, Weinstein H, Liu-Chen LY. Constitutive activation of the mu opioid receptor by mutation of D3.49(164), but not D3.32(147): D3.49(164) is critical for stabilization of the inactive form of the receptor and for its expression. Biochemistry 2001;40:12039–50.
64. Rasmussen SGF, Jensen AD, Liapakis G, Ghanouni P, Javitch JA, Gether U. Mutation of a highly conserved aspartic acid in the beta(2) adrenergic receptor: constitutive activation, structural instability, and conformational rearrangement of transmembrane segment 6. Mol Pharmacol 1999;56:175–84.
65. Haydock K, Weinstein H. Molecular dynamics simulations to model the properties of constitutively active GPCR mutants. Biophys J 1995;68:A255.
66. Cotecchia S. Constitutive activity and inverse agonism at the alpha1adrenoceptors. Biochem Pharmacol 2007;73:1076–83.
67. Cotecchia S, Exum S, Caron MG, Lefkowitz RJ. Regions of the alpha 1-adrenergic receptor involved in coupling to phosphatidylinositol hydrolysis and enhanced sensitivity of biological function. Proc Natl Acad Sci U S A 1990;87:2896–900.
68. Gether U, Lin S, Ghanouni P, Ballesteros JA, Weinstein H, Kobilka BK. Agonists induce conformational changes in transmembrane domains III and VI of the beta(2) adrenoceptor. Embo J 1997;16:6737–47.
69. Parnot C. Systematic identification of mutations that constitutively activate the angiotensin II type 1A receptor by screening a randomly mutated cDNA library with an original pharmacological bioassay. Proc Natl Acad Sci USA 2000;97:7615–20.

70. Parnot C, Miserey-Lenkei S, Bardin S, Corvol P, Clauser E. Lessons from constitutively active mutants of G protein-coupled receptors. Trends Endocrinol Metab 2002;13: 336–43.
71. Ballesteros J, Kitanovic S, Guarnieri F, et al. Functional microdomains in g-protein-coupled receptors – the conserved Arginine-cage motif in the gonadotropin-releasing hormone receptor. J Biol Chem 1998;273:10445–53.
72. Ballesteros JA, Kitanovic S, Guarnieri F et al. Functional microdomains in G protein-coupled receptors: the conserved arginine cage motif in the gonadotropin-releasing hormone receptor. J Biol Chem 1998;273:10445–53.
73. Shapiro DA, Kristiansen K, Weiner DM, Kroeze WK, Roth BL. Evidence for a model of agonist-induced activation of 5-hydroxytryptamine 2A serotonin receptors that involves the disruption of a strong ionic interaction between helices 3 and 6. J Biol Chem 2002;277:11441–9.
74. Huang P, Visiers I, Weinstein H, Liu-Chen LY. The local environment at the cytoplasmic end of TM6 of the mu opioid receptor differs from those of rhodopsin and monoamine receptors: Introduction of an ionic lock between the cytoplasmic ends of helices 3 and 6 by a L6.30(275)E mutation inactivates the mu opioid receptor and reduces the constitutive activity of its T6.34(279)K mutant. Biochemistry 2002;41:11972–80.
75. Dror RO, Arlow DH, Borhani DW, Jensen Mo, Piana S, Shaw DE. Identification of two distinct inactive conformations of the (beta)2-adrenergic receptor reconciles structural and biochemical observations. Proc Natl Acad Sci U S A 2009 – Available Online.
76. Kobilka BK. G protein coupled receptor structure and activation. Biochim Biophys Acta 2007;1768:794–807.
77. Kobilka B, Schertler GF. New G-protein-coupled receptor crystal structures: insights and limitations. Trends Pharmacol Sci 2008;29:79–83.
78. Neve KA, Cox BA, Henningsen RA, Spanoyannis A, Neve RL. Pivotal role for aspartate-80 in the regulation of dopamine D2 receptor affinity for drugs and inhibition of adenylyl cyclase. Mol Pharmacol 1991;39:733–9.
79. Neve KA, Cumbay MG, Thompson KR, Yang R, Buck DC. Modeling and mutational analysis of a putative sodium-binding pocket on the dopamine D2 receptor. Mol Pharmacol 2001;60:373.
80. Mansour A, Meng F, Meador-Woodruff JH, Taylor LP, Civelli O, Akil H. Site-directed mutagenesis of the human dopamine D2 receptor. Eur J Pharmacol 1992;227:205–14.
81. Javitch JA. Mapping the binding-site crevice of the D2 receptor. Adv Pharmacol 1998;42:412–5.
82. Javitch JA. Probing structure of neurotransmitter transporters by substituted-cysteine accessibility method. Neurotransmitter Transporters 1998;296:331–46.
83. Javitch JA, Ballesteros JA, Chen JY, Chiappa V, Simpson MM. Electrostatic and aromatic microdomains within the binding-site crevice of the D2 receptor: contributions of the second membrane-spanning segment. Biochemistry 1999;38:7961–8.
84. Javitch JA, Ballesteros JA, Weinstein H, Chen J. A cluster of aromatic residues in the sixth membrane-spanning segment of the dopamine D2 receptor is accessible in the binding-site crevice. Biochemistry 1998;37:998–1006.
85. Javitch JA, Fu D, Chen J. Residues in the fifth membrane-spanning segment of the dopamine D2 receptor exposed in the binding-site crevice. Biochemistry 1995;34:16433–9.
86. Javitch JA, Fu D, Chen J, Karlin A. Mapping the binding-site crevice of the dopamine D2 receptor by the substituted-cysteine accessibility method. Neuron 1995;14:825–31.
87. Javitch JA, Fu DY, Chen JY. Differentiating dopamine D-2 ligands by their sensitivities to modification of the cysteine exposed in the binding-site crevice. Mol Pharmacol 1996;49:692–8.
88. Javitch JA, Li X, Kaback J, Karlin A. A cysteine residue in the third membrane-spanning segment of the human D2 dopamine receptor is exposed in the binding-site crevice. Proc Natl Acad Sci U S A 1994;91:10355–9.

89. Javitch JA, Shi L, Simpson MM, Chen J, Chiappa V. The fourth transmembrane segment of the dopamine D2 receptor: accessibility in the binding-site crevice and position in the transmembrane bundle. Biochemistry 2000;39:12,190.
90. Fu DY, Ballesteros JA, Weinstein H, Chen JY, Javitch JA. Residues in the seventh membrane-spanning segment of the dopamine D2 receptor accessible in the binding-site crevice. Biochemistry 1996;35:11278–85.
91. Simpson MM, Ballesteros JA, Chiappa V, et al. Dopamine D4/D2 receptor selectivity is determined by a divergent aromatic microdomain contained within the second, third, and seventh membrane-spanning segments. Mol Pharmacol 1999;56:1116–26.
92. Coley C, Woodward R, Johansson AM, Strange PG, Naylor LH. Effect of multiple serine/alanine mutations in the transmembrane spanning region V of the D2 dopamine receptor on ligand binding. Journal of neurochemistry 2000;74:358–66.
93. Ericksen SS, Cummings DF, Weinstein H, Schetz JA. Ligand selectivity of D-2 dopamine receptors is modulated by changes in local dynamics produced by sodium binding. J Pharmacol Exp Thers 2009;328:40–54.
94. Schetz JA, Benjamin PS, Sibley DR. Nonconserved residues in the second transmembrane-spanning domain of the D4 dopamine receptor are molecular determinants of D4-selective pharmacology. Mol Pharmacol 2000;57:144–52.
95. Schetz JA, Sibley DR. Tandem sulfur-containing amino acids are epicritical determinants of dopamine D(2) receptor pharmacology. Eur J Pharmacol 2000;388:R5–R7.
96. Neve KA. Regulation of dopamine D2 receptors by sodium and pH. Mol Pharmacol 1991;39:570.
97. Kortagere S, Gmeiner P, Weinstein H, Schetz JA. Certain 1,4-disubstituted aromatic piperidines and piperazines with extreme selectivity for the dopamine D4 receptor interact with a common receptor microdomain. Mol Pharmacol 2004;66:1491–9.
98. Ortore G, Tuccinardi T, Bertini S, Martinelli A. A theoretical study to investigate D2DAR/D4DAR selectivity; receptor modeling and molecular docking of dopaminergic ligands. J Med Chem 2006;49:1397–407.
99. Mehler EL, Hassan SA, Kortagere S, Weinstein H. Ab initio computational modeling of loops in G-protein-coupled receptors: lessons from the crystal structure of rhodopsin. Proteins 2006;64:673–90.
100. Mehler EL, Periole X, Hassan SA, Weinstein H. Key issues in the computational simulation of GPCR function: representation of loop domains. J Comput Aided Mol Des 2002;15:13.
101. Christopoulos A, Kenakin T. G protein-coupled receptor allosterism and complexing. Pharmacol Rev 2002;54:323–74.
102. de Graaf C, Foata N, Engkvist O, Rognan D. Molecular modeling of the second extracellular loop of G-protein coupled receptors and its implication on structure-based virtual screening. Proteins 2008;71:599–620.
103. Karnik S, Gogonea C, Patil S, Saad Y, Takezako T. Activation of G-protein-coupled receptors: a common molecular mechanism. Trends Endocrinol Metabol 2003;14:431–7.
104. Savarese TM, Wang CD, Fraser CM. Site-directed mutagenesis of the rat m1 muscarinic acetylcholine receptor. Role of conserved cysteines in receptor function. J Biol Chem 1992;267:11439–48.
105. Zeng FY, Soldner A, Schoneberg T, Wess J. Conserved extracellular cysteine pair in the M3 muscarinic acetylcholine receptor is essential for proper receptor cell surface localization but not for G protein coupling. J Neurochem 1999;72:2404–14.
106. Noda K, Saad Y, Graham RM, Karnik SS. The high affinity state of the beta 2-adrenergic receptor requires unique interaction between conserved and non-conserved extracellular loop cysteines. J Biol Chem 1994;269:6743.
107. Lin S, Gether U, Kobilka BK. Ligand stabilization of the beta 2 adrenergic receptor: effect of DTT on receptor conformation monitored by circular dichroism and fluorescence spectroscopy. Biochemistry 1996;35:14,445.

108. Scarselli M, Li B, Kim SK, Wess J. Multiple residues in the second extracellular loop are critical for M3 muscarinic acetylcholine receptor activation. J Biol Chem 2007;282: 7385–96.
109. Zhao MM, Hwa J, Perez DM. Identification of critical extracellular loop residues involved in alpha 1-adrenergic receptor subtype-selective antagonist binding. Mol Pharmacol 1996;50:1118.
110. Wurch T, Pauwels PJ. Coupling of canine serotonin 5-HT(1B) and 5-HT(1D) receptor subtypes to the formation of inositol phosphates by dual interactions with endogenous G(i/o) and recombinant G(alpha15) proteins. J Neurochem 2000;75:1180.
111. Wurch T, Colpaert FC, Pauwels PJ. Chimeric receptor analysis of the ketanserin binding site in the human 5-hydroxytryptamine1D receptor: importance of the second extracellular loop and fifth transmembrane domain in antagonist binding. Mol Pharmacol 1998;54:1088.
112. Menon S, Han M, Sakmar T. Rhodopsin: Structural basis of molecular physiology. Physiol Rev 2001;81:1659–88.
113. Bourne HR, Meng EC. Structure. Rhodopsin sees the light. Science 2000;289:733.
114. Shi L, Javitch JA. The binding site of aminergic G protein-coupled receptors: the transmembrane segments and second extracellular loop. Annu Rev Pharmacol Toxicol 2002;42: 437–67.
115. Shi L, Javitch JA. The second extracellular loop of the dopamine D2 receptor lines the binding-site crevice. Proc Natl Acad Sci U S A 2004;101:440–5.
116. Conner M, Hawtin SR, Simms J, et al. Systematic analysis of the entire second extracellular loop of the V1a vasopressin receptor: key residues, conserved throughout a G-protein-coupled receptor family, identified. J Biol Chem 2007;282:17405–12 .
117. Baneres JL, Mesnier D, Martin A, Joubert L, Dumuis A, Bockaert J. Molecular characterization of a purified 5-HT4 receptor: a structural basis for drug efficacy. J Biol Chem 2005;280:20253–60.
118. Kleinau G, Jaeschke H, Mueller S, Worth CL, Paschke R, Krause G. Molecular and structural effects of inverse agonistic mutations on signaling of the thyrotropin receptor – a basally active GPCR. Cell Mol Life Sci 2008;65:3664–76.
119. White J, Grodnitzky J, Louis J, et al. Dimerization of the class A G protein-coupled neurotensin receptor NTS1 alters G protein interaction. Proc Natl Acad Sci U S A 2007;104:12199–204.
120. Mowbray SL, Petsko GA. The x-ray structure of the periplasmic galactose binding protein from Salmonella typhimurium at 3.0-A resolution. J Biol Chem 1983;258:7991–7.
121. Okamoto T, Sekiyama N, Otsu M, et al. Expression and purification of the extracellular ligand binding region of metabotropic glutamate receptor subtype 1. J Biol Chem 1998;273:13089–96.
122. Chabre M, Lemaire M. Monomeric G-protein-coupled receptor as a functional unit. Biochemistry 2005;44:9395–403.
123. Meyer B, Segura J-M, Martinez K, et al. FRET imaging reveals that functional neurokinin-1 receptors are monomeric and reside in membrane microdomains of live cells. Proc Natl Acad Sci U S A 2006;103:2138–43.
124. Overton M, Blumer K. Use of fluorescence resonance energy transfer to analyze oligomerization of G-protein-coupled receptors expressed in yeast. Methods 2002;27: 324–32.
125. Overton MC, Blumer KJ. G-protein-coupled receptors function as oligomers in vivo. Curr Biol 2000;10:341–4.
126. Overton MC, Blumer KJ. The extracellular N-terminal domain and transmembrane domains 1 and 2 mediate oligomerization of a yeast G protein-coupled receptor. J Biol Chem 2002;277:41463–72.
127. Overton MC, Chinault SL, Blumer KJ. Oligomerization, biogenesis, and signaling is promoted by a glycophorin A-like dimerization motif in transmembrane domain 1 of a yeast G protein-coupled receptor. J Biol Chem 2003;278:49369–77.

128. Overton MC, Chinault SL, Blumer KJ. Oligomerization of G-protein-coupled receptors: lessons from the yeast Saccharomyces cerevisiae. Eukaryot Cell 2005;4:1963–70.
129. Canals M, Lopez-Gimenez JF, Milligan G. Cell surface delivery and structural reorganization by pharmacological chaperones of an oligomerization-defective alpha(1b)-adrenoceptor mutant demonstrates membrane targeting of GPCR oligomers. Biochemical J 2009;417:161–72.
130. Carrillo JJ, Lopez-Gimenez JF, Milligan G. Multiple interactions between transmembrane helices generate the oligomeric {alpha}1b-Adrenoceptor. Mol Pharmacol 2004;66: 1123–37.
131. Fredholm BB, Hokfelt T, Milligan G. G-protein-coupled receptors: an update. Acta Physiol 2007;190:3–7.
132. Lopez-Gimenez JF, Canals M, Pediani JD, Milligan G. The {alpha}1b-adrenoceptor exists as a higher-order oligomer: effective oligomerization is required for receptor maturation, surface delivery, and function. Mol Pharmacol 2007;71:1015–29.
133. Milligan G. Oligomerisation of G-protein-coupled receptors. J Cell Sci 2001;114: 1265–71.
134. Milligan G. G protein-coupled receptor dimerization: function and ligand pharmacology. Mol Pharmacol 2004;66:1–7.
135. Milligan G. GPCR dimerisation: molecular basis and relevance for function and pharmacology. Biochim Biophys Acta 2007;1768:825–35.
136. Milligan G, Anonymous, Behan D, inventors; The University Court of the University of Glasgow, assignee. Materials and methods relating to G-protein coupled receptor oligomers patent US 07405053. 2008.
137. Milligan G, Ramsay D, Pascal G, Carrillo JJ. GPCR dimerisation. Life sciences 2003;74:181–8.
138. Skrabanek L, Murcia M, Bouvier M, et al. Requirements and ontology for a G protein-coupled receptor oligomerization knowledge base. BMC Bioinformatics 2007;8:177.
139. Filizola M, Guo W, Javitch JA, Weinstein H. Dimerization in G-protein coupled receptors: correlation analysis and electron density maps of rhodopsin from different species suggest subtype-specific interfaces. Biophys J 2003;84:269A-70A.
140. Filizola M, Olmea O, Weinstein H. Using correlated mutation analysis to predict the heterodimerization interface of GPCRs. Biophys J 2002;82:2307.
141. Filizola M, Wang S, Weinstein H. Dynamic models of G-protein coupled receptor dimers: indications of asymmetry in the rhodopsin dimer from molecular dynamics simulations in a POPC bilayer. J Comput Aided Mol Des 2006;20, 405–416.
142. Filizola M, Weinstein H. Structural models for dimerization of G-protein coupled receptors: the opioid receptor homodimers. Biopolymers 2002;66:317–25.
143. Filizola M, Weinstein H. The structure and dynamics of GPCR oligomers: a new focus in models of cell-signaling mechanisms and drug design. Curr Opin Drug Discov Devel 2005;8:577–84.
144. Filizola M, Weinstein H. The study of G-protein coupled receptor oligomerization with computational modeling and bioinformatics. FEBS J 2005;272:2926–38.
145. Guo W, Shi L, Filizola M, Weinstein H, Javitch J. From the cover: Crosstalk in G protein-coupled receptors: changes at the transmembrane homodimer interface determine activation. Proc Natl Acad Sci U S A 2005;102:17495–500.
146. Wang XS, Filizola M, Ceruso M, Weinstein H. Rhodopsin dimers: molecular dynamics simulations using discrete representations of the membrane and water environment. Biophys J 2005;88:81A-A.
147. Guo W, Urizar E, Kralikova M, et al. Dopamine D2 receptors form higher order oligomers at physiological expression levels. Embo J 2008;27:2293–304.
148. Hastrup H, Karlin A, Javitch JA. Symmetrical dimer of the human dopamine transporter revealed by cross-linking Cys-306 at the extracellular end of the sixth transmembrane segment. Proc Natl Acad Sci U S A 2001;98:10055–60.

149. McVey M, Ramsay D, Kellett E, et al. Monitoring receptor oligomerization using time-resolved fluorescence resonance energy transfer and bioluminescence resonance energy transfer – The human delta-opioid receptor displays constitutive oligomerization at the cell surface, which is not regulated by receptor occupancy. J Biol Chem 2001;276: 14092–9.
150. Fotiadis D, Liang Y, Filipek S, Saperstein DA, Engel A, Palczewski K. Atomic-force microscopy: rhodopsin dimers in native disc membranes. Nature 2003;421:127–8.
151. Fotiadis D, Jastrzebska B, Philippsen A, Muller D, Palczewski K, Engel A. Structure of the rhodopsin dimer: a working model for G-protein-coupled receptors. Theory and simulation/Macromolecular assemblages – Joel Janin and Michael Levitt/Edward H Egelman and Andrew GW Leslie 2006;16:252–9.
152. Strange P. Oligomers of D2 dopamine receptors: evidence from ligand binding. J Mol Neurosci2005;26:155–60.
153. Park P, Sum CS, Hampson DR, Van Tol HHM, Wells JW. Nature of the oligomers formed by muscarinic m2 acetylcholine receptors in Sf9 cells. Eur J Pharmacol 2001;421: 11–22.
154. Park PSH, Filipek S, Wells JW, Palczewski K. Oligomerization of G protein-coupled receptors: Past, present, and future. Biochemistry 2004;43:15643–56.
155. Paul S.-H. Park JWW. Oligomeric potential of the M2 muscarinic cholinergic receptor. J Neurochem 2004;90:537–48.
156. Pin JP, Neubig R, Bouvier M, et al. International Union of Basic and Clinical Pharmacology. LXVII. Recommendations for the recognition and nomenclature of G protein-coupled receptor heteromultimers. Pharmacol Rev 2007;59:5–13.
157. Bartfai T, Benovic JL, Bockaert J, et al. The state of GPCR research in 2004. Nat Rev Drug Discov 2004;3:574–626.
158. Maggio R, Novi F, Scarselli M, Corsini GU. The impact of G-protein-coupled receptor hetero-oligomerization on function and pharmacology. FEBS J 2005;272:2939–46.
159. Prinster S, Hague C, Hall R. Heterodimerization of G protein-coupled receptors: specificity and functional significance. Pharmacol Rev 2005;57:289–98.
160. Liang Y, Fotiadis D, Filipek S, Saperstein DA, Palczewski K, Engel A. Organization of the G protein-coupled receptors rhodopsin and opsin in native membranes. The J Biol Chem 2003;278:21655–62.
161. Philip F, Sengupta P, Scarlata S. Signaling through a G protein-coupled receptor and its corresponding G protein follows a stoichiometrically limited model. J Biol Chem 2007;282:19203–16.
162. Breitwieser GE. G protein-coupled receptor oligomerization: implications for G protein activation and cell signaling. Circ Res 2004;94:17–27.
163. Lee SP, So CH, Rashid AJ, et al. Dopamine D1 and D2 receptor co-activation generates a novel phospholipase C-mediated calcium signal. The J Biol Chem 2004;279:35671–8.
164. Rashid AJ, So CH, Kong MM, et al. D1-D2 dopamine receptor heterooligomers with unique pharmacology are coupled to rapid activation of Gq/11 in the striatum. Proc Natl Acad Sci U S A 2007;104:654–9.
165. O'Dowd BF, Ji X, Alijaniaram M, et al. Dopamine receptor oligomerization visualized in living cells. J Biol Chem 2005;280:37225–35.
166. Dziedzicka-Wasylewska M, Faron-Gorecka A, Andrecka J, Polit A, Kusmider M, Wasylewski Z. Fluorescence studies reveal heterodimerization of dopamine D1 and D2 receptors in the plasma membrane. Biochemistry 2006;45:8751–9.
167. So CH, Varghese G, Curley KJ, et al. D1 and D2 dopamine receptors form heterooligomers and cointernalize after selective activation of either receptor. Mol Pharmacol 2005;68: 568–78.
168. So CH, Verma V, O'Dowd BF, George SR. Desensitization of the dopamine D1 and D2 receptor hetero-oligomer mediated calcium signal by agonist occupancy of either receptor. Mol Pharmacol 2007;72:450–62.

169. Kong MMC, Fan T, Varghese G, O'Dowd BF, George SR. Agonist-induced cell surface trafficking of an intracellularly sequestered D1 dopamine receptor homo-oligomer. Mol Pharmacol 2006;70:78–89.
170. Milligan G. G-protein-coupled receptor heterodimers: pharmacology, function and relevance to drug discovery. Drug Discov Today 2006;11:541–9.
171. Mannoury la Cour C, Vidal S, Pasteau V, Cussac D, Millan MJ. Dopamine D1 receptor coupling to Gs/olf and Gq in rat striatum and cortex: A scintillation proximity assay (SPA)/antibody-capture characterization of benzazepine agonists. Neuropharmacology 2007;52:1003–14.
172. Jin LQ, Wang HY, Friedman E. Stimulated D-1 dopamine receptors couple to multiple G alpha proteins in different brain regions. J Neurochem 2001;78:981–90.
173. Ming YL, Zhang H, Long LH, Wang F, Chen JG, Zhen XC. Modulation of Ca2+ signals by phosphatidylinositol-linked novel D1 dopamine receptor in hippocampal neurons. J Neurochem 2006;98:1316–23.
174. Ali MK, Bergson C. Elevated intracellular calcium triggers recruitment of the receptor cross-talk accessory protein calcyon to the plasma membrane. J Biol Chem 2003;278: 51654–63.
175. Lidow MS, Roberts A, Zhang L, Koh PO, Lezcano N, Bergson C. Receptor crosstalk protein, calcyon, regulates affinity state of dopamine D1 receptors. Eur J Pharmacol 2001;427: 187–93.
176. Novi F, Millan MJ, Corsini GU, Maggio R. Partial agonist actions of aripiprazole and the candidate antipsychotics S33592, bifeprunox, N-desmethylclozapine and preclamol at dopamine D-2L receptors are modified by co-transfection of D-3 receptors: potential role of heterodimer fort-nation. J Neurochem 2007;102:1410–24.
177. Scarselli M, Novi F, Schallmach E, et al. D2/D3 dopamine receptor heterodimers exhibit unique functional properties. J Biol Chem 2001;276:30308–14.
178. Bolan EA, Kivell B, Jaligam V, et al. D2 receptors regulate dopamine transporter function via an ERK 1/2-dependent and PI3 kinase-independent mechanism. Mol Pharmacol 2007;71:1222–32.
179. Lee F, Pei L, Moszczynska A, Vukusic B, Fletcher P, Liu F. Dopamine transporter cell surface localization facilitated by a direct interaction with the dopamine D2 receptor. The Embo J 2007;26:10.
180. Rocheville M, Lange DC, Kumar U, Patel SC, Patel RC, Patel YC. Receptors for dopamine and somatostatin: formation of hetero-oligomers with enhanced functional activity. Science 2000;288:154–7.
181. Hillion J, Canals M, Torvinen M, et al. Coaggregation, cointernalization, and codesensitization of adenosine A2A receptors and dopamine D2 receptors. J Biol Chem 2002;277:18091–7.
182. Kamiya T, Saitoh O, Yoshioka K, Nakata H. Oligomerization of adenosine A(2A) and dopamine D-2 receptors in living cells. Biochem Biophys Res Commun 2003;306: 544–9.
183. Kearn CS, Blake-Palmer K, Daniel E, Mackie K, Glass M. Concurrent stimulation of cannabinoid CB1 and dopamine D2 receptors enhances heterodimer formation: a mechanism for receptor cross-talk? Mol Pharmacol 2005;67:1697–704.
184. Pin JP, Galvez T, Prezeau L. Evolution, structure, and activation mechanism of family 3/C G-protein-coupled receptors. Pharmacol Ther 2003;98:325–54.
185. Damian M, Martin A, Mesnier D, Pin JP, Baneres JL. Asymmetric conformational changes in a GPCR dimer controlled by G-proteins. Embo J 2006;25:5693–702.
186. Hlavackova V, Goudet C, Kniazeff J, et al. Evidence for a single heptahelical domain being turned on upon activation of a dimeric GPCR. Embo J 2005;24:499–509.
187. Kniazeff J, Bessis AS, Maurel D, Ansanay H, Prezeau L, Pin JP. Closed state of both binding domains of homodimeric mGlu receptors is required for full activity. Nature Struct Mol Biol2004;11:706–13.

188. Chandrashekar J, Hoon MA, Ryba NJP, Zuker CS. The receptors and cells for mammalian taste. Nature 2006;444:288–94.
189. Springael JY, Urizar E, Costagliola S, Vassart G, Parmentier M. Allosteric properties of G protein-coupled receptor oligomers. Pharmacol Ther 2007;115:410–8.
190. El Asmar L, Springael JY, Ballet S, Andrieu EU, Vassart G, Parmentier M. Evidence for negative binding cooperativity within CCR5-CCR2b heterodimers. Mol Pharmacol 2005;67:460–9.
191. Sohy D, Parmentier M, Springael JY. Allosteric transinhibition by specific antagonists in CCR2/CXCR4 heterodimers. J Biol Chem 2007;282:30062–9.
192. Springael JY, Le Minh PN, Urizar E, Costagliola S, Vassart G, Parmentier M. Allosteric modulation of binding properties between units of chemokine receptor homo- and hetero-oligomers. Mol Pharmacol 2006;69:1652–61.
193. Waldhoer M, Fong J, Jones RM, et al. A heterodimer-selective agonist shows in vivo relevance of G protein-coupled receptor dimers. Proc Natl Acad Sci U S A 2005;102:9050–5.
194. Daniels DJ, Lenard NR, Etienne CL, Law PY, Roerig SC, Portoghese PS. Opioid-induced tolerance and dependence in mice is modulated by the distance between pharmacophores in a bivalent ligand series. Proc Natl Acad Sci U S A 2005;102:19208–13.
195. Breit A, Gagnidze K, Devi LA, Lagace M, Bouvier M. Simultaneous activation of the {delta} Opioid Receptor ({delta}OR)/Sensory Neuron-Specific Receptor-4 (SNSR-4) Hetero-Oligomer by the mixed bivalent agonist bovine adrenal medulla peptide 22 activates SNSR-4 but inhibits {delta}OR signaling. Mol Pharmacol 2006;70:686–96.
196. Park PSH, Palczewski K. Diversifying the repertoire of G protein-coupled receptors through oligomerization. Proc Natl Acad Sci U S A 2005;102:8793–4.
197. Maggio R, Vogel Z, Wess J. Coexpression studies with mutant muscarinic adrenergic-receptors provide evidence for intermolecular cross-talk between G-protein-linked receptors. Proc Natl Acad Sci U S A 1993;90:3103–7.
198. Hadac EM, Ji ZS, Pinon DI, Henne RM, Lybrand TP, Miller LJ. A peptide agonist acts by occupation of a monomeric G protein-coupled receptor: Dual sites of covalent attachment to domains near TM1 and TM7 of the same molecule make biologically significant domain-swapped dimerization unlikely. J Med Chem 1999;42:2105–11.
199. Osuga Y, Hayashi M, Kudo M, Conti M, Kobilka B, Hsueh AJW. Co-expression of defective luteinizing hormone receptor fragments partially reconstitutes ligand-induced signal generation. J Biol Chem 1997;272:25006–12.
200. Urizar E, Montanelli L, Loy T, et al. Glycoprotein hormone receptors: link between receptor homodimerization and negative cooperativity. Embo J 2005;24:1954–64.
201. Ji I, Lee C, Jeoung M, Koo Y, Sievert GA, Ji TH. Trans-activation of mutant follicle-stimulating hormone receptors selectively generates only one of two hormone signals. Mol Endocrinol 2004;18:968–78.
202. Whorton MR, Bokoch MP, Rasmussen SGF, et al. A monomeric G protein-coupled receptor isolated in a high-density lipoprotein particle efficiently activates its G protein. Proc Natl Acad Sci U S A 2007;104:7682–7.
203. Bayburt TH, Leitz AJ, Xie G, Oprian DD, Sligar SG. Transducin activation by nanoscale lipid bilayers containing one and two rhodopsins. J Biol Chem 2007;282:14875–81.
204. Ernst OP, Gramse V, Kolbe M, Hofmann KP, Heck M. Monomeric G protein-coupled receptor rhodopsin in solution activates its G protein transducin at the diffusion limit. Proc Natl Acad Sci U S A 2007;104:10859–64.
205. Galvez T. Allosteric interactions between GB1 and GB2 subunits are required for optimal GABAB receptor function. EMBO J 2001;20:2152–9.
206. Guo W, Shi L, Javitch JA. The fourth transmembrane segment forms the interface of the dopamine D2 receptor homodimer. J Biol Chem 2003;278:4385–8.
207. Salom D, Le Trong I, Pohl E, et al. Improvements in G protein-coupled receptor purification yield light stable rhodopsin crystals. J Struct Biol 2006;156:497–504.

208. Salom D, Lodowski DT, Stenkamp RE, et al. Crystal structure of a photoactivated deprotonated intermediate of rhodopsin. Proc Natl Acad Sci U S A 2006;103(44):16123–8.
209. Taylor Ds, Fung HK, Rajgaria R, et al. Mutations affecting the oligomerization interface of G–protein–coupled receptors revealed by a novel denovo protein design framework. Biophys J 2008;94:2470–81.
210. Vohra S, Chintapalli SV, Illingworth CJR, et al. Computational studies of family a and family B GPCRs. Biochem Soc Trans 2007;35:749–54.
211. Hebert TE, Moffett S, Morello JP, et al. A peptide derived from a beta(2)-adrenergic receptor transmembrane domain inhibits both receptor dimerization and activation. J Biol Chem 1996;271:16384–92.
212. Harikumar K, Pinon D, Miller L. Transmembrane segment IV contributes a functionally important interface for oligomerization of the Class II G protein-coupled secretin receptor. J Biol Chem 2007;282:30363–72.
213. Gouldson PR, Reynolds CA. Simulations on dimeric peptides: evidence for domain swapping in G-protein-coupled receptors? Biochem Soc Trans 1997;25:1066–71.
214. Gouldson PR, Snell CR, Bywater RP, Higgs C, Reynolds CA. Domain swapping in G-protein coupled receptor dimers. Protein Eng 1998;11:1181–93.
215. Gouldson PR, Snell CR, Reynolds CA. A new approach to docking in the beta(2)-adrenergic receptor that exploits the domain structure of G-protein-coupled receptors. J Med Chem 1997;40:3871–86.
216. Maggio R, Vogel Z, Wess J. Reconstitution of functional muscarinic receptors by coexpression of amino-terminal and carboxyl-terminal receptor fragments. FEBS Lett 1993;319: 195–200.
217. Stanasila L, Perez J-B, Vogel H, Cotecchia S. Oligomerization of the {alpha}1a- and {alpha}1b-Adrenergic Receptor Subtypes: potential implications in receptor internalization. J Biol Chem 2003;278:40239–51.
218. Hernanz-Falcon P, Rodriguez-Frade JM, Serrano A, et al. Identification of amino acid residues crucial for chemokine receptor dimerization. Nat Immunol 2004;5:216–23.
219. Klco JM, Lassere TB, Baranski TJ. C5a receptor oligomerization – I. Disulfide trapping reveals oligomers and potential contact surfaces in a G protein-coupled receptor. J Biol Chem 2003;278:35345–53.
220. Damien T, Tzvetana LMF, R. RCR. Oligomerization of the fifth transmembrane domain from the adenosine A2A receptor. Protein Sci 2005;14:2177–86.
221. Bouvier M. Oligomerization of G-protein-coupled transmitter receptors. Nat Rev Neurosci 2001;2:274–86.
222. Whorton MR, Jastrzebska B, Park PSH, et al. Efficient coupling of transducin to monomeric rhodopsin in a phospholipid bilayer. J Biol Chem 2007;282:9297–9301.
223. Ridge KD, Marino JP, Ngo T, Ramon E, Brabazon DM, Abdulaev NG. NMR analysis of rhodopsin-transducin interactions. Vision Res 2006;46:4482–92.
224. Ridge KD, Palczewski K. Visual rhodopsin sees the light: Structure and mechanism of G protein signaling. J Biol Chem 2007;282:9297–9301.
225. Brock C, Oueslati N, Soler S, Boudier L, Rondard P, Pin JP. Activation of a dimeric metabotropic glutamate receptor by intersubunit rearrangement. J Biol Chem 2007;282:33000–8.
226. Tateyama M, Abe H, Nakata H, Saito O, Kubo Y. Ligand-induced rearrangement of the dimeric metabotropic glutamate receptor 1[alpha]. Nat Struct Mol Biol 2004;11: 637–42.
227. Farrens DL, Altenbach C, Yang K, Hubbell WL, Khorana HG. Requirement of rigid-body motion of transmembrane helices for light activation of rhodopsin. Science 1996;274: 768–70.
228. Niv MY, Filizola M. Influence of oligomerization on the dynamics of G-protein coupled receptors as assessed by normal mode analysis. Proteins 2008;71: 575–86.

229. Goudet C, Kniazeff J, Hlavackova V, et al. Asymmetric functioning of dimeric metabotropic glutamate receptors disclosed by positive allosteric modulators. J Biol Chem 2005;280:24380–5.
230. Damian M, Mary S, Martin A, Pin JP, Baneres JL. G protein activation by the leukotriene B-4 receptor dimer – Evidence for an absence of trans-activation. J Biol Chem 2008;283: 21084–92.
231. Kniazeff J, Galvez T, Labesse G, Pin JP. No ligand binding in the GB2 subunit of the GABAB receptor is required for activation and allosteric interaction between the subunits. J Neurosci 2002;22:7352–61.
232. Xu H, Staszewski L, Tang H, Adler E, Zoller M, Li X. Different functional roles of T1R subunits in the heteromeric taste receptors. Proc Natl Acad Sci USA 2004;101:6.
233. Filipek S, Krzysko KA, Fotiadis D, et al. A concept for G protein activation by G protein-coupled receptor dimers: the transducin/rhodopsin interface. Photochem Photobiol Sci 2004;3:628–38.
234. Filipek S, Stenkamp RE, Teller DC, Palczewski K. G protein-coupled receptor rhodopsin: a prospectus. Ann Rev Physiol 2003;65:851–79.
235. Jastrzebska B, Fotiadis D, Jang GF, Stenkamp RE, Engel A, Palczewski K. Functional and structural characterization of rhodopsin oligomers. J Biol Chem 2006;281:11917–22.
236. Hamm HE. How activated receptors couple to G proteins. Proc Natl Acad Sci U S A 2001;98:4819–21.
237. Lambright DG, Sondek J, Bohm A, Skiba NP, Hamm HE, Sigler PB. The 2.0 A crystal structure of a heterotrimeric G protein. Nature 1996;379:311–9.
238. Sondek J, Bohm A, Lambright DG, Hamm HE, Sigler PB. Crystal structure of a G-protein beta gamma dimer at 2.1A resolution. Nature 1996;379:369–74.
239. Willardson BM, Pou B, Yoshida T, Bitensky MW. Cooperative binding of the retinal rod G-protein, transducin, to light-activated rhodopsin. J Biol Chem 1993;268:6371–82.
240. Terrillon S, Durroux T, Mouillac B, et al. Oxytocin and vasopressin V1a and V2 receptors form constitutive homo- and heterodimers during biosynthesis. Mol Endocrinol 2003;17:677–91.
241. Terrillon S, Bouvier M. Roles of G-protein-coupled receptor dimerization. EMBO Rep 2004;5:30–4.
242. Banères J-L, Parello J. Structure-based analysis of GPCR function: Evidence for a novel pentameric assembly between the dimeric leukotriene B4 receptor BLT1 and the G-protein. J Mol Biol 2003;329:815–29.
243. Senogles SE, Heimert TL, Odife ER, Quasney MW. A region of the third intracellular loop of the short form of the D2 dopamine receptor dictates Gi coupling specificity. J Biol Chem 2004;279:1601–6.
244. Voss T, Wallner E, Czernilofsky AP, Freissmuth M. Amphipathic alpha-helical structure does not predict the ability of receptor-derived synthetic peptides to interact with guanine nucleotide-binding regulatory proteins. J Biol Chem 1993;268:4637–42.
245. Nanoff C, Koppensteiner R, Yang Q, Fuerst E, Ahorn H, Freissmuth M. The carboxyl terminus of the Galpha-subunit is the latch for triggered activation of heterotrimeric G proteins. Mol Pharmacol 2006;69:397–405.
246. Johnston CA, Siderovski DP. Structural basis for nucleotide exchange on G alpha i subunits and receptor coupling specificity. Proc Natl Acad Sci U S A 2007;104:2001–6.
247. Franco R, Casado V, Cortes A, et al. Basic concepts in G-protein-coupled receptor homo- and heterodimerization. Sci World J 2007;7:48–57.
248. Franco R, Ciruela F, Casado V, et al. Partners for adenosine A1 receptors. J Mol Neurosci: MN 2005;26:221–32.
249. Springael JY, de Poorter C, Deupi X, Van Durme J, Pardo L, Parmentier M. The activation mechanism of chemokine receptor CCR5 involves common structural changes but a different network of interhelical interactions relative to rhodopsin. Cell Signal 2007;19: 1446–56.

250. Kenakin T. Efficacy at G-protein-coupled receptors. Nat Rev Drug Discov 2002;1:103–10.
251. Milligan G, Smith NJ. Allosteric modulation of heterodimeric G-protein-coupled receptors. Trends Pharmacol Sci 2007;28:615–20.
252. Jacoby E, Bouhelal R, Gerspacher M, Seuwen K. The 7Â TM G-protein-coupled receptor target family. Chem Med Chem 2006;1:760–82.
253. Pidasheva S, Grant M, Canaff L, Ercan O, Kumar U, Hendy GN. Calcium-sensing receptor dimerizes in the endoplasmic reticulum: biochemical and biophysical characterization of CASR mutants retained intracellularly. Human Mol Gen 2006;15:2200–9.
254. Calebiro D, de Filippis T, Lucchi S, et al. Intracellular entrapment of wild-type TSH receptor by oligomerization with mutants linked to dominant TSH resistance. Human Mol Gen 2005;14:2991–3002.
255. McElvaine AT, Mayo KE. A dominant-negative human growth hormone-releasing hormone (GHRH) receptor splice variant inhibits GHRH binding. Endocrinology 2006;147:1884–94.

Chapter 4
Dopamine Receptor Subtype-Selective Drugs: D1-Like Receptors

David E. Nichols

Abstract A survey is presented of the development of dopamine D_1 receptor-selective drugs, including agonists and antagonists. It is noted that there are presently no ligands that are specific for the D_1 vs the D_5 receptor isoforms. A general discussion is presented on the structure–activity features of D_1/D_5 selective agents, with the conclusions developed that all known full D_1 agonists must contain a catechol moiety and, in addition, require the presence of a hydrophobic moiety (typically a phenyl ring) in the region adjacent to the beta side chain carbon of the embedded dopamine fragment. This latter structural feature is so crucial that when added to a noncatechol ergoline it gave a D_1-selective partial agonist. Present evidence indicates that D_1 agonists may be therapeutically useful in the treatment of Parkinson's disease, as well as improving cognition and working memory in schizophrenia and age-related cognitive decline. No D_1 agonist has yet been commercialized, and that seems largely due to the difficulties of oral bioavailability for catechol-containing drugs.

Keywords D_1 · D_5 · Apomorphine · Phenylbenzazepine · Isochroman · Dihydroxynomifensine · Dihydrexidine · ABT-431 · Dinapsoline · Dinoxyline · Doxanthrine

4.1 Introduction

The first simple classification of dopamine receptors into D_1 and D_2 families in 1979 was based on the observation that D_1 receptors are positively coupled to adenylate cyclase, whereas the activation of D_2 receptors resulted in an inhibitory response or had no effect on adenylate cyclase [1]. Dopamine receptors are divided into two main families: the D1-like family, which includes the D_{1A} and D_{1B} (also referred

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to as D_1 and D_5), and the D2-like family, composed of the D_{2long}, D_{2short}, D_3, and D_4 receptor isoforms. Of all the dopamine receptors, the D_1 is the most prevalent in the brain, with a proposed role in a variety of CNS functions, including motor control and memory-related processes. Antagonist radiolabeling has shown highest D_1 receptor density in the frontal cortex, mainly in the nucleus accumbens and olfactory tubule, but also in the substantia nigra pars compacta [2, 3]. In the peripheral nervous system it is present in the kidney and cardiovascular system [4]. All of the dopamine receptors are members of the family A G-protein-coupled receptor family (GPCR). These receptors exist as a bundle of seven alpha-helical transmembrane segments embedded in the target cell membrane.

The D_1 and D_5 receptors were first cloned in the early 1990s [5, 6]. The D_1 receptor consists of 446 amino acids, whereas the D_5 is comprised of 477 amino acids in humans and 475 in rats. These receptors are 82% homologous in the transmembrane spanning helices and 100% in the region where orthosteric ligands are thought to bind. Although we shall discuss certain molecular features of the receptor later in this chapter, a more complete discussion of the molecular biology of this seven transmembrane helix family is provided in other chapters of this book; the focus of this chapter will be on selective dopamine D_1 drugs and the structure–activity relationships of these molecules.

It should be noted at the outset, however, that there is presently no agonist or antagonist ligand that is specific, or even selective, for the D_1 vs the D_5 receptor isoforms; all presently available "D_1 ligands," including both agonists and antagonists, have similar affinity and potency at both the D_1 and D_5 receptor isoforms. Hence, it has not been possible to employ traditional pharmacological approaches to elucidate distinct roles in brain function for these two closely related receptors. Therefore, the development of ligands specific for these two receptor isoforms still remains of extremely high importance today.

4.2 Apomorphine

The first drug known with D_1 agonist properties was apomorphine (Fig. 4.1), although this pharmacological property of the molecule was not recognized for many years. Its ready access by simple acid treatment of morphine allowed extensive studies and led eventually to its experimental therapeutic use in humans for a wide variety of ailments and conditions, including coughing, erectile dysfunction, alcohol and opiate addiction, schizophrenia and, later, Parkinson's disease [7, 8]. Studies

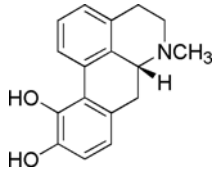


Fig. 4.1 The structure of apomorphine

of its binding in nervous tissue led to the recognition of "apomorphine receptors," which would eventually become known as dopamine receptors [9].

N-alkylation of apomorphine yields ligands with improved D_2 affinity and reduced D_1 affinity, the *N*-propyl analog (NPA) being the optimum modification for maximizing D2-like selectivity [10, 11]. This observation appears to be a general property that extends to most dopaminergic molecules and has been referred to as the "propyl effect" [12, 13], and groups longer than propyl tend to be detrimental for D_2 receptor affinity and potency. The size of the *N*-alkyl group in dopaminergic ligands proves to be one determinant of high D_1 receptor affinity and agonist activity, and as a general rule, a primary or secondary amine within the structure is optimal for D_1 activity.

Apomorphine is characterized as a mixed D_2/D_1 agonist and was briefly marketed in the United States (Apokyne©) as a rescue medication for the "off" motor effects observed in late-stage Parkinson patients treated with levodopa. In view of the fact that no other D2-like agonist has anti-Parkinson efficacy comparable to apomorphine, its D_1 agonist character [14] is likely responsible for its greater therapeutic effect [15].

4.3 1-Phenyl-3-Benzazepines

The first compound discovered to possess high D_1 receptor selectivity was SKF38393 (Fig. 4.2), initially tested for its peripheral effects [16] but assessed soon thereafter for a central action and found to stimulate striatal adenylate cyclase [17]. The discovery of SKF38393 truly represented a breakthrough for dopamine research, as the molecule is remarkably selective for D_1 receptors, with affinities of 18 and 9,300 nM at the D_1 and D_2 receptors, respectively [18]. The absolute configuration of the more active enantiomer was established as R, as shown in Fig. 4.2 [19].

SKF38393 was characterized as an agonist ligand, but early studies found that it displayed little antiparkinsonian potential [20, 21]. Unfortunately, these findings were used to argue against the relevance of the D_1 receptor as a target for the treatment of Parkinson's disease, an erroneous perception that still persists in many quarters today. It was ultimately realized, however, that the partial agonist character of SKF38393 [17] was likely responsible for its lack of efficacy in Parkinson's disease. Nevertheless, great research attention was focused on this molecule and the structural characteristics that were responsible for its high affinity and selectivity at D1-like receptors.

Following the discovery of SKF38393, it was found that compounds with a halogen replacing the hydroxyl at the 7-position and an *N*-methylated nitrogen were extremely potent and selective D_1/D_5 antagonists. The most notable example is the chloro compound SCH23390, a highly D_1-selective antagonist (Fig. 4.2; D_1 K_i = 0.12 nM; D_2 K_i = 1,210 nM) [22, 23]. Most of the pharmacological characterization of D_1 receptors has been performed with a tritiated form of SCH23390,

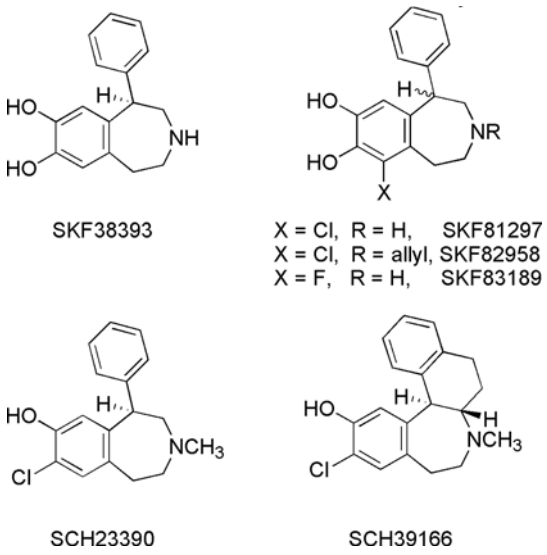


Fig. 4.2 1-Phenylbenzazepine D_1-selective ligands

which remains the ligand of choice for radioligand binding assays. This ligand has potent in vivo antagonist activity and virtually all of the studies of the role of the D_1 receptor in various behaviors also have been carried out using SCH23390. The only alternative approach to studies of the D_1 receptor has been the use of genetic mice that are null for the D_1 receptor.

Several analogs of SKF38393 also have remarkable selectivity for the D_1 receptor. Adding a halogen to the catechol ring led to an increase in D_1 affinity, yielding the selective ligands SKF81297 and SKF83189 [18, 24]. The activity of all these compounds resides in the illustrated (+)-enantiomers (Fig. 4.2). Despite their high degree of selectivity, functional assays of these agonists have generally revealed a lack of full intrinsic activity. That is, they are not able to stimulate cyclic AMP (cAMP) production to the same extent as dopamine itself in cells expressing D_1 receptors. A notable exception is SKF82958, which induces a robust activation of D_1 receptors [25].

The crystal structure of SKF38393 revealed that the pendant phenyl substituent occupies an equatorial position, twisted orthogonal to the plane of the catechol ring, and the ethylamino side chain is locked into a "*gauche*" conformation [26]. As we shall see later, both of these structural features may account for the inability of this ligand to stimulate adenylate cyclase optimally (only ca. 30–50% vs dopamine). It is unknown, of course, what conformation the molecule adopts when it binds to the receptor. Molecular modeling has indicated the existence of several low-energy conformational states, with the orientation of the pendant phenyl ring ranging between orthogonal and coplanar relationships with respect to the catechol ring plane [27]. Not surprisingly, the X-ray crystal solution of the conformation of SCH23390 also revealed a structure similar to that of SKF38393 [26].

Tethering the pendant phenyl ring of SCH23390 into a *trans*-fused tetrahydronaphthalene led to SCH39166, a potent D_1 antagonist with the pendant phenyl ring constrained in a conformation that extends it in the equatorial direction and with a slight β-phenyl ring-plane twist (Fig. 4.2) [26]. Although of somewhat lower D_1 affinity ($K_i = 3.3$ nM) than SCH23390, this conformation still has a high degree of complementarity to the D_1 binding site, suggesting that the active conformation of SCH23390 may be one where the pendant phenyl ring approaches coplanarity with the hydroxylated ring (but is not actually coplanar with it). Based on the structural similarities between SKF38393 and SCH23390, the dihydroxy analog of SCH39166 might have been expected to have high D_1 receptor affinity. Surprisingly, however, it had 1,000-fold lower affinity than the chlorohydroxy analog! Although this unexpected finding apparently has not raised interest, it may suggest that, despite the apparent structural similarity between SKF38393 and SCH23390, these molecules could be binding to the receptor in a fundamentally different way.

4.4 4-Phenyltetrahydroisoquinolines

Nomifensine (Fig. 4.3) was developed in the late 1960s by Hoechst AG as a potential antidepressant with weak dopaminergic properties [28, 29]. Studies of its potential hydroxylated metabolites indicated that 3′,4′-dihydroxynomifensine had significant dopaminergic agonist effects at the renal "DA1" receptor [30]. We subsequently demonstrated that its des-amino analog THIQ retained these dopamine agonist properties [12] and, further, that *N*-propylation of this compound abolished its D_1 activity, establishing a key difference in the SAR of D_1 and D_2 agonists, namely that *N*-alkylation is detrimental to D_1 affinity and functional activity. As noted earlier under the discussion on apomorphine, *N*-alkylation of dopamine agonists generally reduces activity at D_1 receptors, whereas it enhances activity at D2-like receptors.

Replacing the phenyl ring in THIQ with a thiophene gave a bioisosteric molecule (SKF89626) with properties virtually identical to THIQ [31, 32]. This class of compounds had efficacy comparable to dopamine in stimulating cAMP production. It will also be noted that the pendant phenyl moieties in these congeners, like the phenylbenzazepines, still possess a large degree of rotational freedom.

Dihydroxynomifensine was also resolved into its two enantiomers and the absolute configuration solved by X-ray crystallography [33]. The more active isomer had the *S* absolute configuration, shown in Fig. 4.3. It will be recognized that this stereochemistry corresponds to the active stereochemistry of the 1-phenylbenzazepines at the carbon atom where the pendant phenyl moiety is attached (the β-position of the "dopamine moiety"). Dandridge et al. [34] proposed a conceptual model of the D_1 receptor to accommodate this stereochemistry, as well as to be compatible with the earlier model of McDermed et al. [35].

The appearance of a "β-phenyldopamine" motif in all of these ligands led us to study β-phenyldopamine itself, and we subsequently proposed a β-phenyldopamine pharmacophore as a requirement for activation of D_1 receptors [36]. We compared

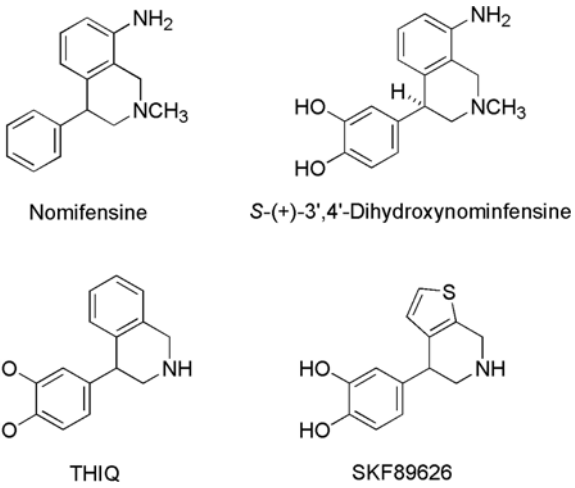


Fig. 4.3 Tetrahydroisoquinoline-type D_1 agonists

the ability of dopamine, β-methyldopamine, and β-phenyldopamine to activate the D_1 receptor in rat retina. Racemic β-methyldopamine had only one-twentieth the potency of dopamine in this assay, whereas β-phenyldopamine had one-half the potency of dopamine and was 10-fold more potent than β-methyldopamine. Clearly, the pendant β-phenyl ring dramatically enhanced potency, compared to the simple β-methyl, and suggested that this structural feature was a key determinant of D_1 activity in all of these molecules.

4.5 Benzo[*a*]phenanthridines

The demonstrated D_1 agonist activity of 4-(3′,4′-dihydroxyphenyl)-tetrahydroisoquinoline (THIQ), coupled with the well-known dopaminergic activity of simple 2-aminotetralins, led us to tether the THIQ structure into a tetracyclic benzo[*a*]phenanthridine, dihydrexidine (DHX), the first high-potency, full-efficacy selective dopamine D_1 agonist [37, 38]. DHX had modest 10-fold selectivity for D_1 over D_2 receptors. The analogous benzo[*f*]isoquinoline, lacking the "β-phenyl" moiety, lacked D_1 activity, demonstrating again that the interaction of the pendant phenyl moiety with some accessory binding region within the D_1 receptor was crucial for high activity. In this same report, the *cis*-fused compound (*cis*-DHX) also was examined and found to be inactive, demonstrating that full agonists should be relatively planar molecules (Fig. 4.4).

In a later study, the enantiomers of DHX were resolved, and the (+)-isomer, with the absolute stereochemistry shown, was the most active [39]. Thus, these studies all confirmed the requirement for a similar absolute stereochemistry at the chiral carbon that is the site of attachment of the appended "β-phenyl" moiety in DHX, SKF38393, and 3′,4′-dihydroxynomifensine.

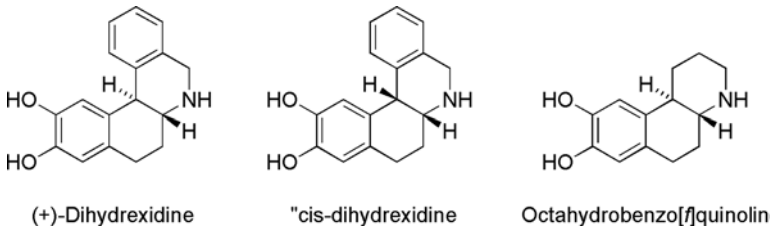


Fig. 4.4 The structure of dihydrexidine (DHX), its inactive *cis* isomer, and DHX with the appended phenyl ring removed, the inactive octahydrobenzo[*f*]quinoline

DHX produced a profound reduction of *N*-methyl-4-phenyl-1,2,5,6-tetrahydropyridine (MPTP)-induced parkinsonian symptoms in male African green monkeys [40]. In a small clinical trial, however, three of four human subjects experienced marked hypotension when the drug was rapidly administered intravenously [41]. This effect was likely the result of potent activation of renal D_1 receptors [42]. The fourth patient, with the highest measured blood plasma levels of DHX, displayed a significant improvement of Parkinson's disease (PD) symptoms. Not surprisingly, the D_1 antagonist SCH23390 blocked the behavioral effects of DHX in rodents, whereas the D_2 antagonist remoxipride did not [43]. In contrast to the failure of the partial D_1 agonist SKF38393 to show efficacy in PD, these proof-of-principle results in both humans and a nonhuman primate model of PD clearly demonstrated the need for a full D_1 agonist to achieve an optimal antiparkinsonian effect [44].

The presence of both catechol hydroxyl groups seems to be necessary for the high affinity of DHX; however, the *meta*-hydroxyl has been shown to be most important. Removal of the *para*-hydroxyl results in a more than a 20-fold loss in D_1 affinity, whereas the absence of the *meta*-hydroxyl resulted in a more than 200-fold loss of affinity [45]. Low-energy conformations of the *trans*-diastereomer (DHX) situate the conformationally restricted β-phenyl ring plane at an angle of approximately 56° above the plane of the catechol ring [46, 47].

Substitutions on the β-phenyl ring of DHX led to important structure–activity information for this series [48]. Most notably, substitution at the 2-position with a methyl group, as in 2-methyldihydrexidine (Fig. 4.5), led to a significant increase in D_1:D_2 selectivity, resulting in a greater than 5-fold loss in D_2 affinity with no significant change in its D_1 affinity. Substitution with an ethyl group at this position also led to a highly D_1-selective compound.

Although DHX passed through preclinical toxicology studies and was taken further into the drug development process, it so far has not been successfully commercialized, for at least two reasons. First and foremost, DHX is a catechol and has less than 5% oral bioavailability, which is considered to be too low to be suitable for an orally administered commercial drug. Second, DHX has a very short duration of action, something that also could be related to the rapid metabolic processes that cause the low oral availability.

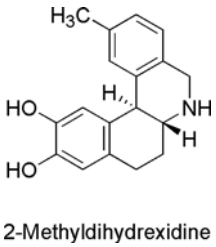


Fig. 4.5 The structure of 2-methyldihydrexidine, an analog with increased D_1:D_2 selectivity

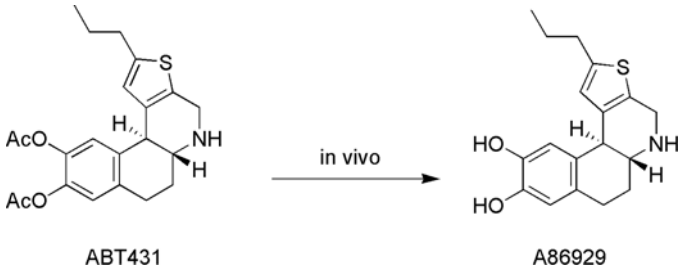


Fig. 4.6 The structure of ABT-431, an *O,O*-diacetyl prodrug of the Abbott D_1 agonist A86929

A structurally related thiophene bioisostere of DHX developed by Abbott Laboratories, A-86929 (Fig. 4.6), had pharmacological properties almost identical to DHX [49–51]. Its *O,O*-diacetyl prodrug, ABT-431, was shown to have efficacy comparable to levodopa in a small trial in Parkinson patients [52, 53], but due to poor pharmacokinetic properties of this drug, further development was abandoned. ABT-431 also was reported to produce dose-related dyskinesias in patients who had already developed levodopa-related dyskinesias [53].

4.6 Abbott Isochromans

The compounds in this family, with examples shown in Fig. 4.7, were first synthesized by Abbott laboratories and are extremely potent and selective D_1 dopamine agonists with high intrinsic activity [54–57]. The postulated requirement for the *trans*-β-phenyldopamine structure is not strictly fulfilled in this series; however, this work demonstrated that large nonaromatic hydrophobic substituents, such as the adamantyl (A77636) [58], may be substituted for the phenyl (A68930) [56, 59, 60]. Removing this bulky substituent results in a marked decrease in both D_1 and D_2 affinities. A large difference in affinities is observed in the isochromans with *cis* vs *trans* stereochemistry, with the latter being almost inactive. In the *cis* series, the potency resides mainly in the (+)-enantiomer, as shown in Fig. 4.7.

The phenyl-substituted isochroman A68930 (Fig. 4.7) has the highest D_1 binding affinity of the series (D_1 K_i = 3.1 nM, D_2 K_i = 776 nM), comparable to the affinity of the cyclohexyl derivative, which displayed somewhat higher selectivity

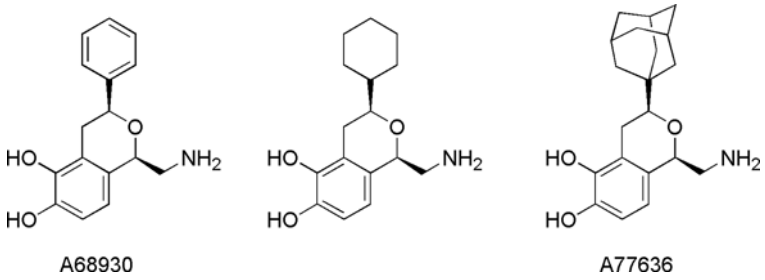
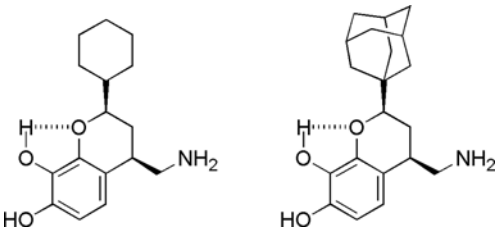


Fig. 4.7 Abbott isochroman D_1 agonist ligands

(D_1 K_i = 5.4 nM, D_2 K_i = 1,120 nM). A prominent analog of the series was the adamantyl compound A77636 (D_1 K_i = 31.7 nM, D_2 K_i = 1,290 nM), making evident the tolerance of the D_1 accessory binding region for bulky hydrophobic groups. It can be recognized that the isochromans do contain an embedded β-substituted dopamine moiety, albeit the group attached to the "β-position" is not necessarily a phenyl group. As contrasted to the benzo[*a*]phenanthridines, however, this moiety, a phenyl or bulky hydrophobic group, also is displaced two atoms away from the β-side chain position, suggesting that the accessory binding site in the receptor has some flexibility or is larger than a single phenyl ring.

Fig. 4.8 Chroman molecules with markedly reduced D_1 agonist activity



Although the oxygen atom in the isochromans is not essential for activity, moving the oxygen to give chromans (Fig. 4.8) led to a very dramatic loss of D_1 activity [61]. For example, the cyclohexyl and adamantyl congeners shown above had D_1 affinities of 500 and 2,500 nM, respectively, and were only 3- to 15-fold D_1 selective, whereas the corresponding isochromans in Fig. 4.3 had affinities of 2.4 and 3.5 nM, respectively, and were 400- to 540-fold D_1 selective [61]. Complete removal of the oxygen atom from the heterocyclic ring gives potent and highly selective D_1 agonists [62], suggesting that there is no requirement for the oxygen atom in the Abbott isochroman compounds.

The most likely explanation for this finding would seem to be the formation of an intramolecular hydrogen bond between the chroman oxygen and the adjacent ring OH group that disrupts the hydrogen-bonding network that must form when the ligand binds to the receptor. This postulated hydrogen bond is illustrated in Fig. 4.8 as a dashed line. If this hypothesis is true, something we are now testing, it suggests

a specific hydrogen-bonding scheme for agonist ligands within the D_1 receptor that is probably not identical within the D_2 receptor.

4.7 Dinapsoline

A second tethering strategy that retained the β-phenyldopamine pharmacophore (and yielded a ligand geometry similar to DHX) led to another full D_1 agonist named dinapsoline (Fig. 4.9; DNS) that is as potent as DHX with respect to its D_1 pharmacology, although it is less D_1 vs D_2 selective [63, 64]. Interestingly, dinapsoline is a catechol, but has a much longer in vivo action than DHX [65], and was also found to have somewhat higher oral bioavailability. These observations suggest that certain molecular features can attenuate the undesirable physicochemical properties of a catechol with respect to oral bioavailability and plasma half-life. DNS was also shown to have marked and long-lasting effects in the 6-OH-DA rotating rat model, as well as efficacy in an MTPT-lesioned marmoset model of Parkinson's disease. In the MPTP-lesioned rat model of Parkinson's disease, it was shown that repeated daily dosing for 4 days with A77636 produced behavioral tolerance, whereas DNS administered once or twice daily failed to produce tolerance [65]. The authors suggested that the tolerance was likely related to the duration of D_1 receptor occupancy.

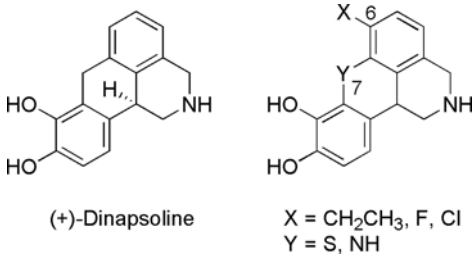
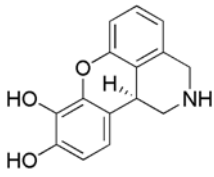


Fig. 4.9 Dinapsoline and selected substituted analogs

A series of substituted DNS derivatives was studied to develop preliminary structure–activity relationships [66]. Replacing the 7-methylene bridge ("Y") with either nitrogen or sulfur (Fig. 4.9) greatly diminished D_1 activity and in general gave D_2-selective compounds. Interestingly, the 6-ethyl-substituted compound ($X = CH_2CH_3$) had twice the D_1 affinity of DNS itself. The 6-fluoro-DNS analog had about one-half the affinity of DNS, but surprisingly was 10-fold selective for the D_2 receptor. When a chlorine was placed at the 6-position, the molecule had nearly 100-fold selectivity for the D_2 receptor. When considered in conjunction with the finding that 2-methyl-DHX had enhanced D_1 selectivity, these results suggest the presence of a region in the D_1 and D_2 receptors that may be exploited to alter D_1:D_2 selectivity.

4.8 Dinoxyline

The oxygen bioisostere of dinapsoline, named dinoxyline (DNX) (Fig. 4.10), again was a full dopamine D_1 agonist, but in this case the ligand was not D_1 selective, and possessed high affinity at all five dopamine receptor isoforms [67, 68]. Nothing further has been reported on this ligand since its original discovery. It is somewhat puzzling, however, that DNX has high D_1 affinity and potency, whereas the chromans discussed earlier (Fig. 4.8) do not. The presence of the pendant phenyl ring must somehow compensate for changes in the hydrogen-bonding scheme when DNX binds. The two phenyl rings, through resonance overlap with the heterocyclic ring oxygen, also would be expected to reduce the electron density on the bridging oxygen, and this reduced density would decrease the strength of the intramolecular hydrogen bond. Thus, the serine residue(s) that normally hydrogen bond to the catechol "meta" OH group would not have to compete with the formation of an intramolecular hydrogen bond.

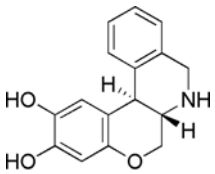


Dinoxyline

Fig. 4.10 The structure of dinoxyline, an agonist that activates all five dopamine receptor isoforms

4.9 Doxanthrine

Most recently, an oxygen bioisostere of DHX was prepared named doxanthrine (DOX) (Fig. 4.11) [69]. The absolute configuration of the more active (+)-enantiomer of DOX had the anticipated stereochemistry, identical to DHX and DNS. In contrast to DHX, however, doxanthrine had much higher selectivity for the D_1 vs the D_2 receptor, making it perhaps one of the best of the newer D_1 "β-phenyldopamine"-type agonist ligands. In porcine striatal homogenates, the affinities at the D1-like and D2-like receptors for racemic DHX and DOX were 21 and 240 nM vs 22 and 3,700 nM, respectively, indicating a D_1/D_2 selectivity of 168 for DOX vs only 11 for DHX. In cloned human D_1 receptors (+)-DOX had an EC50 for enhancing cAMP accumulation of 29 nM, whereas the (–)-isomer was not a full agonist and had an EC50 of 1,100 nM. Surprisingly, the (–)-isomer proved to be an α_{2C}-adrenergic receptor agonist with potency at least comparable to clonidine (EC50 4.4 nM vs 17 nM for clonidine)! In the DOX series, however, and in contrast to both the DHX and DNS series, substituents on the pendant phenyl ring did not enhance D_1 selectivity, but in fact decreased D_1 activity significantly [70]. Additional studies are underway in the author's laboratory to characterize this ligand further.



Doxanthrine

Fig. 4.11 The structure of doxanthrine, a potent and selective dopamine D_1 agonist

4.10 Aminomethylfluorenes

In 1986, workers at Smith, Kline & French reported the synthesis of a series of phenethylamines that exploited the features of two known dopaminergic ligands, the biaryl ring system of apomorphine and the β-phenyldopamine skeleton of SKF38393 [71]. This fluorene series (Fig. 4.12) yielded two compounds active at D_1 receptors: aminomethylfluorenetriol, which had modest affinity at D_1 receptors ($K_i = 43$ nM), and the des-hydroxy analog aminomethylfluorenediol, which displayed 9-fold lower affinity ($K_i = 380$ nM).

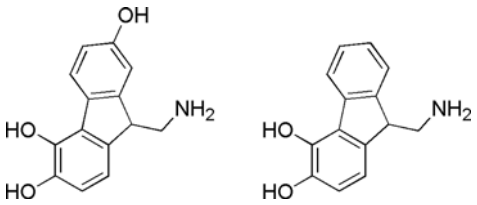


Fig. 4.12 Aminomethyl fluorenetriol (*left*) and aminomethyl fluorenediol

The D_1 affinities of these fluorene "β-phenyldopamine" analogs demonstrate that a fully coplanar β-phenyl ring can also lead to good receptor complementarity, at least with respect to affinity. Given the flexibility of the ethylamino side chain, however, the positioning of the ammonium moiety with respect to the coplanar β-substituent remains unknown [47].

4.11 Defining the D_1 Agonist Pharmacophore

Fundamentally, all full D_1 agonists discovered to date have a dopamine structure embedded within them, in a beta-rotameric orientation. For most of the known full D_1 receptor agonist molecules, the basic pharmacophoric structure can be envisioned as a β-substituted dopamine fragment. The pendant β-moiety is most typically a phenyl or aryl ring, but as evident from the Abbott isochromans, this

group can simply be a large hydrophobic moiety. The isochromans also illustrate that the location of the accessory binding region is not precisely fixed but has some variation with respect to the location of the appended hydrophobic moiety.

4.11.1 The Embedded Dopamine Fragment

The ethylamine side chain of dopamine is freely rotating and theoretically may adopt many different conformations in solution (or at the receptor). Cannon, in 1975, first designated the two possible *trans* conformational extremes of dopamine as "alpha" and "beta" [72]. Apomorphine incorporates an "alpha" rotamer, as do most D_2 agonist ligands, but all of the other full D_1 agonists contain a "beta" rotamer. Freeman et al. [35] originally developed a conceptual model that could account for the stereochemistries of 2-aminotetralines with either an alpha- or a beta-embedded side chain.

If we consider all of the foregoing discussion, we can envision the essential features of the D_1 agonist pharmacophore as shown in Fig. 4.13. Although the pendant phenyl ring in DHX and the phenyl ring in Abbott A68930 do not appear to reside in identical space, in fact the centroids of the pendant phenyl rings are located nearly the same distance from the key "meta" hydroxy, at a distance of 6.8–7 Å.

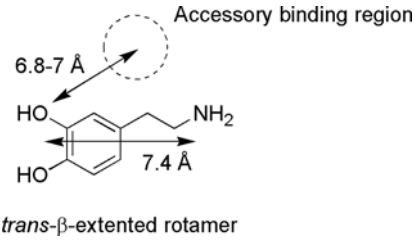


Fig. 4.13 The dopamine D_1 agonist pharmacophore

The dopamine fragment within the agonist should be in a *trans*-extended beta-rotameric form, with the distance between the meta hydroxy and the amino group being about 7.4 Å. In rigid analogs, it also has been suggested that the protonated electron pair on the nitrogen atom should be directed in a pseudoequatorial orientation [13]. Computational studies by Froimowitz were consistent with this hypothesis [73]. If all of these essential requirements are met, then the published evidence suggests that a molecule that contains these elements should have dopamine D_1 receptor agonist activity.

4.11.2 Design Limitations: The Catechol Moiety

Probably the most important structural feature, and unfortunately the one that has limited the development of "druggable" selective dopamine D_1 agonists, is the requirement for a catechol function in the molecule. To date, no full dopamine D_1

agonist has been discovered that does not possess a catechol moiety. All catechols do not have the same pharmacokinetic and metabolic properties; nevertheless, when drug companies consider the problems of developing a catechol-containing drug, they decline in favor of noncatechol alternatives. Despite modern formulation strategies and novel approaches to prodrug techniques, the historical failure to develop a catechol as a drug reinforces the notion that catechols are not good drug candidates.

Studies with monohydroxy analogs of apomorphine have shown the importance of the catechol moiety in binding at both D2- and D1-like receptors. Removal of the 10-hydroxy group, which yields 11-hydroxyaporphine, increased D_1 affinity by about 10-fold [74]. By contrast, removal of the 11-hydroxy caused a marked loss of dopaminergic activity [75]. Similar observations in other series of dopaminergic compounds have established the predominant importance of the hydroxy that is "*meta*" to the ethylamine side chain fragment, whereas the "*para*"-hydroxy has a lesser contribution to binding but is necessary for full agonist activity. For example, replacing the para-hydroxy (10-OH) in DHX with a hydrogen results in antagonist or partial agonist properties, whereas replacement by a methoxy, halogen, or a methyl results in antagonistic properties. Removing the meta OH (11-OH) gave an inactive compound [45].

There is one example of a D_1-selective compound that lacks a catechol: the ergoline CY208-243 developed by Sandoz (Fig. 4.14) [76]. It has been suggested that the pyrrole portion of the ergoline molecule can serve as a replacement for the catechol moiety [77], and that theory has been supported both by the synthesis of bicyclic and tricyclic partial ergolines that retain dopaminergic activity [78] and also by theoretical calculations showing similarities between the molecular electrostatic fields around simple models of apomorphine and ergolines [79].

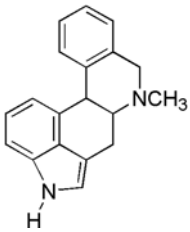


Fig. 4.14 The structure of the noncatechol ergoline partial D_1 agonist CY208-243

Like the benzo[*a*]phenanthridines, one can point to a β-phenyl moiety within CY208-243 as the basis for enhanced D_1 activity. Nonetheless, CY208-243, like SKF38393, failed to show efficacy in PD [80], and again that result is likely related to its partial agonist character at the D_1 receptor. It might be noted, however, that CY208-243 also has an *N*-methyl alkyl group, which is known in all other series of dopamine D_1 agonists to attenuate D_1 activity and enhance D_2 activity. Consistent with this reasoning, the reported intrinsic activity of CY208-243 for activating bovine retinal adenylate cyclase is only 57%, whereas the intrinsic activity of its *N*-des-methyl analog is reported as 82%, indicating that the latter compound is superior as a D_1 agonist. Unfortunately, no further results were reported

for the des-methyl analog of this compound, and it may well be that it could have therapeutic potential.

4.11.3 Relative Orientation of the Catechol and Pendant Phenyl Rings

Although the presence of the β-substituent appears necessary for high affinity and potency in D_1 agonists, there are studies that demonstrate the need for a fairly specific spatial orientation of this ring, relative to the catechol ring. The two measures of this relationship can be envisioned as angles measured as ring-plane torsion and ring-plane twist, as shown in Fig. 4.15. In general, the ring-plane torsion should place the appended accessory ring in a plane at about an angle of 50–60° from the plane of the catechol ring [25]. DHX, dinapsoline, and doxanthrine are exemplary of this active conformation.

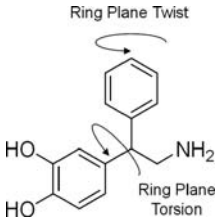


Fig. 4.15 Angles that define the relative orientations of the aromatic rings in β-phenyldopamine-type D_1 agonists

A study by Negash et al. [47] examined congeners where the C ring was expanded to seven atoms, and these molecules proved to be inactive. An example of this series is shown in Fig. 4.16. This molecule distorts not only the *trans*-beta-rotamer dopamine fragment but also the orientation of the appended β-phenyl moiety.

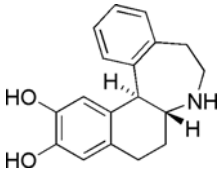


Fig. 4.16 Inactive C-ring expanded dihydrexidine analog

Snyder et al. [27] used a methylene or an ethyl bridge to tether the pendant phenyl ring of SKF38393 to the catechol ring, molecules shown in Fig. 4.17. The methylene tether forces the β-phenyl moiety into a virtually coplanar orientation with respect to the catechol ring, but reduces the conformational mobility of the azepine ring. It had about 50-fold lower D_1 receptor affinity than SKF38393. Tethering the pendant phenyl ring with an ethyl bridge restores some conformational mobility to the azepine ring, but forces the β-phenyl moiety into an

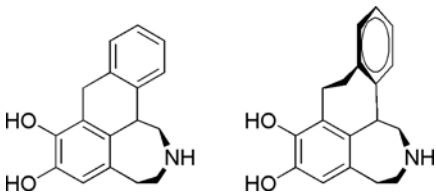


Fig. 4.17 Rigid dihydrexidine analogs with reduced D_1 binding affinity

orientation that is nearly orthogonal to the catechol ring. This analog had about 34-fold lower D_1 affinity than SKF38393, slightly better than the methyl-tethered compound.

These results are all consistent with the requirement that the β-phenyl moiety must reside in a *nearly* coplanar arrangement with the catechol ring and that the ethylamine side chain must to be in an extended *trans*-beta-rotamer orientation. The full agonist properties of DHX, DNS, DNX, and DOX, compared to the partial agonist activity of SKF38393, also suggest that this conformation is important for full receptor activation, but is not a requirement for high D_1 affinity.

4.11.4 Linking the Conceptual Model to the 3D Receptor Structure

Molecular modeling and mutagenesis studies of the D_1 receptor indicate that Asp 103 in TM3 is probably responsible for binding the protonated nitrogen of DA, whereas serines 198, 199, and 202 are involved in binding to the catechol hydroxyls [81, 82]. As noted earlier, the amino acid sequence of the D_1 and D_5 receptors in the orthosteric ligand binding domain is 100% identical.

The lack of an X-ray crystal structure for the D_1 receptor has compelled the use of analogies to other GPCRs as the best recourse for understanding the structural characteristics of the D_1 receptor binding site. The crystal structure of bovine rhodopsin, a GPCR distantly related to monoamine GPCRs, and more recently the β_2-adrenergic receptor [83], has enabled computationally derived hypotheses positing that the dopamine binding site is located between helices 3, 5, and 6, which traverse the cellular membrane.

In the β_2-adrenergic receptor, site-directed mutagenesis experiments have identified a key conserved residue, Asp 113, that has been proposed to be involved in an electrostatic interaction with the protonated amine of catecholamines [84, 85]. The cognate residue is conserved in all monoamine GPCRs and corresponds to Asp 103 in the D_1 receptor. Based on solid evidence using monohydroxy derivatives of DHX and serine-to-alanine mutants of these residues, as well as earlier experiments with the β_2-adrenergic receptor, Nichols, Mailman, and coworkers (unpublished results) have proposed that Ser 202 of the fifth transmembrane domain interacts via a hydrogen bond with the *para*-hydroxy and that Ser 198 and Ser 199 interact with the *meta*-hydroxyl of DHX. This information forms the basis for an examination of the 3D binding of agonists into the D_1 receptor.

Figure 4.18 is a sequence alignment for the D_1, D_5, and D_2 receptors. Residues in the D_5 receptor that are not identical to those in the D_1 receptor are shown in boldface type. The sequences of intracellular loop 3 (IL3), connecting the bottoms of helices 5 and 6, have been omitted for clarity. Sequence identity in the transmembrane regions is about 86%, but if the differences in TMs 1, 2, 4, and 8, which are too distant to affect agonist binding directly, are excluded, then the sequence identity in the transmembrane regions where the orthosteric agonist ligand binds is much higher, about 96%, and sequence similarity approaches 100%. It is apparent that the sequence similarity/identity in the orthosteric binding domain, principally in TMs 3, 5, 6, and 7, is so high that it will probably be impossible to design a molecule with D_1 vs D_5 selectivity that exploits this region of the receptor. The N-terminal portions of extracellular loop 2 (EL2), however, are sufficiently divergent that receptor-specific ligand design might be possible by differentially engaging residues in this loop. For example, there are several tryptophan residues in EL2 of the D_5 receptor that are absent in the D_1 sequence. Indeed, this portion of EL2 in the D_1 receptor contains no aromatic amino acids.

The 3D structure of the receptor, derived from homology models, can be reconciled with the earlier conceptual models, attesting to the insight provided by extensive experimental work over many years by numerous investigators. A number of the proposed features in the receptor can be identified, especially the catechol binding residues and the amine binding site. Figure 4.19 presents a conceptual "cartoon" model that incorporates all of the elements known to be factors in the structure–activity relationships of D_1 agonists.

Figure 4.20 presents a parallel illustration, but with virtual docking of doxanthrine into a homology model of the D_1 receptor based on the crystal structure of the β_2-adrenergic receptor. This figure illustrates the residues that account for the functional properties of the receptor and reflects the elements of the conceptual model that were used to drive the design of new D_1 ligands. In particular, serine residues 198 and 199 appear to be involved in hydrogen-bonding interactions with the more critical "meta" hydroxy group of the catechol moiety, and serine 202 appears to engage the "para"-hydroxy.

Aspartate $103^{(3.32)}$ forms a salt bridge with the amino group of the ligand, a feature common to all monoamine G-protein-coupled receptors (GPCRs). In the virtual docking studies, Asp 103 approaches the protonated amine from an equatorial direction, an idea that first originated with the recognition that *N*-alkylation was detrimental to D_1 receptor activation, as the lower energy of an equatorial *N*-alkyl [73] would force the protonated electron pair into a pseudoaxial orientation and disfavor the equatorial approach of Asp 103.

Phenylalanine residues $288^{(6.51)}$ and $289^{(6.52)}$ are thought to interact with the aromatic ring of the agonist ligand, with F6.52 believed to be the most important. These residues form pi stacking complexes with the aromatic ring of the agonist ligand. The region of "steric occlusion" below the ligand is less well defined, but probably consists of a sterically crowded area with contributions from a variety of residues, including Phe203 in helix 5. Overall, viewed from the extracellular side, the orthosteric agonist binding site is a relatively narrow groove or slot in the protein, stretching somewhat linearly between helices 3 and 5.

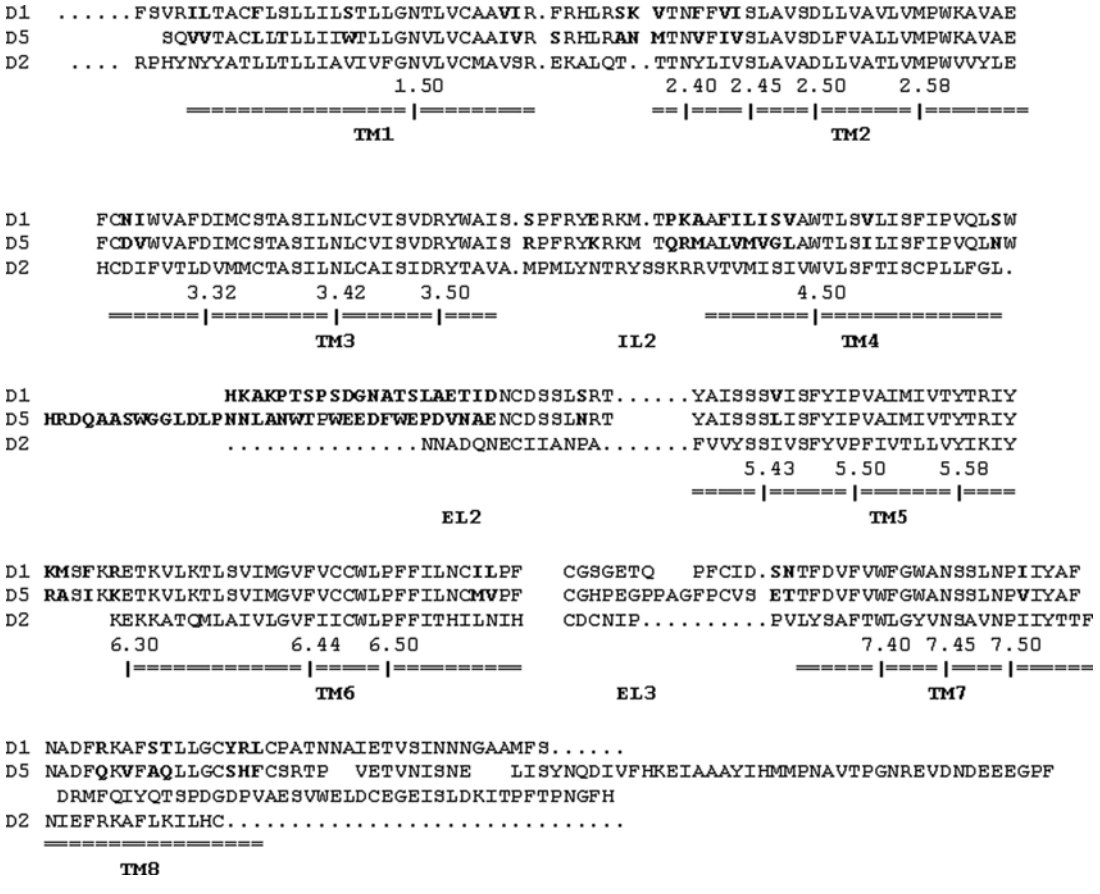


Fig. 4.18 Sequence alignments of the D_1, D_5, and D_2 receptors

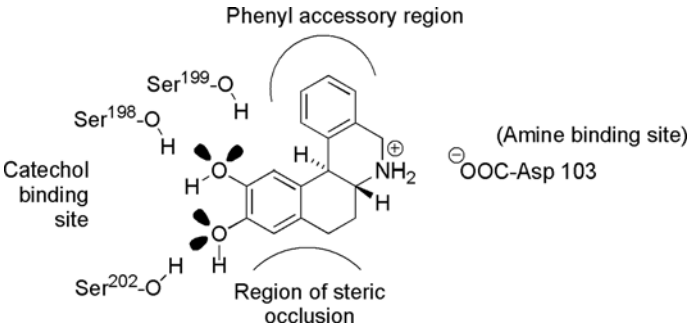


Fig. 4.19 Conceptual model of the D_1 agonist binding site

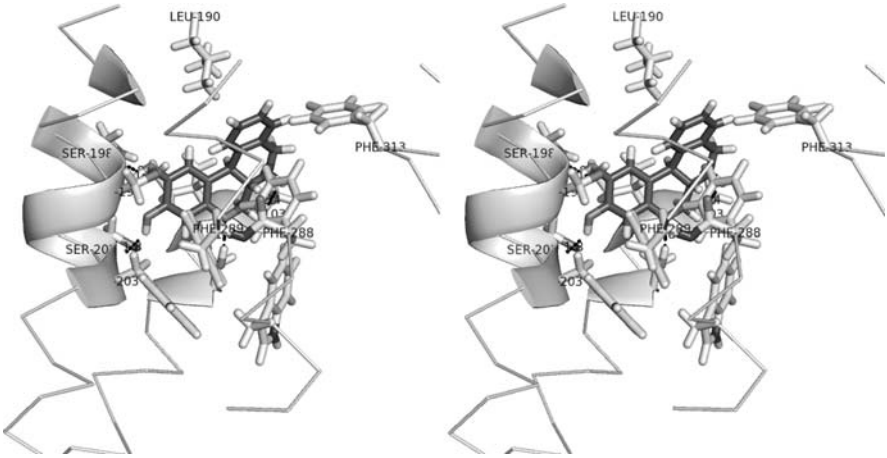


Fig. 4.20 Stereopair view (cross eyed) of doxanthrine (*dark* central molecule) docked into a homology model of the D_1 receptor. The view is with TM3 in the foreground and TM5 in the right rear. The ligand is in approximately the same orientation as in the conceptual model shown in Fig. 4.19

A structural feature that has not previously been explored is the role of Ser $107^{(3.36)}$, which is one turn below the essential D103. Our finding that doxanthrine had higher selectivity for D_1 vs D_2 receptors was initially difficult to explain. After we had carried out virtual docking studies, however, it was evident that Ser 107 could probably hydrogen bond to the heterocyclic oxygen atom in doxanthrine. Yet, the D_1 affinity of doxanthrine was essentially identical to that of its carbon analog, dihydrexidine; it is the loss of affinity of doxanthrine at the D_2 receptor that gives doxanthrine its higher D_1 receptor selectivity. We ultimately realized that the

heterocyclic oxygen atom in the chroman ring of doxanthrine would be solvated in aqueous solution. Binding in the receptor would involve desolvation of that oxygen atom and a corresponding energy penalty as a result. For doxanthrine, this energy cost could be compensated for by the hydrogen bond with Ser 107, but no similar gain could be realized when DHX bound because it has a carbon atom adjacent to Ser 107. In the D_2 receptor the cognate residue is a cysteine, with much weaker hydrogen bonding potential, as well as a larger van der Waals radius. Thus, we had stumbled onto a very interesting indirect way to enhance D_1 selectivity that was not originally anticipated.

This difference at residue 3.36, a serine in the D_1 receptor, and a cysteine in the D_2 receptor, also could account for the loss of D_1 selectivity in dinapsoline, compared to DHX. Whereas dihydrexidine has an ethyl bridge in this region that will experience greater steric interference with the larger cysteine than with a serine, dinapsoline has no molecular component in this region, and thus the presence of the cysteine in the D_2 receptor does not create the same extent of steric impediment to binding for DNS.

As elaborated earlier at some length, one of the most important features of the D_1 receptor is its "β-phenyl accessory region" or accessory ring binding site. Unfortunately, the identity of this region has not yet been established. As discussed earlier, it clearly plays a very important role in conferring high D_1 affinity and, in catechols, full agonist activity. Virtual docking of doxanthrine into a homology model of the D_1 receptor does suggests some possible players, however. Phe288$^{(6.51)}$ is one obvious choice, except that it appears more important for antagonist binding in other GPCRs where it has been examined. A critical consideration, however, is the fact that the D_2 receptor has identical phenylalanine residues at these positions, so it is very difficult to conclude that Phe288 is a key part of the accessory binding region. By contrast, Phe313 in TM7 is particularly attractive as part of the accessory region because the corresponding residue in the D_2 receptor is a tyrosine. If this residue comprises part of the accessory binding region, it seems possible that there could be two explanations for its effect on D_1 vs D_2 selectivity. First, the para-OH of the tyrosine residue could project toward the pendant phenyl ring of ligands such as DHX or DOX. That would prevent an edge-to-face type of pi stacking interaction suggested by the virtual docking presented in Fig. 4.21. A second possible explanation is that the tyrosine in the D_2 receptor engages a residue in helix 2 or 3, pulling it away from an interaction with the agonist ligand. At the present time the importance of this residue remains unknown, and there are no mutagenesis studies to suggest its role in the dopamine D_1 receptor. It addition, it appears likely that a residue in extracellular loop 2 (EL2) may be a component of the accessory binding region, possibly serving as one of the pieces of "bread," with the ligand accessory ring being the "meat" of a sandwich. One possibility for this role is Leu190. One study has been published where it was reported that mutation of four residues in EL2 (DSS**L**) containing this amino acid led to a mutant receptor with a significant 25-fold loss of affinity for SCH23390 [86]. No data were reported for binding of an agonist ligand and there is no report of a receptor with the single mutation of L190.

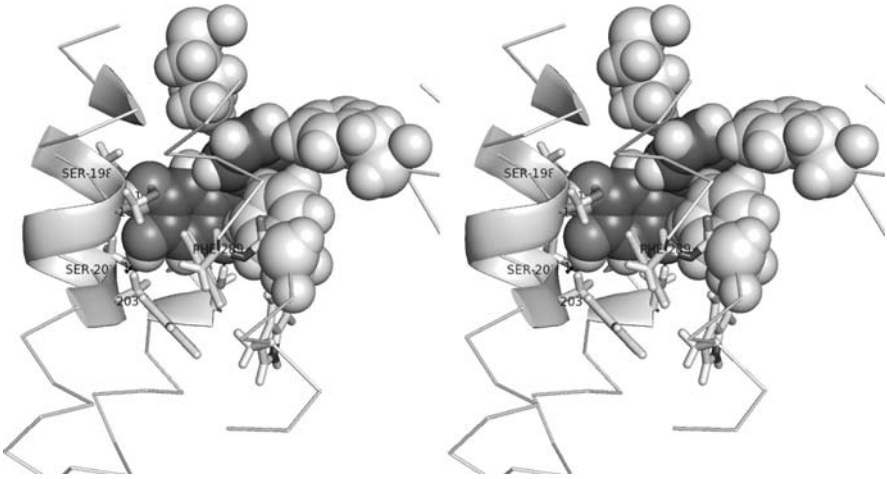


Fig. 4.21 Doxanthrine docked into a homology model of the D_1 receptor, in the same orientation as shown in Fig. 4.20, but with the ligand and key residues shown as space-filled representations to illustrate the potential accessory binding region

4.12 The Future

We clearly know quite a bit about the structure–activity relationships of dopamine D_1 full agonists and about functional topography of the receptor. The D_1 receptor remains of very high importance, as it has been identified as a target for a number of potential disease states, the most important ones at the present time being Parkinson's disease and memory and cognition enhancement, especially in schizophrenia. It seems quite likely that a "druggable" full D_1 agonist would be a commercially successful therapeutic agent. What chiefly prevents that from happening at the present time is the generally low oral bioavailability of catechol-containing drugs, which includes all of the present generation D_1 agonists. The fact that CY208-243 is not a catechol suggests that novel agonists may be discovered that are not limited by the problems associated with a catechol moiety. Nonetheless, the absence of a successful dopamine D_1 agonist clinical candidate also seems to have prevented major pharmaceutical companies from addressing the challenge of discovering a bioavailable full D_1 agonist that can be brought to market. One must have some hope that this situation will change before long, as there is every reason to believe that a D_1 full agonist should have a variety of very useful therapeutic indications.

References

1. Kebabian JW, Calne DB. Multiple receptors for dopamine. Nature 1979;277:93–6.
2. Dubois A, Savasta M, Curet O et al. Autoradiographic distribution of the D1 agonist [3H]SKF 38393, in the rat brain and spinal cord. Comparison with the distribution of D2 dopamine receptors. Neuroscience 1986;19:125–37.

3. Savasta M, Dubois A, Scatton B. Autoradiographic localization of D1 dopamine receptors in the rat brain with [3H]SCH 23390. Brain Res 1986;375:291–301.
4. Amenta F, Ferrante F, Ricci A. Pharmacological characterisation and autoradiographic localisation of dopamine receptor subtypes in the cardiovascular system and in the kidney. Hypertens Res 1995;18(Suppl 1):S23–S27.
5. Monsma FJ, Jr., Mahan LC, McVittie LD et al. Molecular cloning and expression of a D_1 dopamine receptor linked to adenylyl cyclase activation. Proc Natl Acad Sci U S A 1990;87:6723–7.
6. Sunahara RK, Guan HC, O'Dowd BF et al. Cloning of the gene for a human dopamine D_5 receptor with higher affinity for dopamine than D_1. Nature 1991;350:614–9.
7. Haq IU, LeWitt PA, Fernandez HH. Apomorphine therapy in Parkinson's disease: a review. Expert Opin Pharmacother 2007;8:2799–809.
8. Subramony JA. Apomorphine in dopaminergic therapy. Mol Pharm 2006;3:380–5.
9. Horowski R. A history of dopamine agonists. From the physiology and pharmacology of dopamine to therapies for prolactinomas and Parkinson's disease – a subjective view. J Neural Transm 2007;114:127–34.
10. Koch MV, Cannon JG, Burkman AM. Centrally acting emetics. II. Norapomorphine and derivatives. J Med Chem 1968;11:977–81.
11. Menon MK, Clark WG, Neumeyer JL. Comparison of the dopaminergic effects of apomorphine and (-)-N-n-propylnorapomorphine. Eur J Pharmacol 1978;52:1–9.
12. Jacob JN, Nichols DE, Kohli JD et al. Dopamine agonist properties of N-alkyl-4-(3,4-dihydroxyphenyl)-1,2,3,4- tetrahydroisoquinolines. J Med Chem 1981;24:1013–5.
13. Nichols DE. The development of novel dopamine agonists. In: Kaiser C, Kebabian JW, editors. Dopamine Receptors. Washington, D.C.: American Chemical Society; 1983 pp. 201–218.
14. Hedberg MH, Linnanen T, Jansen JM et al. 11-Substituted (R)-aporphines: synthesis, pharmacology, and modeling of D-2A and 5-HT_{1A} receptor interactions. J Med Chem 1996;39:3503–13.
15. Mailman R, Huang X, Nichols DE. Parkinson's disease and D_1 dopamine receptors. Curr Opin Investig Drugs 2001;2:1582–91.
16. Pendleton RG, Samler L, Kaiser C et al. Studies on renal dopamine receptors with a new agonist. Eur J Pharmacol 1978;51:19–28.
17. Setler PE, Sarau HM, Zirkle CL et al. The central effects of a novel dopamine agonist. Eur J Pharmacol 1978;50:419–30.
18. Andersen PH, Jansen JA. Dopamine receptor agonists: selectivity and dopamine D1 receptor efficacy. Eur J Pharmacol 1990;188:335–47.
19. Kaiser C, Dandridge PA, Garvey E et al. Absolute stereochemistry and dopaminergic activity of enantiomers of 2,3,4,5-tetrahydro-7,8-dihydroxy-1-phenyl-1H-3-benzazepine. J Med Chem 1982;25:697–703.
20. Braun A, Fabbrini G, Mouradian MM et al. Selective D-1 dopamine receptor agonist treatment of Parkinson's disease. J Neural Transm 1987;68:41–50.
21. Weinstock J, Hieble JP. The chemistry and pharmacology of 3-benzazepine derivatives. Drug Future 1985;10:645–97.
22. Cross AJ, Marshal RD, Johnson JA et al. Preferential inhibition of ligand binding to calf striatal dopamine D1 receptors by SCH 23390. Neuropharmacology 1983;22:1327–9.
23. Iorio LC, Barnett A, Leitz FH et al. SCH 23390, a potential benzazepine antipsychotic with unique interactions on dopaminergic systems. J Pharmacol Exp Ther 1983;226:462–8.
24. Neumeyer JL, Kula NS, Bergman J et al. Receptor affinities of dopamine D1 receptor-selective novel phenylbenzazepines. Eur J Pharmacol 2003;474:137–40.
25. Mottola DM, Laiter S, Watts VJ et al. Conformational analysis of D_1 dopamine receptor agonists: pharmacophore assessment and receptor mapping. J Med Chem 1996;39:285–96.
26. Berger JG, Chang WK, Clader JW et al. Synthesis and receptor affinities of some conformationally restricted analogues of the dopamine D1 selective ligand (5R)-8-chloro-2,3,4,5-tetrahydro-3-methyl-5-phenyl- 1H-3-benzazepin-7-ol. J Med Chem 1989;32:1913–21.

27. Snyder SE, Aviles-Garay FA, Chakraborti R et al. Synthesis and evaluation of 6,7-dihydroxy-2,3,4,8,9,13b-hexahydro-1H- benzo[6,7]cyclohepta[1,2,3-ef][3]benzazepine, 6,7-dihydroxy-1,2,3,4,8,12b-hexahydroanthr[10,4a,4-cd]azepine, and 10-(aminomethyl)-9,10- dihydro-1,2-dihydroxyanthracene as conformationally restricted analogs of beta-phenyldopamine. J Med Chem 1995;38:2395–409.
28. Kinney JL. Nomifensine maleate – A new 2nd-generation antidepressant. Clin Pharm 1985;4:625–36.
29. Costall B, Kelly DM, Naylor RJ. Nomifensine: a potent dopaminergic agonist of antiparkinson potential. Psychopharmacologia 1975;41:153–64.
30. Kohli JD, Goldberg LI. Effects of 3′,4′-dihydroxynomifensine on the dopamine vascular receptor. J Pharm Pharmacol 1980;32:225–6.
31. Andersen PH, Nielsen EB, Scheel-Kruger J et al. Thienopyridine derivatives identified as the first selective, full efficacy, dopamine D1 receptor agonists. Eur J Pharmacol 1987;137:291–2.
32. Riggs RM, Nichols DE, Foreman MM et al. Specific dopamine D-1 and DA1 properties of 4-(mono- and - dihydroxyphenyl)-1,2,3,4-tetrahydroisoquinoline and its tetrahydrothieno[2,3-c]pyridine analogue. J Med Chem 1987;30:1454–8.
33. Dandridge PA, Kaiser C, Brenner M et al. Synthesis, resolution, absolute stereochemistry, and enantioselectivity of 3′,4′-dihydroxynomifensine. J Med Chem 1984;27:28–35.
34. Dandridge PA, Kaiser C, Brenner M et al. Synthesis, resolution, absolute stereochemistry, and enantioselectivity of 3′,4′-dihydroxynomifensine. J Med Chem 1984;27:28–35.
35. McDermed JD, Freeman HS, Ferris RM. Enantioselectivity in the binding of (+) and (-)-2-amino-6,7-dihydroxy-1,2,3,4-tetrahydronapthalene and related agonists to dopamine receptors. In: Usdin E, Kopin IJ, Barchas J, editors. Catecholamines: Basic and Clinical Frontiers. New York: Pergamon Press; 1978 pp. 568–570.
36. Riggs RM, McKenzie AT, Byrn SR et al. Effect of beta-alkyl substitution on D-1 dopamine agonist activity: absolute configuration of beta-methyldopamine. J Med Chem 1987;30: 1914–8.
37. Lovenberg TW, Brewster WK, Mottola DM et al. Dihydrexidine, a novel selective high potency full dopamine D-1 receptor agonist. Eur J Pharmacol 1989;166:111–3.
38. Mottola DM, Brewster WK, Bennet J et al. In vivo and in vitro actions of DHBP, a unique agonist for both D_1 and D_2 dopamine receptors. Faseb J 1989;3:A379.
39. Knoerzer TA, Nichols DE, Brewster WK et al. Dopaminergic benzo[a]phenanthridines: resolution and pharmacological evaluation of the enantiomers of dihydrexidine, the full efficacy D1 dopamine receptor agonist. J Med Chem 1994;37:2453–60.
40. Taylor JR, Lawrence MS, Redmond DE, Jr. et al. Dihydrexidine, a full dopamine D1 agonist, reduces MPTP-induced parkinsonism in monkeys. Eur J Pharmacol 1991;199:389–91.
41. Blanchet PJ, Fang J, Gillespie M et al. Effects of the full dopamine D1 receptor agonist dihydrexidine in Parkinson's disease. Clin Neuropharmacol 1998;21:339–43.
42. Kohli JD, Horn PT, Glock D et al. Dihydrexidine: a new potent peripheral dopamine D1 receptor agonist. Eur J Pharmacol 1993;235:31–5.
43. Darney KJ, Jr., Lewis MH, Brewster WK et al. Behavioral effects in the rat of dihydrexidine, a high-potency, full-efficacy D1 dopamine receptor agonist. Neuropsychopharmacol 1991;5:187–95.
44. Lawrence MS, Redmond DE, Jr., Elsworth JD et al. The D1 receptor antagonist, SCH 23390, induces signs of parkinsonism in African green monkeys. Life Sci 1991;49:L229–34.
45. Brewster WK, Nichols DE, Watts VJ et al. Evaluation of cis- and trans-9- and 11-hydroxy-5,6,6a,7,8,12b-hexahydrobenzo[a]phenanthridines as structurally rigid, selective D1 dopamine receptor ligands. J Med Chem 1995;38:318–27.
46. Brewster WK, Nichols DE, Riggs RM et al. trans-10,11-dihydroxy-5,6,6a,7,8,12b-hexahydrobenzo[a]phenanthridine: a highly potent selective dopamine D1 full agonist. J Med Chem 1990;33:1756–64.
47. Negash K, Nichols DE, Watts VJ et al. Further definition of the D1 dopamine receptor pharmacophore: synthesis of trans-6,6a,7,8,9,13b-hexahydro-5H-benzo[d]naphth[2,1-b]azepines as rigid analogues of beta-phenyldopamine. J Med Chem 1997;40:2140–7.

48. Knoerzer TA, Watts VJ, Nichols DE et al. Synthesis and biological evaluation of a series of substituted benzo[a]phenanthridines as agonists at D1 and D2 dopamine receptors. J Med Chem 1995;38:3062–70.
49. Michaelides MR, Hong Y, DiDomenico SJ et al. (5aR,11bS)-4,5,5a,6,7,11b-hexahydro-2-propyl-3-thia-5-azacyclopent-1- ena[c]-phenanthrene-9,10-diol (A-86929): a potent and selective dopamine D1 agonist that maintains behavioral efficacy following repeated administration and characterization of its diacetyl prodrug (ABT-431). J Med Chem 1995;38:3445–7.
50. Shiosaki K, Jenner P, Asin KE et al. ABT-431: the diacetyl prodrug of A-86929, a potent and selective dopamine D1 receptor agonist: in vitro characterization and effects in animal models of Parkinson's disease. J Pharmacol Exp Ther 1996;276:150–60.
51. Michaelides MR, Hong Y, Didomenico S et al. Substituted hexahydrobenzo[f]thieno[c]quinolines as dopamine D1- selective agonists: synthesis and biological evaluation in vitro and in vivo. J Med Chem 1997;40:1585–99.
52. Rascol O, Blin O, Thalamas C et al. ABT-431, a D1 receptor agonist prodrug, has efficacy in Parkinson's disease. Ann Neurol 1999;45:736–41.
53. Rascol O, Nutt JG, Blin O et al. Induction by dopamine D1 receptor agonist ABT-431 of dyskinesia similar to levodopa in patients with Parkinson disease. Arch Neurol 2001;58: 249–54.
54. Michaelides MR, Schoenleber R, Thomas S et al. Synthesis and pharmacological evaluation of 1-(aminomethyl)-3,4-dihydro- 5-hydroxy-1H-2-benzopyrans as dopamine D1 selective ligands. J Med Chem 1991;34:2946–53.
55. DeNinno MP, Schoenleber R, Asin KE et al. (1R,3S)-1-(aminomethyl)-3,4-dihydro-5,6-dihydroxy-3-phenyl-1H-2- benzopyran: a potent and selective D1 agonist. J Med Chem 1990;33:2948–50.
56. DeNinno MP, Schoenleber R, MacKenzie R et al. A68930: a potent agonist selective for the dopamine D1 receptor. Eur J Pharmacol 1991;199:209–19.
57. DeNinno MP, Schoenleber R, Perner RJ et al. Synthesis and dopaminergic activity of 3-substituted 1-(aminomethyl)- 3,4-dihydro-5,6-dihydroxy-1H-2-benzopyrans: characterization of an auxiliary binding region in the D1 receptor. J Med Chem 1991;34:2561–9.
58. Kebabian JW, Britton DR, DeNinno MP et al. A-77636: a potent and selective dopamine D1 receptor agonist with antiparkinsonian activity in marmosets. Eur J Pharmacol 1992;229: 203–9.
59. Kebabian JW, Briggs C, Britton DR et al. A68930: a potent and specific agonist for the D_1 dopamine receptor. Am J Hypertens 1990;3:40S–2S.
60. Kebabian JW, DeNinno MP, Schoenleber R et al. A68930: a potent agonist specific for the dopamine D1 receptor. Neurochem Int 1992;20(Suppl):157S–60S.
61. Laban U. Synthesis of 4-aminomethylchromans: novel dopamine D_2-selective agonists. PhD thesis, Purdue University, West Lafayette, IN; 2004.
62. Chemel BR, Bonner LA, Watts VJ et al. D_1 versus D_2 dopamine receptor selectivity is determined by intramolecular hydrogen bonding patterns in catechol-containing novel dopamine analogues. Soc Neurosci Abstr 2007;33:351.1.
63. Ghosh D, Snyder SE, Watts VJ et al. 9-Dihydroxy-2,3,7,11b-tetrahydro-1H-naph[1,2,3-de]isoquinoline: a potent full dopamine D1 agonist containing a rigid-beta-phenyldopamine pharmacophore. J Med Chem 1996;39:549–55.
64. Sit SY, Xie K, Jacutin-Porte S et al. (+)-Dinapsoline: An efficient synthesis and pharmacological profile of a novel dopamine agonist. J Med Chem 2002;45:3660–8.
65. Gulwadi AG, Korpinen CD, Mailman RB et al. Dinapsoline: characterization of a D(1) dopamine receptor agonist in a rat model of Parkinson's disease. J Pharmacol Exp Ther 2001;296:338–44.
66. Sit SY, Xie K, Jacutin-Porte S et al. Synthesis and SAR exploration of dinapsoline analogues. Bioorg Med Chem 2004;12:715–34.
67. Grubbs RA. Synthesis of novel dopaminergic ligands: a bioisosteric approach. PhD thesis, Purdue University, West Lafayette, IN;2000.

68. Grubbs RA, Lewis MM, Owens-Vance C et al. 8,9-Dihydroxy-1,2,3,11b-tetrahydrochromeno[4,3,2,-de]isoquinoline (dinoxyline), a high affinity and potent agonist at all dopamine receptor isoforms. Bioorg Med Chem 2004;12:1403–12.
69. Cueva JP, Giorgioni G, Grubbs RA et al. *trans*-2,3-Dihydroxy-6a,7,8,12b-tetrahydro-6*H*-chromeno[3,4-*c*]isoquinoline: Synthesis, resolution, and preliminary pharmacological characterization of a new dopamine D_1 receptor full agonist. J Med Chem 2006;49:6848–57.
70. Cueva JP. Inquiry into the structural and conformational requirements of β-phenyldopamine-type ligands for selective dopamine D_1 receptor activation. PhD thesis, Purdue University, West Lafayette, IN; 2008.
71. Ladd DL, Weinstock J, Wise M, Gessner GW, Sawyer JL, Flaim KE. Synthesis and dopaminergic binding of 2-aryldopamine analogues: phenethylamines, 3-benzazepines, and 9-(aminomethyl)fluorenes. J Med Chem 1986; 29(10):1904–12.
72. Cannon JG. Chemistry of dopaminergic agonists. Adv Neurol 1975;9:177–83.
73. Froimowitz M, Bellott EM, Jr. Structural factors that distinguish dopamine D1 and D1 agonists. J Mol Model 1995;1:36–45.
74. Csutoras C, Zhang A, Zhang KH et al. Synthesis and neuropharmacological evaluation of R(-)-N-alkyl-11-hydroxynoraporphines and their esters. Bioorg Med Chem 2004;12:3553–9.
75. Neumeyer JL, Reinhard JF, Dafeldecker WP et al. Aporphines 14. Dopaminergic and antinociceptive activity of aporphine derivatives – synthesis of 10-hydroxyaporphines and 10-hydroxy-N-normal-propylnoraporphine. J Med Chem 1976;19:25–9.
76. Seiler MP, Hagenbach A, Wuthrich HJ et al. trans-hexahydroindolo[4,3-ab]phenanthridines ("benzergolines"), the first structural class of potent and selective dopamine D1 receptor agonists lacking a catechol group. J Med Chem 1991;34:303–7.
77. Nichols DE. Structural correlation between apomorphine and LSD: involvement of dopamine as well as serotonin in the actions of hallucinogens. J Theor Biol 1976;59:167–77.
78. Bach NJ, Kornfeld EC, Jones ND et al. Bicyclic and tricyclic ergoline partial structures. Rigid 3-(2- aminoethyl)pyrroles and 3- and 4-(2-aminoethyl)pyrazoles as dopamine agonists. J Med Chem 1980;23:481–91.
79. Kocjan D, Hadzi D. Conformationally restricted dopamine congeners–a molecular mechanics-based study. J Pharm Pharmacol 1983;35:780–5.
80. Temlett JA, Quinn NP, Jenner PG et al. Antiparkinsonian activity of CY 208-243, a partial D-1 dopamine receptor agonist, in MPTP-treated marmosets and patients with Parkinson's disease. Mov Disord 1989;4:261–5.
81. Pollock NJ, Manelli AM, Hutchins CW et al. Serine mutations in transmembrane V of the dopamine D1 receptor affect ligand interactions and receptor activation. J Biol Chem 1992;267:17780–6.
82. Tomic M, Seeman P, George SR et al. Dopamine D1 receptor mutagenesis: role of amino acids in agonist and antagonist binding. Biochem Biophys Res Commun 1993;191: 1020–7.
83. Cherezov V, Rosenbaum DM, Hanson MA et al. High-resolution crystal structure of an engineered human 2-Adrenergic G protein coupled receptor. Science 2007;318:1258–65.
84. Strader CD, Sigal IS, Candelore MR et al. Conserved aspartic acid residues 79 and 113 of the beta-adrenergic receptor have different roles in receptor function. J Biol Chem 1988;263:10267–71.
85. Strader CD, Fong TM, Tota MR et al. Structure and function of G protein-coupled receptors. Ann Rev Biochem 1994;63:101–32.
86. Lan H, Du R and CJ, Teeter MM, Neve KA. Structural determinants of pharmacological specificity between D_1 and D_2 dopamine receptors. Mol Pharmacol 2006;69:185–94.

Chapter 5
Dopamine Receptor Subtype-Selective Drugs: D2-Like Receptors

Olaf Prante, Miriam Dörfler, and Peter Gmeiner

Abstract Drugs that are known to activate or block dopamine receptors are widely used for the treatment of a number of severe diseases. In most cases, dopaminergic drugs preferentially interact with the subtypes of the D2 family (D_2, D_3, D_4). However, only minor selectivity has been observed between D_2, D_3, and D_4. Nevertheless, in recent years D_3 and D_4 subtype-selective agonists, partial agonists, and antagonist have been developed. The most interesting structural features required for high selectivity and affinity are presented as well as structure–activity relationship (SAR) studies. Moreover, the use of subtype-selective radioligands is discussed.

Keywords D2-like receptors · subtype selectivity · aminotetralins · phenylpiperazines · radioligands · D_3-receptors · D_4-receptors

5.1 Drugs on the Market and Classical Pharmacological Tools

Drugs that are known to activate or block D2-like dopamine receptors are widely used for the treatment of a number of severe diseases. Thus, dopamine receptor agonists (Fig. 5.1) such as apomorphine **1**, the ergoline derivatives lisuride **2**, bromocriptine **3**, and pergolide **5** as well as the bicyclic dopamine bioisosteres rotigotine **6**, pramipexole **7**, and ropinirole **8** are important for the treatment of Parkinson's disease and dyskinesia. Bromocriptine **3** is also employed as a prolactin inhibitor, whereas apomorphine **1** has emetic effects and has been described for the treatment of erectile dysfunction [1]. Pramipexole **7** is also approved for the treatment of restless legs syndrome [2]. Quinelorane **9**, quinpirole **10**, and PD128907 **4** are accepted as valuable pharmacological tools for the characterization of responses mediated by D2-like receptors.

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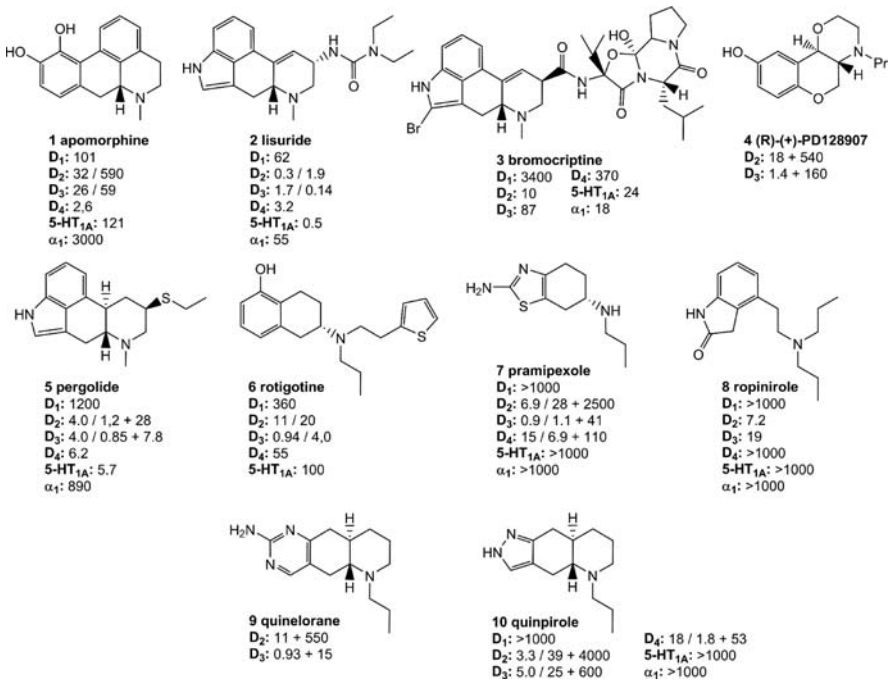


Fig. 5.1 Dopamine receptor agonists, K_i values in nM ($K_{i\,high} + K_{i\,low}$)

Dopamine receptor antagonists (Fig. 5.2) are used for the treatment of schizophrenia and anxiety. The pharmacological agents in clinical use are divided into classical and atypical antipsychotics [3]. The classical family of antipsychotics consists of the structural class of phenothiazines (such as chlorpromazine **11**), thioxanthenes (such as chlorprothixene **12**), butyrophenones (such as haloperidol **13**), and diphenylbutyl piperidines (such as pimozide **15**). These drugs are able to reduce the positive symptoms of schizophrenia [3]. In contrast to classical antipsychotics displaying extrapyramidal side effects [3], atypical derivatives are less likely to produce these side effects and address not only positive but also negative symptoms [3]. Major representatives for atypical antipsychotics are clozapine **17** and olanzapine **18**, binding not only dopamine D_4- and D_2-receptors but also serotonergic 5-HT_2- and muscarinic receptors as well as the adrenergic α_1- and the histamine H_1-receptors [3]. Risperidone **19** additionally displays a mixed binding profile [3]. Aripiprazole **16** is described as a system stabilizer for dopaminergic and serotonergic systems [4], exerting agonist effects at the presynaptic and antagonist effects at the postsynaptic dopamine D_2-receptors [5, 6]. Prominent examples of the family of methoxybenzamides are sulpiride **20**, amisulpride **21**, raclopride **22**, nemonapride **23**, nafadotride **24** which are used as antipsychotic drugs and valuable pharmacological tools. Tritiated spiperone **14** is well established as a standard radioligand for binding assays.

Figure 5.1 and Fig. 5.2 clearly indicate that the established dopamine receptor agonists and antagonists are relatively unselective and, in many cases, recognize not

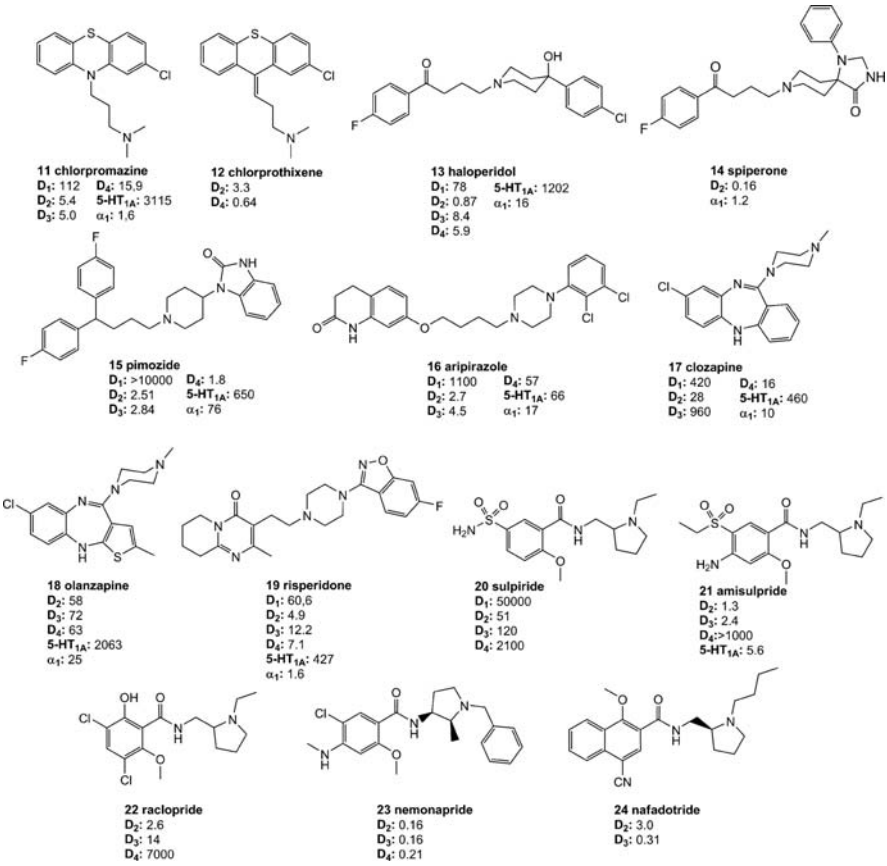


Fig. 5.2 Dopamine receptor antagonists, K_i values in nM

only the various subtypes of the dopamine receptor but also related biogenic amine receptors such as serotonergic or adrenergic receptors [1]. Nonselective dopaminergic drugs may possess therapeutic advantages in some cases, provided that effects synergistic to the dopaminergic response enhance the antipsychotic efficacy [7, 8]. To improve the pharmacological profiles of dopamine receptor-targeting drugs, selective dopamine receptor ligands have been developed in recent years. These efforts have been especially concentrated on the D_3- and the D_4-subtypes, since the D_2-subtype is associated with side effects, such as extrapyramidal dysfunction [9].

5.2 D_3-Selective Ligands

The design of D_3-receptor ligands with high selectivity over the related subtypes D_2 and D_4 proved to be difficult, since the binding site crevices of these subtypes are very similar [10, 11]. Nevertheless, medicinal chemists succeeded in developing even highly selective agonists and antagonists which are summarized below.

5.2.1 Aminotetralins and Analogs

5.2.1.1 Aminotetralins

The neurotransmitter dopamine is able to adopt both the α- and β-conformation according to Cannon (Fig. 5.3) [12]. Structure–activity relationship studies showed that only the *meta*-hydroxy function of the catechol system is necessary for dopaminergic activity and *N*-alkyl substituents, especially *n*-propyl groups, increase binding affinity. Formal conformational rigidization of the ethylene chain led to the structural family of aminotetralins **27** (Fig. 5.4).

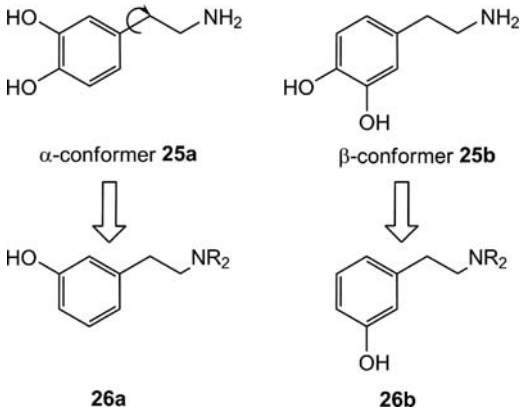


Fig. 5.3 Dopamine conformers

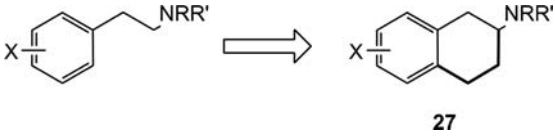
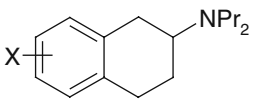


Fig. 5.4 Design of aminotetralins

Two alternatives of bridging the dopamine structure are possible resulting in formation of the 5,6- and the 6,7-dihydroxy derivatives which represent the β- and α-conformer of dopamine, respectively. Interestingly, the 5,6-dihydroxy derivative shows significantly higher dopaminergic potency [13, 14]. Among the monohydroxylated dipropylaminotetralins (DPATs), the 7-hydroxy substituted derivative (7-OH-DPAT; **27b**) displays the highest D_3-selectivity [15], when the (*R*)-(+)-enantiomer turned out to be significantly more active [16]. On the other hand, the (*S*)-isomer of 5-OH-DPAT **27a** reveals higher potency and a mixed D_2/D_3-affinity profile (Table 5.1) [16]. Karlsson et al. described substantial D_2-agonist activity for the (*S*)-enantiomer and weak D_2-antagonist properties for the (*R*)-enantiomer [17]. Interestingly, the unsubstituted DPAT also exhibits configuration-specific affinity profiles for receptors of the D_2-family and the 5-HT_{1A}-receptor [18].

SAR data investigating the effect of the nitrogen substitution pattern indicated that one of the two substituents is represented in the best way by a propyl substituent, consistent with receptor modeling studies identifying a propyl cleft as an important

Table 5.1 Binding affinities of 5-OH- and 7-OH-DPAT;K_i Values in nM



	X	D_{2L}	D_3	D_2/D_3
(S)-27a	(*S*)-(–)-5–OH	6	0.54	11
(R)-27b	(*R*)-(+)-7–OH	56	0.57	98

part of the binding pocket [15]. Structural variation of the second nitrogen substituent led to the attachment of terminal π-systems as realized within compounds **28** [19, 20] and **29** [21] (Fig. 5.5). 7-OH-PIPAT (**28**) is a D_3-selective analog of 7-OH-DPAT, whereas 5-OH-PIPAT shows higher D_3-affinity but significantly lower selectivity over D_2 [19, 20]. The binding profile of another prominent aminotetralin, rotigotine **6,** is very similar to that of dopamine with respect to D_1, D_2, and D_3 (Fig. 5.1) [21, 22].

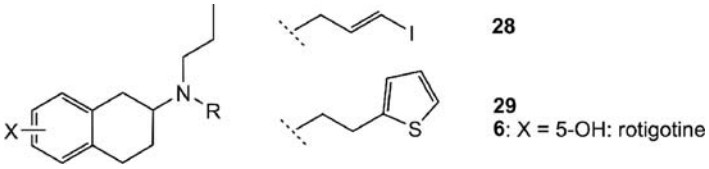


Fig. 5.5 Side chain variations in aminotetralins

5.2.1.2 DPAT Bioisosteres

Since the aromatic hydroxyl function of OH-DPAT analogs is rapidly inactivated by conjugation with glucuronic acid and elimination via the kidney, the oral bioavailability of OH-DPATs is low and the duration of maximum efficacy is short [23]. To attenuate rapid metabolization an effective bioisosteric replacement has been performed [23].

Heterocyclic Bioisosteres

A bioisosteric replacement of the hydroxyphenyl substructure by substituted and unsubstituted 6- and 5-membered heteroarenes was investigated and resulted in compounds with different affinity and selectivity patterns (Fig. 5.1; Fig. 5.6). Thus, quinelorane **9** and quinpirole **10** (Fig. 5.1) display substantial D_3-selectivity over D2 [9, 24–29]. Pramipexole **7** (Fig. 5.1) behaves as a D_2-, D_3-, and D_4-receptor agonist preferentially recognizing D_3 [26, 29–31]. Pramipexole **7** is also described as a selective or preferential autoreceptor agonist [32]. Interestingly, replacement of the secondary amine structure by primary amine leads to a loss of activity [33]. The pyridine analogs **30a** and **30b** are known for their significant D_3-selectivity and autoreceptor agonist properties [23]. The dopaminergic agents FAUC 54 **31**

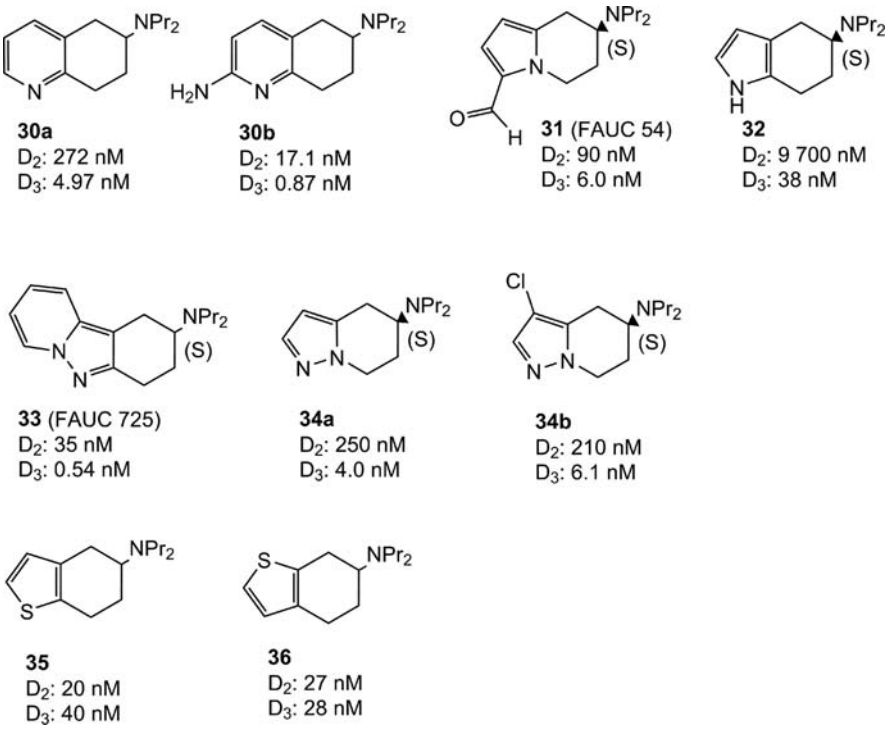


Fig. 5.6 Binding affinities of several bioisosteric DPATs, K_i values in nM ($K_{i\,high}$ for **31**, **33**, **34a**, **34b**)

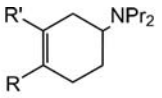
[34], FAUC 725 **33** [35], and **34a** [36] show single-digit nanomolar and subnanomolar D_3-affinities and substantial agonist effects in mitogenesis experiments. On the other hand, the ligands **32** [37] and **34b** [36] turned out to be partial agonists when compared with the full agonist quinpirole.

The tetrahydrobenzothiophene derivatives **35** and **36** are described as agonists displaying an improved bioavailability in comparison with 5-OH-DPAT **27a** [38, 39].

Non-aromatic Bioisosteres

Until very recently, an aromatic partial structure representing the catechol function of dopamine was regarded as crucial for the pharmacophore described by the McDermed model [13]. Very recently, it has been demonstrated that such a π-system can also be provided by non-aromatic analogs. Chemical syntheses, pharmacological investigations, and computational studies lead to enynes, dienes, and endiynes of type **37** that display high affinity to dopamine receptors of the D2-like family (Fig. 5.7) [40–43].

In detail, the terminal enynes FAUC 73 (**37a**) and FAUC 88 (**37b**) reveal higher affinity than the trimethylsilyl substituted analogs [42]. The ethenyl analog FAUC



37a (FAUC 73)	**37b** (FAUC 88)	**37c** (FAUC 206)
R = acetylene, R' = H	R = R' = acetylene	R = ethylene, R' = H
D_2: 250 + 12 000 nM	D_2: 54 + 2 600 nM	D_2: 99 + 6 400 nM
D_3: 5.2 + 590 nM	D_3: 3.2 + 49 nM	D_3: 5.6 + 430 nM
D_4: 22 + 380 nM	D_4: 6.3 + 420 nM	D_4: 260 + 3 100 nM

Fig. 5.7 Binding affinities of several enynes, K_i values in nM ($K_{i\ high}$ + $K_{i\ low}$)

206 (**37c**) shows a similar binding profile as FAUC 73 (**37a**) with an improved selectivity over the D_4 subtype [41]. Functional investigations were performed for FAUC 73 (**37a**) and FAUC 88 (**37b**) displaying substantial intrinsic activity [42].

5.2.2 Aminoindans

Formal restriction of the aminotetralin six-membered ring to a five-membered ring leads to the structural family of aminoindans **38** (Fig. 5.8). As described for the aminotetralins the substitution pattern at the aromatic substructure and the amino substituents play an essential role for the dopaminergic activity. Thus, the 4-hydroxy substituted dipropyl aminoindan **38b** displays combined D_2- and 5-HT_{1A}-affinity, reflecting the anology both to the dopaminergic active 5-OH-DPAT and to the selective 5-HT_{1A}-ligand 8-OH-DPAT (Table 5.2). On the other hand, the 5-hydroxy isomer **38a** displays an approximately threefold selectivity for D_3-receptors [44]. Interestingly, introduction of methoxy substituents leading to the bioactive agent U 99194 (**38c**) results in an improved D_3-selectivity but, on the other hand, a complete loss of intrinsic activity [44, 45].

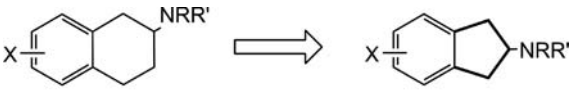
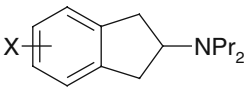


Fig. 5.8 Design of aminoindans

Table 5.2 Binding affinities of several DPAIs,K_i Values in nM



	X	D_2	D_3	D_2/D_3
38a	5-OH	53	14	3.7
38b	4-OH	5.2	42	0.12
38c	5,6-di-OMe	992	31	32

5.2.3 Arylcarboxamidobutyl Substituted Aminotetralins and Analogs Thereof

The exchange of an *n*-propyl side chain of the family of DPATs by an *N*-butyl-4-biphenylamido moiety results in an improvement of D_3-affinity and selectivity [46]. Depending on the residue X within structure **39** (Fig. 5.9), the compounds behave as agonists or antagonists [46].

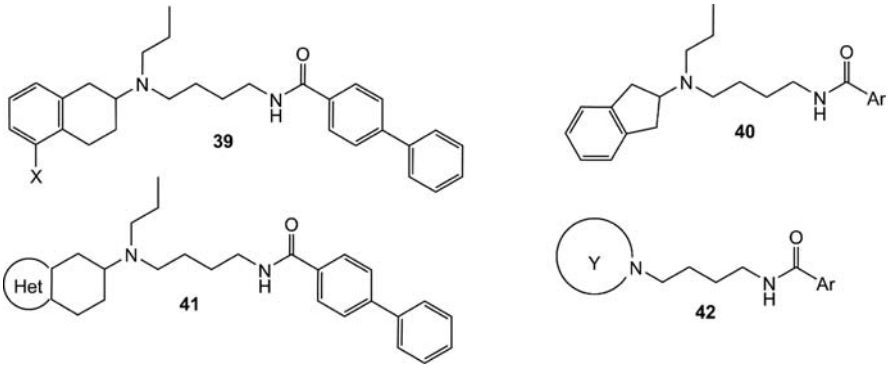


Fig. 5.9 Binding affinities of several amides with butylene linkers, K_i values in nM

As described for DPATs, heterocyclic bioisosteres are also known for the amide analogs. The compounds with the general structure **41** show high D_3-affinity and selectivity (Fig. 5.9) [47].

Also for this class of compounds the replacement of the aminotetralin substructure by aminoindan (**40**) proved to be a successful replacement [48]. Since compounds of type **39** can be metabolized in vivo by depropylation, the amino function was integrated into a ring system leading to compounds of the general structure **42** [48–51]. Besides dopamine receptor ligands with flexible linker chains, conformationally rigidized ethyl-*trans*-cyclohexyl derivatives have been described (**L6**, Fig. 5.13) [52–55].

5.2.3.1 2-Methoxybenzamides and Analogs Thereof

Based on the classical representatives sulpiride **20**, amisulpride **21**, and raclopride **22** (Fig. 5.2), variations of the substituents Y and Z (Fig. 5.10) lead to an improvement of D_3-selectivity. Besides this, the phenyl ring was replaced by a naphthyl system resulting in an increased D_3-selectivity over D_2 and both σ-receptor subtypes [56, 57]. Bioisosteric replacement of the amide structure by certain 5-membered heteroarenes was exploited to increase D_3-affinity and selectivity. Besides pyrroles **44** and imidazoles **45** as amide bioisosteres (Fig. 5.10) further 5-membered heterocycles such as oxazoles and oxadiazoles were investigated [58–61].

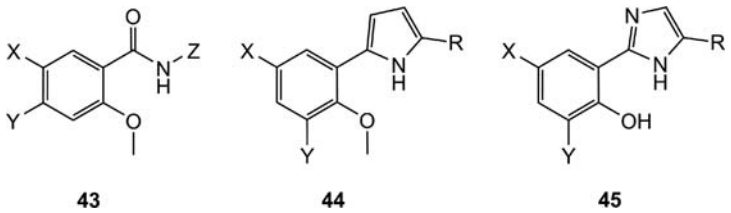
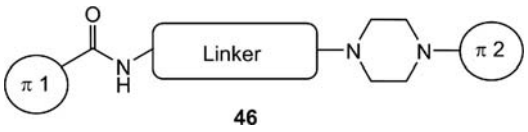


Fig. 5.10 Benzamides and bioisosteres

5.2.4 Phenylpiperazines

Dopamine receptor ligands of the class of phenylpiperazines contain an aryl carboxamide which is connected via a linker to a 4-aryl substituted piperazine unit (Fig. 5.11).

Fig. 5.11 General formula of phenylpiperazines



5.2.4.1 Variations at π2

The aromatic residue **π2** as a part of the structure **46** is usually represented by a substituted phenyl ring (Fig. 5.12). D_3-selectivity is increased when the substituent is shifted from the *para*- to the *meta*- to the *ortho*-position [62]. Very frequently, 2-methoxy- and 2,3-dichlorophenylpiperazines (**π2b** and **π2c**) are used when the latter usually results in higher D_3-affinity compared to **π2a** [63] or methoxy-substituted derivatives of type **π2b** [64, 65]. Structural hybrids with 2-methoxy- and 3-chloro-substitution of type **π2d** show binding properties ranging between **π2b** and **π2c** [66, 67]. Furthermore, 2,3-dimethyl substituted compounds of type **π2e** with high D_3-selectivity over D_2, D_4, 5-HT_{1A} and α_1 [68], and heterocyclic derivatives of type **π2f** have been described [69, 70].

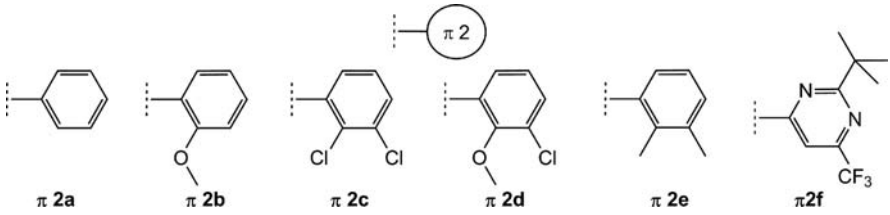


Fig. 5.12 Aromatic residues of type **π 2**

5.2.4.2 Variations of the Linker Unit

As suitable linker units, saturated or unsaturated aliphatic and cyclic carbon-based systems are used (Fig. 5.13) when a saturated butyl linker of type **L1** results in the highest D_3 binding affinity [63, 64, 68, 70, 71]. Exchange of the saturated linker by a *trans*-butenyl system (**L2**) leads to antagonists [72]. Similar binding properties are described for phenylpiperazines with 3-cyclopropyl spacer of type **L4** [73]. On the other hand, D_2- and D_3-affinity is strongly reduced for butenyl linkers (**L3**) [72]. Replacement of the butyl chain by a cyclohexyl ring **L5** results in reduced D_3-affinity [74]. However, if the cyclohexyl moiety is combined with 2-methylene units (**L6**), D_3-affinity and selectivity increases [75]. A number of disubstituted phenyl systems have also been investigated [76].

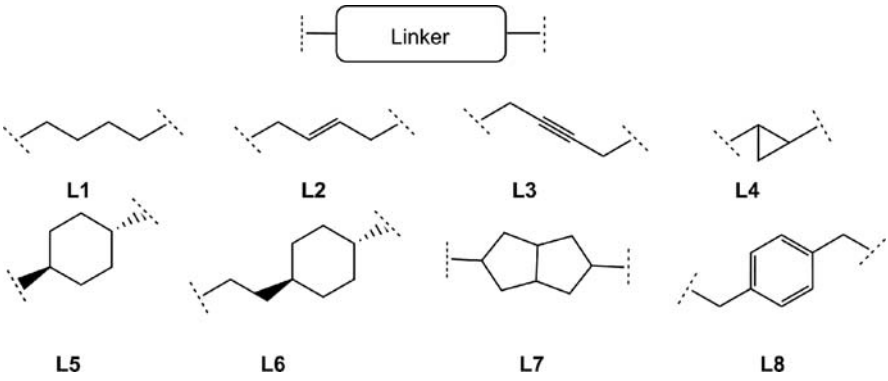


Fig. 5.13 Linker

5.2.4.3 Variations at π1

For the structural unit **π1** a great number of different carbo- and heterocyclic fused and monocyclic aromatic units was employed (Fig. 5.14). Besides substituted phenyl and biphenyl systems (**46a-c**), fused carbo- and heterocyclic rings were investigated [68, 71, 72, 77]. The most prominent representatives of this class are the D_3-selective partial agonist BP-897 (**46d**), that has been successfully used for the treatment of cocaine abuse [78], and the tricyclic antagonists NGB 2849 (**46f**) and NGB 2904 (**46 g**) [79]. For the 2,3-dichloro analog of BP-897 (**46e**) the D_3-selectivity over D_2 and D_4 is further improved [68].

A great number of heterocyclic units have been investigated when high D_3-affinity and selectivity was determined. For substituted pyrazolopyridines, affinity decreases with the substitution positions $5 > 2 = 6 > 3 > 4 > 7$ [64, 80]. Interesting binding profiles are also displayed by the indoles (**46o**) [81], the benzofurans (**46 k**, **46 l**), and the benzothiophenes (**46m**, **46n**) [64]. Comparison of FAUC 365 (**46n**) and FAUC 346 (**46m**) shows significantly higher D_3-selectivity for the dichloro derivative **46n** [64]. On the other hand, **46m** (FAUC 346) shows partial agonist properties, whereas **46n** (FAUC 365) is a neutral antagonist [64].

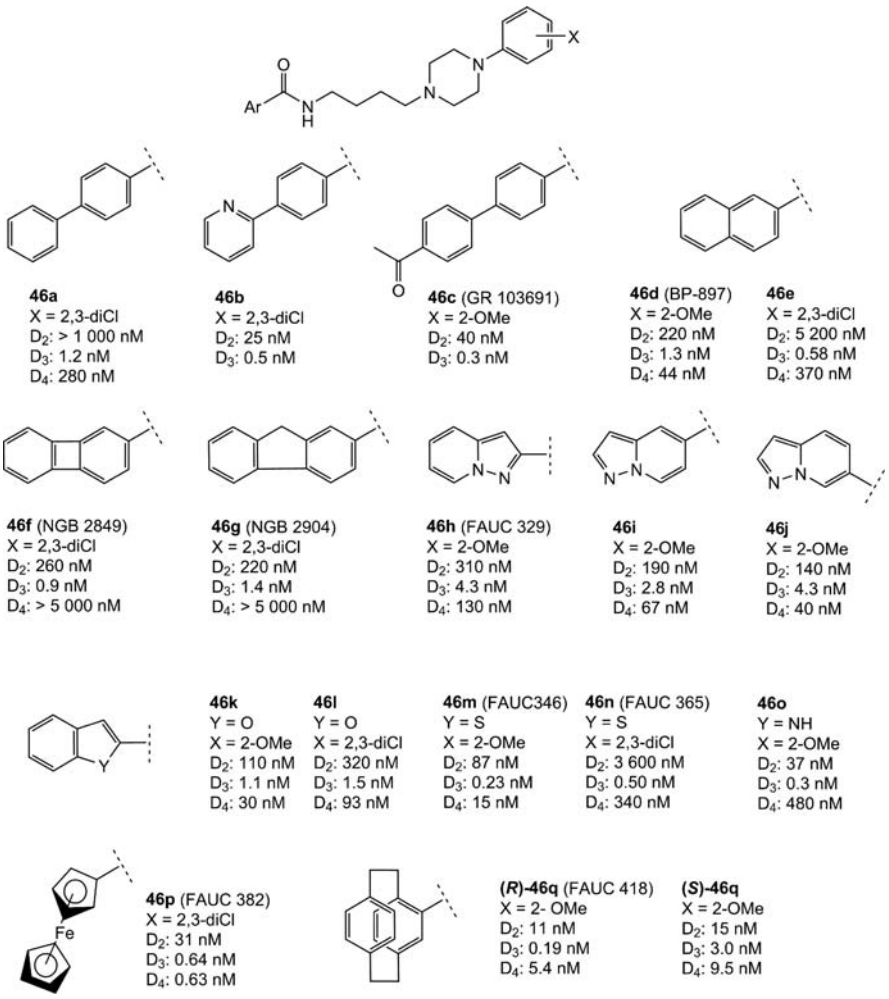


Fig. 5.14 Binding affinities of several phenylpiperazines with butyl linker, K_i values in nM

Interestingly, bilayered systems involving metallocene or paracyclophane derivatives could be established as a novel type of dopamine receptor ligands. Depending on the type of metal that is bound between the two cyclopentadienyl systems, different binding profiles can be observed when partial agonist properties were found [66]. On the other hand, the paracyclophane derivatives show neutral antagonist properties and selective binding dependent on the planar chirality of the paracyclophane system [67].

5.2.5 Structural Hybrids

Combination of the aminotetralin or pramipexole moiety with the phenylpiperazine unit resulted in a hybrid structure of type **47** and **48**, respectively (Fig. 5.15). Both

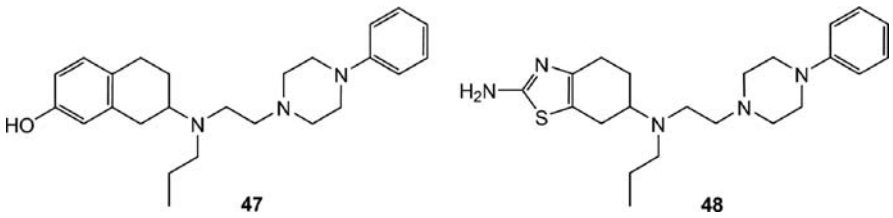


Fig. 5.15 Hybrids

compounds are partial agonists with high intrinsic activity, single-digit nanomolar D_3-affinity, and high selectivity over D_2 [82, 83].

5.2.6 D_3-Selective Radioligands

Employing autoradiography, D_3-receptors can be distinguished from D_2 receptors by using both selective dopaminergic antagonists and agonists. The preferential expression of D_3-receptors in limbic areas, such as the ventromedial shell of the nucleus accumbens and the islands of Calleja, has been repeatedly demonstrated in the rat using the tritiated radioligand [^{3}H]7-OH-DPAT [84–87]. Autoradiography studies with (+)-[^{3}H]PD128907 (Fig. 5.1) yield a quite similar cerebral distribution in the human while additionally revealing D_3-receptors in the human neocortex [88–90]. Moreover, in vivo studies suggest a role of the D_3-receptor in cognition and motivated behavior [89, 90], and that D_3 inhibition activates the mesocorticolimbic dopaminergic system [29]. The relevance of disturbances of D_3-receptor mediated neurotransmission in psychiatric disease is coincidently justifying the urgent need for selective D_3 imaging agents allowing the non-invasive detection of receptor disturbances (compare Chapter 13 of this book). Positron emission tomography (PET) has already gained growing importance for in vivo imaging of disturbances of D2-like receptor densities using the nonselective D_2/D_3 radioligands [^{11}C]raclopride [91] and [^{18}F]fallypride [92] (Fig. 5.16). Analogs of these most commonly used

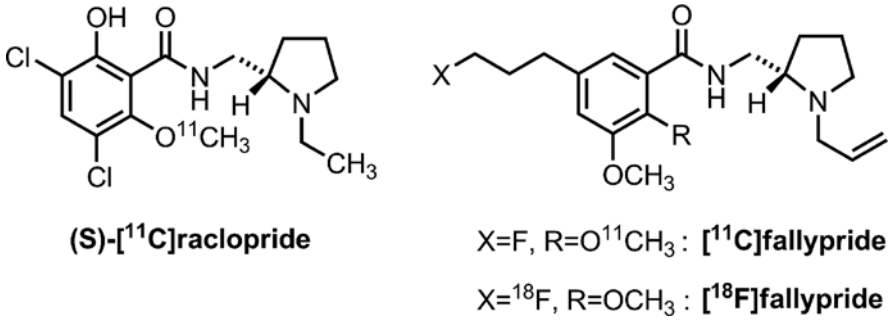


Fig. 5.16 D_2/D_3 radioligands for PET

PET radioligands have also been developed [93, 94]. The lack of bioavailable D_3-subtype-selective PET ligands excluding cross talk with the strongly related subtypes D_2 and D_4 hampers the non-invasive investigation of the physiological role of D_3. Various efforts have been made to develop selective D_3 PET imaging agents, including the preparation of ^{11}C-labeled imidazo[2,1-*b*]thiazolylpiperazine derivative RGH-1756 and the substituted aminotetralin [^{11}C]GR218231 (Fig. 5.17) [95–97]. The D_3-selective lead compound FAUC 365 has also been labeled with radioiodide [98], F-18 [65], or C-11 [99], yielding analogs or the ^{11}C-labeled parent compound, respectively, with high in vitro affinity and D_3 subtype selectivity. However, the above-mentioned D_3 radioligand candidates have not yet been successfully used for in vivo imaging, partly due to rapid efflux from the brain, or disappointing binding characteristics in vivo or in autoradiography experiments. Recent progress has been made by the use of comparative molecular field analysis (CoMFA) and comparative molecular similarity indices analysis (CoMSIA) guided approaches to predict D_3 radioligand selectivities [100, 101]; however the bioavailability of the resulting new candidates (**50a-d**, Fig. 5.17), which are derived from BP-897 and FAUC 346 (Fig. 5.14), remains to be elucidated. Thus, the future discovery of highly selective radioligands as PET tracers is critical for obtaining new insights into the role of the D_3-receptor subtype in the pathophysiology of various psychiatric diseases.

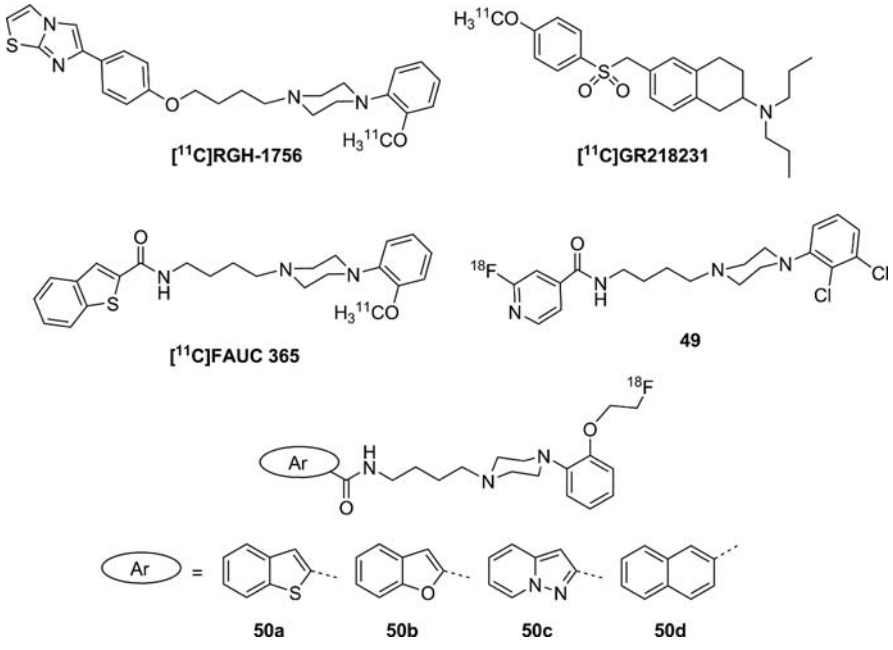


Fig. 5.17 Subtype-selective D_3 radioligands for PET

5.3 D_4-Selective Ligands

The interest in selective D_4-receptor ligands was initially driven by the finding that the atypical neuroleptic drug clozapine preferentially binds to the D_4-receptor [102, 103]. Up to now, several candidates have been evaluated in vitro and in vivo, including agonists as putative agents for the treatment of sexual dysfunction and attention deficit hyperactivity disorder and antagonists as antipsychotic drugs focussing on the treatment of neuropsychiatric disorders, such as schizophrenia, as well as selective imaging agents for the D_4-receptor subtype. The medicinal chemistry governing these developments has been separately summarized in recent reviews [104–106]. The crucial structural requirement for selective D_4 ligands consists of a basic nitrogen represented by a piperazine or piperidine core flanked by two aromatic rings with an optimal distance. A pharmacophore model for several structurally diverse D_4 antagonists and CoMFA studies has been successfully used to guide the design of novel selective D_4 ligands in recent years [107–109].

5.3.1 Selective D_4 Agonists

One of the first selective D_4 agonists, PD-168077 (Fig. 5.18), was discovered by Glase et al. [110]. These authors examined a large series of *N*-methylpiperazinyl benzamides with *ortho*-substituent (2-Cl, 2-CN, and 2-OMe) at the phenylpiperazine under variation of the substitution pattern of the benzamide phenyl core. In comparison with 2-methoxy and 2-cyano derivatives, the 2-chloro compounds reveal decreased D_4 selectivity in binding studies using CHO K1 transfected cells. Introduction of a methyl group in the 3-position of the 2-cyano-phenylpiperazinyl compound yields PD-168077 with substantial D_4 affinity ($K_i(hD_4) = 8.7$ nM) and excellent subtype selectivity (>300-fold over D_3 and > 400-fold over D_2). Stimulated mitogenesis in D_4-transfected CHO pro-5 cells shows an EC_{50} of 17 nM and 80% intrinsic activity compared with quinpirole as a reference dopamine receptor agonist. The high D_4-receptor affinity of PD-168077 has also been confirmed for the human D_4 alleles $hD_{4.2}$ (6.0 nM), $hD_{4.4}$ (22.3 nM), and $hD_{4.7}$ (29.0 nM) [111]. PD-168077 has been studied in vivo in a number of pharmacological studies in rats, including behavioral studies and memory tests. The compound induces an improved memory performance [112], but dyskinesia was noticed that was insensitive to the D_4 antagonists L-745,870 or haloperidol [113], and thus could be ascribed to non-dopaminergic binding sites of PD-168077. The proerectile effect of PD-168077 when administered locally in the paraventricular nucleus (PVN) of the hypothalamus of male rats [114] or s.c [111] provides evidence for the role of D_4-receptors mediating erectile function.

Since PD-168077 shows limited stability toward acidic medium [110], Matulenko et al. aimed at the corresponding retroamides, of which they synthesized a series of *ortho*-, *meta*-, and *para*-phenylpiperazinyl substitutes candidates [115]. Among the series of derivatives, only the 2-cyano derivative (A-369508, Fig. 5.18)

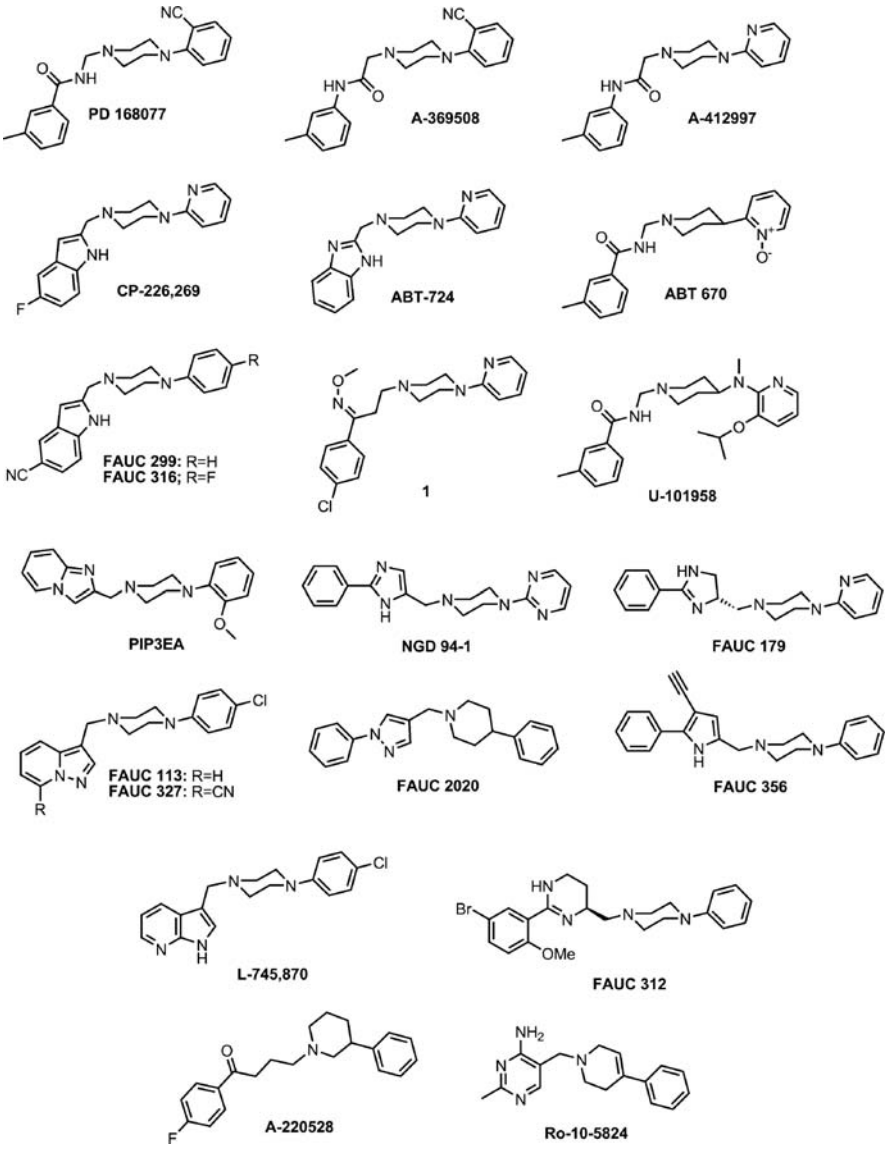


Fig. 5.18 Selective D_4 ligands: partial agonists

shows equivalent efficacy ($EC_{50} = 7.5$ nM) and increased D_4 potency ($K_i(D_4) = 2.7$ nM) compared to PD-168077 as studied in a calcium flux assay using HEK 293 cells co-transfected with $hD_{4.4}$ and $G\alpha_{qo5}$. Replacement of the 2-cyano group by other electron-withdrawing groups, such as fluorine or nitro, causes slightly decreased potency ($EC_{50} = 18$ nM or 13 nM, respectively), whereas changing the

ortho-substituent to a *meta*- or *para*-position leads to total loss in agonist activity. Noteably, some of these derivatives (3-Me, 4-Me, 3-OMe, and 4-F) retain their D_4 binding affinity (K_i: 8–70 nM) and exhibit functional antagonism. Substitution on the aryl acetamide portion of 3-cyanopyridin-2-yl derivatives also results in compounds with good D_4 potency, but the intrinsic efficacy is slightly lower when compared to A-369508.

Recently, a series of structurally related analogs of PD-168077 has been developed by replacement of the amide group with a methylene-oxime moiety [116]. Introduction of a 2-pyridine ring revealed to be superior for D_4 agonist activity of the most potent candidate **1** shown in Fig. 5.18. This derivative shows poor D_4 binding selectivity over D_2 ($K_i(D_4) = 38.2$ nM; $D_2/D_4 = 1.7$); however, it shows no efficacy at concentrations up to 10 μM in an assay using HEK-293 cells co-transfected with human D_{2L} and chimeric $G\alpha_{qo5}$ and its activity in the rat penile erection model is three times higher than that of the nonselective D2-like agonist apomorphine. Thus, this compound is a D_4 agonist and a moderately potent D_2 antagonist.

The indole CP-226,269 (Fig. 5.18), another selective D_4 agonist bearing a 2-pyridinylpiperazinyl moiety ($K_i(D_4) = 6.0$ nM; $K_i(D_2) > 600$ nM), has been described contemporary with PD-168077 by Zorn et al. [117]. Competition studies using recombinant human D_4 variants $hD_{4.2}$, $hD_{4.4}$, and $hD_{4.7}$ revealed even higher receptor affinities than PD-168077 with K_i values of 2.4 nM, 3.6 nM, and 5.6 nM, respectively [111]. Despite a lack of affinity to human D_{2L} receptors, it has been demonstrated that CP-226,269 binds to the rat D_2 receptor with moderate affinity ($EC_{50} = 55$ nM) [118], which complicates the interpretation of its in vivo pharmacology in rats.

The benzimidazole derivative ABT-724 (Fig. 5.18), also bearing the 2-pyridinylpiperazinyl moiety and thus closely related to CP-226,269, has been developed by Stewart et al. [119]. Interestingly, these authors notice a distinct "*ortho*-effect" when comparing *para*-, *meta*-, and *ortho*-substituted phenylpiperazinyl derivatives with the corresponding 2-, 3-, and 4-pyridyl compounds with reference to binding affinity to hD_4 and efficacy measured by a calcium flux assay using HEK 293 cells co-transfected with $hD_{4.4}$ and $G\alpha_{qo5}$. A total loss of D_4 affinity and efficacy is observed for the 4-pyridyl compound, whereas the 2-pyridyl (ABT-724) and the 2-chlorophenyl derivative display satisfactory D_4 potency and efficacy. This study indicates that the phenylpiperazine moiety plays a key role in determining D_4-receptor efficacy [119, 120]. The EC_{50} of ABT-724 is 12.4 nM, intrinsic activity is 61%, and receptor selectivity has been confirmed by screening 70 different neuroreceptors [121]. Using the agonist D_4 radioligand [^{3}H]A-369508 (Fig. 5.20) and membrane preparations expressing $hD_{4.2}$, $hD_{4.4}$, and $hD_{4.7}$, receptor-binding experiments reveal K_i values of 47–64 nM. ABT-724 has been further evaluated in vivo, confirming the proerectile activity of the compound when given s.c. at very low doses (0.03 μmol/kg) to conscious rats, an effect that is blocked by haloperidol and clozapine, but not by the peripheral dopaminergic antagonist domperidone, indicating CNS activity of ABT-724.

More recently, ABT-670 has been discovered by further structural optimization of ABT-724 introducing a (*N*-oxy-2-pyridinyl)piperidine template (Fig. 5.18),

which exhibits comparable efficacy and improved oral bioavailability in rat, dog, and monkey in comparison with ABT-724 [122].

A-412997 (Fig. 5.18) is a piperidine analog derived from the retroamide series of D_4 agonists [118]. In comparison with PD-168077 and CP-226,269, A-412997 ($K_i(hD_{4.4}) = 7.9$ nM; K_i (rD_4) = 12.1 nM) shows a better selectivity profile, with no affinity (> 1,000 nM) for other dopamine receptors and reveals potent full D_4 agonism in functional assays ($EC_{50} = 28.4$ nM, intrinsic efficacy: 83% related to 10 μM dopamine). A-412997 shows rapid blood–brain barrier penetration, induces penile erection in a conscious rat model with an effective dose of 0.1 μmol/kg, and also induces cognitive enhancement properties in a rat model of ADHD and short-term memory [123].

An extensive series of phenylpiperazines with an indole or pyrazolo[1,5-*a*]pyridine core unit has been studied by Gmeiner et al. [124–127]. The majority of these derivatives are partial D_4 agonists as demonstrated by stimulated mitogenesis assays on CHO cells expressing the human $D_{4.2}$ receptor. Using a solid-phase supported synthesis, the influence of electropositive and electronegative substituents at C-2 of the indole core was investigated, showing that such modifications do not affect D_4 affinity or subtype selectivity [124, 127]. These studies reveal that a 5-cyano and the phenylpiperazinylmethyl substitution in 2-position of the indole core is most favorable for high D_4 affinity and substantial D_4 subtype selectivity. Among the series of indoles (Fig. 5.18), the phenylpiperazine FAUC 299 shows D_4 affinity in the subnanomolar range ($K_i = 0.52$ nM), and the corresponding *para*-fluoro derivative FAUC 316 displays high D_4 affinity ($K_i = 1.0$ nM) combined with substantial D_4 subtype selectivity of more than 8,600-fold over the other dopamine subtypes. FAUC 299 and FAUC 316 reveal partial agonism with EC_{50} values of 1.5 nM and 9.4 nM, respectively, and a relative partial agonist effect of 30–35% compared to the maximal effect of the unselective agonist quinpirole [124].

Similarly, replacing the indole moiety by conformationally restricted benzamide bioisosteres, such as a dihydroimidazole ring, led to the discovery of FAUC 179 (Fig. 5.18) [128]. The binding experiments show a biphasic curve, providing a high-affinity D_4 binding site ($K_i = 0.95$ nM) and a low-affinity binding site (51 nM), being comparable to the properties of quinpirole. The D_4 selectivity of FAUC 179 is more than 7,000-fold over the other D2-like receptors. The affinity toward serotonergic sites (5-HT_{1A}: 45 nM; 5-HT_2: 870 nM) is moderate to low, as determined by displacement studies on porcine brain homogenates using selective tritiated radioligands. FAUC 179 is finally characterized as a selective D_4 partial agonist ($EC_{50} = 31$ nM; efficacy: 42% relative to the reference quinpirole).

Alternative bioisosteres of the dihydroimidazole core unit of FAUC 179 have also been reported, resulting in partial agonists with retained D_4 affinity and efficacy including the ethenylpyrrole FAUC 356 (Fig. 5.18) [129] or the tetrahydropyrimidine FAUC 312 [130]. The latter reveals high D_4 affinity ($K_i = 1.5$ nM), superior subtype selectivity ($K_i > 10$ μM for the other D2-like receptors), and 83% intrinsic agonist activity ($EC_{50} = 50$ nM).

The series of pyrazolo[1,5-*a*]pyridine-based ligands demonstrate high D_4 affinity (1.5–2.2 nM) and selectivity [125, 126]. Starting from pyrazolo[1,5-*a*]pyridine,

which is readily available by 1,3-dipolar cycloaddition, or its 3-carboxylic ester, an extended series of 3-substituted phenylpiperazinyl derivatives, including FAUC 113 (Fig. 5.18), has been achieved by Löber et al. [126]. This study also indicates that a negative potential below and above the 5-membered ring of the heterocycle appeared to be crucial for high D_4 affinity, whereas an enlarged negative region below the ring appears to be responsible for substantial D_4 selectivity, since this molecular property obviously is not tolerated by the other dopaminergic receptors. Interestingly, non-bulky substituents in the 7-position of the pyrazolo[1,5-*a*]pyridine core are well tolerated by the D_4-receptor. The 7-iodo [131], 7-methyl, 7-acetylenyl, 7-carbaldehyde, and 7-cyano (FAUC 327, Fig. 5.18) derivative shows K_i values of 1.2–2.7 nM for the D_4-receptor [125]. The latter has been subsequently shown to stimulate mitogenesis in CHO-D_4-expressing cells inducing an intrinsic effect of 31% with an EC_{50} of 1.5 nM. Applying a methyl group as a substituent within the piperazine template, the *R*-isomer is a partial D_4 agonist with an EC_{50} of 6.2 nM, being five times more potent than the *S*-isomer [132].

As a structurally closely related analog of the pyrazolo[1,5-*a*]pyridines, the 2-methoxyphenylpiperazinyl derivative of imidazo[1,2-*a*]pyridine (PIP-3EA, Fig. 5.18) has been investigated by Enguehard-Gueiffier et al. [133]. This compound is a potent and selective D_4 partial agonist ($K_i = 2.8$ nM, $D_4/D_2 > 350$; $D_4/D_3 > 1{,}300$) with EC_{50} of 4.5 nM and an intrinsic activity of 57% (based on quinpirole) as determined in a [^{35}S]GTPγS binding assay, whereas the 4-chlorophenylpiperazinyl analog shows weak agonist properties and the 3,4-dichloro derivative even reveals D_4 antagonism. PIP-3EA has been further studied in vivo. Applying systematic, intracerebroventricular (0.1–20 μg) or direct injection into the paraventricular nucleus (PVN) of the hypothalamus, PIP-3EA mediates penile erection in rats, being as potent as PD-168077 when given into the PVN, but more potent when given systematically [134]. More recently, Succu et al. provided evidence that the stimulation of dopamine receptors in the PVN, including the D_4 subtype, caused oxytocin release in extra-hypothalamic rat brain areas, which in turn is known to modulate the activity of mesolimbic dopaminergic neurons that are involved in rewarding effects of sexual activity [135].

As a structurally related analog of haloperidol, A220528 (Fig. 5.18) was found by conducting a high-throughput screen of an in-house library of compounds [136]. The 3-phenylpiperidine derivative (A220528, Fig. 5.1) and the respective thiazolyl analog are potent and selective D_4 agonists ($EC_{50} = 51$ and 34 nM; $IC_{50}(D_2) > 2$ μM) exhibiting full agonism (91 and 94%, related to dopamine) as determined by a calcium flux assay on HEK 293 cells co-expressing $hD_{4.4}$ and $G\alpha_{q05}$ using a fluorometric imaging plate reader (FLIPR assay).

Another high-throughput screening approach was successful for the discovery of Ro-10-5824, a member of the biaryl type class of D_4 selective ligands [137]. The compound displays high D_4 affinity binding with a K_i of 5.2 nM, a 250-fold D_4-selectivity versus hD_3-receptors and > 1,000-fold selectivity over the other dopamine receptor subtypes. GTPγS-binding assays in CHO-$D_{4.4}$ cell membranes reveal an EC_{50} of 205 nM and a relative activation level of 36%, characterizing

Ro-10-5824 as a selective partial D_4 agonist (Fig. 5.18). The compound has been tested in vivo for locomotor activity in a dose–response study and increases novel object exploration by C57BL/6 J male mice using a dose of 10 mg/kg.

Employing a modified "click chemistry" method [138] or reductive amination of the respective *N*-phenylpyrazole carbaldehyde, Löber et al. provide access to another series of biaryl analogs of NGD 94-1 including FAUC 2020 (Fig. 5.18) and its 2-fluoro- and 2-methoxyphenylpiperazinyl analogs [139]. These compounds exhibit extraordinarily high D_4 affinities with K_i values in the subnanomolar range combined with > 50-fold D_4 preference over the other dopamine subtypes ($K_i(hD_4) = 0.59$ nM for FAUC 2020; $K_i(hD_4) = 0.7$ nM (2-fluorophenyl analog); $K_i(hD_4) = 0.28$ nM (2-methoxyphenyl)). In comparison with the corresponding pyrrole template, the pyrazole or a triazole scaffold increases D_4 affinity by a factor of 20. FAUC 2020 proves to be a partial agonist displaying intrinsic activities of 40 % and EC_{50} of 5.6 nM using GTPγS binding and mitogenesis assays on D_4-receptor-expressing CHO cells. However, discrepancies between D_4 selectivity in binding experiments and functional assays have been noticed.

5.3.2 Selective D_4 Antagonists

Several compounds originally reported to act as D_4 antagonists were revealed by subsequent investigations [140, 141] to be partial agonists, such as the selective D_4 ligands L-745,870 ($K_i(hD_4)$=0.43 nM, > 2,000-fold selectivity over other dopamine subtypes, > 1,000-fold selective over 5-HT_2 [142, 143]), U-101958 ($K_i(hD_4) =$ 1.4 nM, > 460-fold selectivity over other dopamine subtypes [144]), and NGD 94-1 ($K_i(hD_4) = 3.6$ nM, $K_i(D_2) = 2230$ nM, $K_i(5\text{-}HT_{1A}) = 180$ nM [145]) (Fig. 5.18). Interestingly, the efficacy was only apparent in cells with high receptor density in the in vitro assays [146]. Since an increased D_4-receptor density in postmortem schizophrenia brain tissue was reported by Seeman et al. [147], it is tempting to speculate that this in vivo situation could also result in partial agonist activity of these compounds in vivo, which may help explain the lack of efficacy of these compounds in clinical trials aiming at the treatment of schizophrenia [148, 149].

In the search for D_4-receptor antagonists, Löber et al. studied the influence of the linkage position of the (4-(4-chlorophenyl)piperazin-1-yl)methyl moiety to the pyrazolo[1,5-a]pyridine on the functional activity of the resulting potential D_4 ligand [150]. Linkage in the 7-position of the heterocycle leads to loss of D_4 affinity, whereas in the 4-position moderate D_4 affinity of the resulting ligand with $K_i =$ 64 nM for D_4 is observed. However, the corresponding 2-substituted (FAUC 213, (Fig. 5.19)), 3-substituted (FAUC 113, Fig. 5.18), and 6-substituted compounds equally demonstrate high D_4 affinities with K_i values of 2.3, 3.6, and 3.1 nM. Among these, the regioisomer FAUC 213 is a neutral D_4 antagonist as shown by ligand-induced mitogenesis experiments, with substantial D_4 selectivity. It was further demonstrated that the intrinsic activity of the regioisomers investigated

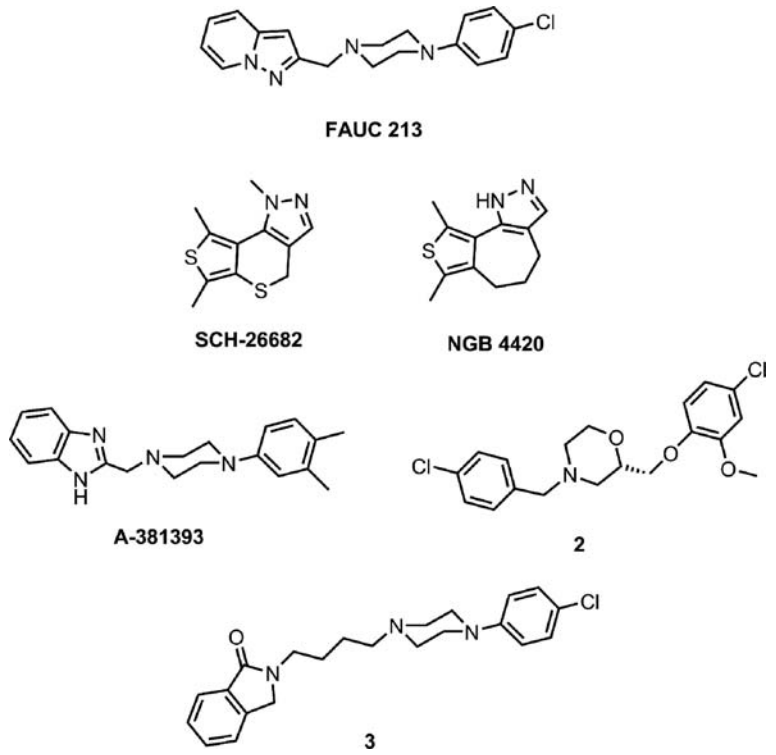


Fig. 5.19 Selective D_4 ligands: neutral antagonists

depends on the ability of the heterocyclic unit to interact with both elements of the D_4 binding-site crevice, the aromatic microdomain in TM6, and a serine residue in TM5. FAUC 213 has been further studied in behavioral and neurochemical animal models of schizophrenia in vivo, exhibiting atypical antipsychotic properties [151]. Although FAUC 213 holds promise for further pharmacological experiments, it remains to be elucidated whether interfering receptors, such as adrenergic or serotonergic, could influence the in vivo efficacy of FAUC 213.

A series of similar azaindoles with high D_4 potency ($K_i(hD_4) = 1.4–4.7$ nM) and D_4 selectivity are also characterized as D_4 antagonists, and due to the methoxy substituents in some analogs, their C-11-labeled analogs are proposed as radioligands for in vivo imaging studies [152].

Varying the aromatic core unit, D_4 selective pyrido[1,2-*a*]pyrazine antagonists have also been reported, including CP-293,019 ($K_i(hD_4) = 3.4$ nM, $K_i(hD_2) > 3$ μM), which inhibits apomorphine-induced hyperlocomotion in rats after oral dosing [153].

As a derivative bearing a benzenesulfonamide, U-101387 displays moderately high affinity and selectivity for the D_4-receptor in vitro ($K_i = 10$ nM), lacking measurable affinity for other dopamine, noradrenaline, serotonin and histamine

receptors (K_i > 2,000 nM) [154, 155]. U-101387 also displays favorable oral bioavailability, good brain penetration and is without effect in behavioral animal tests predictive of extrapyramidal and neuroendocrine side effects. U-101387 has been introduced in a clinical trial including 467 schizophrenia patients, where U-101387 was, however, ineffective for the treatment of patients with schizophrenia [156]. Recently, U-101387 has been used to inhibit the effects of dopamine, noradrenaline, and serotonin on G-protein-regulated inwardly rectifying potassium channels (GIRK1) in frog oocytes co-expressing D_4-receptors, thus indicating that D_4 can be pharmacologically stimulated by any of the three major central monoamines [157].

With a unique fused thiophene structure, SCH 26682 (Fig. 5.19) has been found by a compound library screening approach and further structural optimization has been performed leading to the corresponding 2,5-dimethylthiophene NGB 4420 with a K_i of 12 nM and good D_4 selectivity of 170-fold over D_2 and > 1,000-fold over D_1, D_3, and D_5 [158]. The [^{35}S]GTPγS binding assay using $hD_{4.2}$ receptor-expressing cells suggests that NGB 4420 functions as a neutral antagonist.

Nakane et al. identified the benzoimidazole A-381393 (Fig. 5.19) as a structurally related bioisostere of L-745,870 with potent D_4 binding (K_i = 1.5 nM) combined with high selectivity (> 2,700-fold) over the other dopamine receptor subtypes [159]. Contrary to L-745,870, A-381393 does not show any intrinsic activity in $D_{4.4}$ and $G\alpha_{q05}$ co-expressing HEK cells (measured by Ca^{2+}-flux) and significantly blocks agonist-induced GTP binding. Moreover, A-381393 also inhibits D_4 agonist (PD-168077)-induced penile erection in the male rat animal model, successfully demonstrating D_4 antagonist properties of A-381393 both in vitro and in vivo. In addition, the selective D_4-receptor antagonist A-381393 reduces c-Fos expression in the PVN below control levels suggesting a tonic dopamine D_4-receptor activation under basal conditions in vivo [160].

The morpholine template as an bioisostere of the piperazine core has been introduced by Audouze et al., revealing high D_4 affinity ($K_i(D_4)$ = 2.0–4.5 nM), especially for the *S*-isomer of a 2-methoxy-4-chlorophenoxy substituted compound (**2**, Fig. 5.19), whereas the *R*-isomer is inactive [161]. A predictive 3D-QSAR model also shows that the size of the morpholine or 1,4-oxazepane ring is a prerequisite for high D_4 affinity.

Alternative structural features of D_4-selective candidates have been reported by others, including benzylpiperazines bearing an azaindole core [162], benzamides bearing a benzylpyrrolidine scaffold [163, 164], or compounds with structurally rigid pyrimidinylpiperazines [165]. More recently, novel lactam derivatives have been prepared and evaluated for binding affinity and functional activity by Awadallah et al., including the indolinone derivative **3** (Fig. 5.19) that displays superior D_4 affinity (K_i = 0.04 nM) and a > 43,000-fold selectivity over hD_2 receptor [166]. Antagonistic activity of this series of compounds was confirmed using a calcium fluorescence assay. These series of candidates could be helpful in further developments of D_4-selective antagonists as potential treatments for psychiatric disorders.

5.3.3 Selective D_4 Radioligands

Most of the D_4 radioligand-binding studies were performed employing the indirect method described by Seeman et al. [147] that involves the subtraction of binding sites measured with the selective D_2/D_3 radioligand [^{3}H]raclopride from total D2-like binding sites defined by the unselective radioligand [^{3}H]nemonapride. This methodology has been used in various autoradiography studies [167–169] and has been extended by the use of cold raclopride (1μM) in an assay system with [^{3}H]nemonapride, which appears to be more adequate when estimating D_4-receptor overall population in the mouse brain [170].

Isotopically labeled radioligands derived from the above described selective D_4 ligands have been used for in vitro studies on D_4-receptor expression in tissue by autoradiography [171, 172]. A detailed study by Primus et al. using ^{3}H-labeled NGD 94-1 reveals region-specific binding that appeared to be low in density (< 20 fmol/mg) in the rat brain [172]. Highest D_4-receptor density is detected in rat hippocampus, lateral septal nucleus, medial preoptic area, and entorhinal cortex, with negligible binding in the rat striatum. In several human brain regions, including hippocampus, hypothalamus, dorsal medial thalamus, entorhinal cortex, prefrontal cortex, and lateral septal nucleus, high-affinity [^{3}H]NGD 94-1 binding has been determined (approx. 30 fmol/mg). Again, binding was low, if not absent, in striatal human brain regions.

Matulenko et al. synthesized the tritiated D_4 agonist A-369508 (Fig. 5.20) by reaction of a suitable brominated precursor with tritium gas in the presence of a sensitive cyano group, obtaining the agonist radioligand in adequate specific activity for saturation binding experiments [173]. [^{3}H]A-369508 shows high affinity to $hD_{4.2}$ (1.7 nM), $hD_{4.4}$ (4.0 nM), and $hD_{4.7}$ (1.2 nM), and also binds to rat D_4-receptors (K_d = 4.4 nM) [174]. The D_4-receptor-subtype selectivity of [^{3}H]A-369508 is greater than 400-fold (D_1, > 2,475-fold; D_{2L}, 434-fold; D_3, 1,022-fold; D_5 > 2,475-fold) and over 70 other potentially interfering neurotransmitter receptors were tested. Among these, only moderate affinity to the 5-HT_{1A} receptor (K_i = 1219 nM) is worth mentioning. Therefore, [^{3}H]A-369508 is useful as an agonist radioligand in competition-binding experiments and in autoradiography studies.

As recently reviewed [104], there is an intriguing interest in the development of radiolabeled selective D_4 ligands suitable for in vivo imaging of D_4-receptor densities by positron emission tomography (PET) to further explore the relevance of this molecular target for various neurobehavioral and psychiatric disorders, such as schizophrenia. Early PET studies using the nonselective D_2/D_3 radioligand [^{11}C]raclopride (Fig. 5.16) failed to detect dopamine receptor disturbances in drug-naive patients [175], whereas the $D_2/D_3/D_4$ affine radioligand [^{11}C]spiperone markedly indicates an increase in dopamine receptors in similar patients [176]. These findings suggest elevated D_4-receptor densities in vivo, as confirmed by Seeman et al. for postmortem schizophrenia brain tissue some years later [147]. Consequently, various attempts toward the development of subtype-selective D_4 radioligands labeled with the positron emitters ^{18}F and ^{11}C have been reported. The first study on a putative D_4 radioligand was reported by Bender et al. dealing

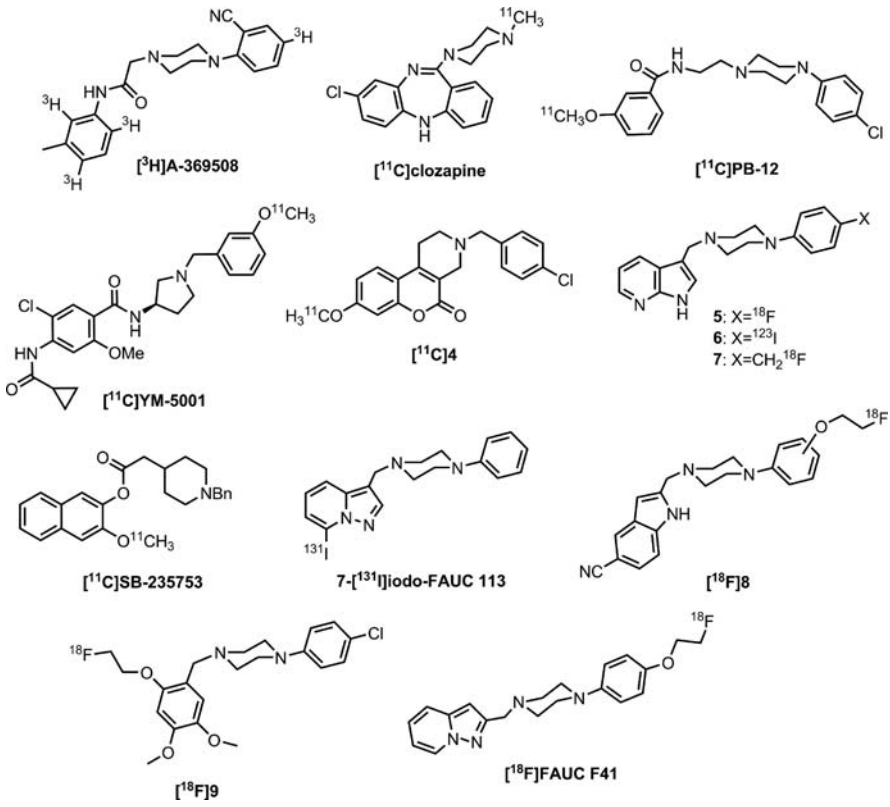


Fig. 5.20 Subtype-selective D_4 radioligands

with the synthesis of [^{11}C]clozapine (Fig. 5.20) [177]. Using [^{11}C]methyl iodide in DMSO, norclozapine was labeled in 70% with a specific activity in the range of 92–130 GBq/μmol. Biodistributional experiments in NMRI mice showed preferential brain uptake of [^{11}C]clozapine in the frontal cortex, which was retained for up to 40 min.

Later on, Boy et al. applied [^{11}C]SDZ-GLC756 for imaging of D_4-receptors in the primate brain by PET, which included the need for D_1, D_2, D_3, and D_5 receptor blockade by preinjection of SCH23390 and raclopride [178], indicating D_4-receptor localization in the frontal cortex and neocortex by this subtractive approach.

When searching for a selective PET ligand, without the need for pharmacological blocking of interfering receptors, methoxybenzamide analogs of PD-168077, such as [^{11}C]PB-12 (Fig. 5.20), reported as highly potent and selective D_4 ligands [179, 180], were labeled with [^{11}C]methyl triflate [181, 182]; however, no specific binding was detected in the monkey and rat brain.

The same group provided evidence that a ^{11}C-labeled tetrahydrochromeno[3,4-*c*]pyridin-5-one (**[^{11}C]4**, Fig. 5.20), originally reported as a selective D_4 antagonist

[183], preferentially binds to sigma-1 receptors in vivo as demonstrated by blocking studies with pentazocine [184]. Closely related ^{18}F-labeled analogs have also been synthesized [185, 186] and one candidate showed highest uptake in frontal cortex and medulla of the rat brain [185].

^{18}F- and ^{123}I-labeled derivatives of pyrrolo[2,3-*b*]pyridines L-745,870 and L-750,667 (**5**–**7**, Fig. 5.20) [162, 187–190] were also evaluated in animal models and determined not to be suitable for in vivo imaging of the D_4-receptor. In addition, D_4 radioligand candidates for PET have been derived from naphthyl-based D_4 ligands [191], such as ^{11}C-labeled SB-235753 (Fig. 5.20) [192], revealing homogeneous distribution in the rat brain suggesting non-specific tracer uptake.

In terms of the selective D_4 ligands FAUC 316, FAUC 113, and FAUC 213 (Fig. 5.18, Fig. 5.19), the syntheses of radiolabeled analogs have been studied by Prante et al., including 7-iodo-FAUC 113 [131, 193], ^{18}F-labeled 5-cyano-indoles (**[^{18}F]8**) [194], benzylpiperazines (**[^{18}F]9**) [195], and an extended series of ^{18}F-labeled pyridinyl- and (2-fluoroethoxy)phenyl substituted pyrazolo[1,5-*a*]pyridines [196] (Fig. 5.20). Among these, the *para*-(2-fluoroethoxy)phenyl candidates revealed superior D_4 selectivity over D_2 (> 2,300-fold) combined with inverse agonism at D_4 as studied in mitogenesis experiments. The ^{18}F-labeled D_4 inverse agonist FAUC F41 (Fig. 5.20) revealed a promising binding pattern in vitro and ex vivo, reflecting the known D_4-receptor distribution, as determined by autoradiography in comparison with [^{3}H]nemonapride (1μM raclopride) [196]. ^{18}F-labeled FAUC F41 binding was blocked by FAUC 213 and eticlopride in vitro, while co-injection of rats with L-750,667 blocked binding of FAUC F41 in distinct regions of the rat brain, including the gyrus dentate region of the hippocampus, hypothalamus, the medial habenular nucleus, central medial thalamic nucleus, septum, and cortical areas, whereas low binding was detected in the striatum [196, 197]. Although FAUC F41 holds promise for further in vivo studies, a suitable selective D_4 PET ligand for human use still remains to be established.

References

1. Boeckler F. Competition for the “gold standard” L-Dopa? Receptor mediated Parkinson’s therapy. Pharm Unserer Zeit 2006;35:204–16.
2. Merlino G, Serafini A, Robiony F, Valente M, Gigli GL. Clinical experience with pramipexole in the treatment of restless legs syndrome. Expert Opin Drug Metab Toxicol 2008;4:225–35.
3. De Oliveira IR, Juruena MF. Treatment of psycosis: 30 years of progress. J Clin Pharm Ther 2006;31:523–34.
4. Grady MA, Gasperoni TL, Kirkpatrick P. Aripiprazole. Nat Rev Drug Discov 2003;2:427–8.
5. Lawler CP, Piroleau C, Lewis MM, et al. Interactions of the novel anitpsychotic aripiprazole (OPC-14597) with dopamine and serotonin receptor subtypes. Neuropsychopharmacology 1999;20:612–27.
6. Burris KD, Molski TF, Xu C, et al. Aripiprazole, a novel anitpsychotic, is a high-affinity partial agonist at human dopamine D_2 receptors. J Pharmacol Exp Ther 2002;302: 381–9.

7. Laruelle M, Frankle WG, Narendran R, Kegeles LS, Abi-Dargham A. Mechanism of action of antipsychotic drugs: from dopamine D_2 receptor antagonism to glutamate NMDA facilitation. Clin Ther 2005;27(Suppl A):S16–24.
8. Steiner G, Bach A, Bialojan S, et al. D_4/5-HT_{2A} Receptor antagonists: LU-111995 and other potential new antipsychotics in development. Drugs Future 1998;23: 191–204.
9. Sokoloff P, Andrieux M, Besancon R, et al. Pharmacology of human dopamine D_3 receptor expressed in a mammalian cell line: comparison with D_2 receptor. Eur J Pharmacol 1992;225:331–7.
10. Boeckler F, Gmeiner P. The structural evolution of dopamine D_3 receptor ligands: structure-activity relationships and selected neuropharmacological aspects. Pharmacol Ther 2006;112:281–333.
11. Boeckler F, Lanig H, Gmeiner P. Modeling the similarity and divergence of dopamine D_2-like receptors and identification of validated ligand-receptor complexes. J Med Chem 2005;48:694–709.
12. Cannon JG. Structure-activity relationships of dopamine agonists. Annu Rev Pharmacol Toxicol 1983;23:103–30.
13. McDermed JD, McKenzie GM, Phillips AP. Synthesis and pharmacology of some 2-aminotetralins. Dopamine receptor agonists. J Med Chem 1975;18:362–7.
14. Cannon JG, Lee T, Goldman HD, Costall B, Naylor RJ. Cerebral dopamine agonist properties of some 2-aminotetralin derivatives after peripheral and intracerebral administration. J Med Chem 1977;20:1111–6.
15. Malmberg A, Nordvall G, Johansson AM, Mohell N, Hacksell U. Molecular basis for the binding of 2-aminotetralins to human dopamine D_{2A} and D_3 receptors. Mol Pharmacol 1994;46:299–312.
16. van Vliet LA, Tepper PG, Dijkstra D, et al. Affinity for dopamine D_2, D_3, and D_4 receptors of 2-aminotetralins. Relevance of D_2 agonist binding for determination of receptor subtype selectivity. J Med Chem 1996;39:4233–7.
17. Karlsson A, Björk L, Pettersson C, Andén N-E, Hacksell U. (R)- and (S)-5-Hydroxy-2-(dipropylamino)tetralin (5-OH DPAT): Assessment of optical purities and dopaminergic activities. Chirality 1990;2:90–5.
18. Yu H, Liu Y, Malmberg A, Mohell N, Hacksell U, Lewander T. Differential serotoninergic and dopaminergic activities of the (*R*)- and the (*S*)-enantiomeres of 2-(di-*n*-propylamino)tetralin. Eur J Pharmacol 1996;303:151–62.
19. Chumpradit S, Kung M-P, Kung HF. Synthesis and optical resolution of (R)- and (S)-*trans*-7-Hydroxy-2-[N-propyl-N-(3′-iodo-2′-propenyl)amino]tetralin: a new D3 dopamine receptor ligand. J Med Chem 1993;36:4308–12.
20. Chumpradit S, Kung M-P, Vessotskie J, Foulon C, Mu M, Kung HF. Iodinated 2-aminotetralins and 3-amino-1-benzopyrans: ligands for dopamine D2 and D3 receptors. J Med Chem 1994;37:4245–50.
21. Cusack NJ, Peck JV. N-0923. Dopamine D_2 agonist. Drugs Future 1993;18:1005–8.
22. Reynolds NA, Wellington K, Easthope SE. Rotigotine in Parkinson's disease. CNS Drugs 2005;19:973–81.
23. Glase SA, Corbin AE, Pugsley TA, Heffner TG, Wise LD. Synthesis and dopaminergic activity of pyridine analogs of 5-hydroxy-2-(di-*n*-propylamino)tetralin. J Med Chem 1995;38:3132–7.
24. Sokoloff P, Giros B, Martres M-P, Bouthenet M-L, Schwartz J-C. Molecular cloning and characterization of a novel dopamine receptor (D_3) as a target for neuroleptics. Nature 1990;347:146–51.
25. Tang L, Todd RD, Heller A, O'Malley KL. Pharmacological and functional characterization of D_2, D_3 and D_4 dopamine receptors in fibroblast an dopaminergic cell lines. J Pharmacol Exp Ther 1994;268:495–502.

26. Kreiss DS, Bergstrom DA, Gonzales AM, Huang K-X, Sibley DR, Walters JR. Dopaminergic receptor agonist potencies for inhibition of cell firing correlate with dopamine D_3 receptor binding affinities. Eur J Pharm 1995;277:209–14.
27. Burris KD, Pacheco MA, Filtz TM, Kung M-P, Kung HF, Molinoff PB. Lack of discrimination by agents for D_2 and D_3 dopamine receptors. Neuropsychopharmacology 1995;12:335–45.
28. Millan MJ, Peglion J-L, Vian J, et al. Functional correlates of dopamine D_3 receptor activation in the rat in vivo and their modulation by selective antagonist, (+)-S 14297: I. Activation of postsynaptic D_3 receptors mediates hypothermia, whereas blockade of D_2 receptors elicits prolactin secretion and catalepsy. J Pharmacol Exp Ther 1995;275:885–98.
29. Levant B. The D_3 dopamine receptor: Neurobiology and potential clinical relevance. Pharmacol Rev 1997;49:231–52.
30. Mierau J, Schneider FJ, Ensinger HA, Chio CL, Lajiness ME, Huff RM. Pramipexole binding and activation of cloned and expressed dopamine D_2, D_3 and D_4 receptors. Eur J Pharmacol; Molecular Pharmacology Section 1995;290:29–36.
31. Perachon S, Schwartz J-C, Sokoloff P. Functional potencies of new antiparkinsonian drugs at recombinant human dopamine D_1, D_2 and D_3 receptors. Eur J Pharmacol 1999;366:293–300.
32. Mierau J, Schingnitz G. Biochemical and pharmacological studies on pramipexole, a potent and selective dopamine D_2 receptor agonist. Eur J Pharmacol 1992;215:161–70.
33. Schneider CS, Mierau J. Dopamine autoreceptor agonists: Resolution and pharmacological activity of 2,6-diaminotetrahydrobenzothiazole and an aminothiazole analogue of apomorphine. J Med Chem 1987;30:494–8.
34. Lehmann T, Hübner H, Gmeiner P. Dopaminergic 7-aminotetrahydroindolizines: Exchiral pool synthesis and preferential D_3 receptor binding. Bioorg Med Chem Lett 2001;11:2863–6.
35. Löber S, Hübner H, Gmeiner P. Fused azaindole derivatives: Molecular design, synthesis and in vitro pharmacology leading to the preferential dopamine D_3 receptor agonist FAUC 725. Bioorg Med Chem Lett 2002;12:2377–80.
36. Elsner J, Boeckler F, Heinemann FW, Hübner H, Gmeiner P. Pharmacophore-guided drug discovery investigations leading to bioactive 5-aminotetrahydropyrazolopyridines. Implications for the binding mode of heterocyclic dopamine D_3 receptor agonists. J Med Chem 2005;48:5771–9.
37. Bergauer M, Hübner H, Gmeiner P. Practical ex-chiral-pool methodology for the synthesis of dopaminergic tetrahydroindoles. Tetrahedron 2004;60:1197–204.
38. Dijkstra D, Rodenhuis N, Vermeulen ES, Pugsley TA, Wise LD, Wikström HV. Further characterization of structural requirements for ligands at the dopamine D2 and D3 receptor: exploring the thiophene moiety. J Med Chem 2002;45:3022–31.
39. Rodenhuis N, Timmerman W, Wikström HV, Dijkstra D. Thiophene analogs of naphthoxazines and 2-aminotetralins: bioisosteres with improved relative oral bioavailability, as compared to 5-OH-DPAT. Eur J Pharmacol 2000;394:255–63.
40. Hübner H, Haubmann C, Utz W, Gmeiner P. Conjugated enynes as nonaromatic catechol bioisosteres: Synthesis, binding experiments, and computational studies of novel dopamine receptor agonists recognizing preferentially the D3 subtype. J Med Chem 2000;43:756–62.
41. Lenz C, Boeckler F, Hübner H, Gmeiner P. Analogues of FAUC 73 revealing new insights into the structural requirements of nonaromatic dopamine D3 receptor agonists. Bioorg Med Chem 2004;12:113–7.
42. Lenz C, Haubmann C, Hübner H, Boeckler F, Gmeiner P. Fancy bioisosteres: synthesis and dopaminergic properties of the endiyne FAUC 88 as a novel non-aromatic D3 agonist. Bioorg Med Chem 2005;13:185–91.
43. Lenz C, Boeckler F, Hübner H, Gmeiner P. Fancy bioisosteres: Synthesis, SAR, and pharmacological investigations of novel nonaromatic dopamine D3 receptor ligands. Bioorg Med Chem 2005;13:4434–42.

44. Haadsma-Svensson SR, Cleek KA, Dinh DM, et al. Dopamine D_3 receptor antagonists. 1. Synthesis and structure-activity relationships of 5,6-dimethoxy-*N*-alkylaryl-substituted 2-aminoindanes. J Med Chem 2001;44:4716–32.
45. Waters N, Svensson K, Haadsma-Svensson SR, Smith MW, Carlsson A. The dopamine D_3 receptor: A postsynaptic receptor inhibitory on rat locomotor activity. J Neural Transmission 1993;94:11–9.
46. Boyfield I, Coldwell MC, Hadley MS, et al. A novel series of 2-aminoteralins with high affinity and selectivity for the dopamine D_3 receptor. Bioorg Med Chem Lett 1997;7:1995–8.
47. Avenell KY, Boyfield I, Hadley MS, et al. Heterocyclic analogues of 2-aminotetralins with high affinity and selectivity for the dopamine D_3 receptor. Bioorg Med Chem Lett 1999;9:2715–20.
48. Mach UR, Hackling AE, Perachon S, et al. Development of novel 1,2,3,4-tetrahydroisoquinoline derivatives and closely related compounds as potent and selective dopamine D_3 receptor ligands. ChemBioChem 2004;5:508–18.
49. Austin NE, Avenell KY, Boyfield I, et al. Novel 1,2,3,4-tetrahydroisoquinolines with high affinity and selectivity for the dopamine D_3 receptor. Bioorg Med Chem Lett 1999;9:179–84.
50. Avenell KY, Boyfield I, Coldwell MC, et al. Fused aminotetralins: novel antagonists with high selectivity for the dopamine D_3 receptor. Bioorg Med Chem Lett 1998;8:2859–64.
51. Dubuffet T, Newman-Tancredi A, Cussac D, et al. Novel benzopyrano[3,4-*c*]pyrrole derivatives as potent and selective dopamine D_3 receptor antagonists. Bioorg Med Chem Lett 1999;9:2059–64.
52. Austin NE, Avenell KY, Boyfield I, et al. Novel 2,3,4,5-tetrahydro-1*H*-3-benzazepines with high affinity and selectivity for the dopamine D_3 receptor. Bioorg Med Chem Lett 2000;10:2553–5.
53. Austin NE, Avenell KY, Boyfield I, et al. Design and synthesis of novel 2,3-dihydro-1*H*-isoindoles with high affinity and selectivity for the dopamine D_3 receptor. Bioorg Med Chem Lett 2001;11:685–8.
54. Macdonald GJ, Branch CL, Hadley MS, et al. Design and synthesis of *trans*-3-(2-(4-((3-(3-(5-methyl-1,2,4-oxadiazolyl))-phenyl)carboxamido)cyclohexyl)ethyl)-7-methylsulfonyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine (SB-414796): a potent and selective dopamine D_3 receptor antagonist. J Med Chem 2003;46:4952–64.
55. Stemp G, Ashmeade T, Branch CL, et al. Design and synthesis of *trans*-*N*-[4-[2-(6-cyano-1,2,3,4-tetrahydroisoquinolin-2-yl)ethyl]cyclohexyl]-4-quinolinecarboxamide (SB-277011): a potent and selective dopamine D_3 receptor antagonist with high oral bioavailability and CNS penetration in the rat. J Med Chem 2000;43:1878–85.
56. Sautel F, Griffon N, Sokoloff P, et al. Nafadotride, a potent preferential dopamine D_3 receptor antagonist, activates locomotion in rodents. J Pharmacol Exp Ther 1995;275:1239–46.
57. Huang Y, Luedtke RR, Freeman RA, Wu L, Mach RH. Synthesis and structure-activity relationships of naphthamides as dopamine D_3 receptor ligands. J Med Chem 2001;44:1815–26.
58. Bolton D, Boyfield I, Coldwell MC, et al. Novel 2,5-disubstituted-1 *H*-pyrroles with high affinity for the dopamine D_3 receptor. Bioorg Med Chem Lett 1996;6:1233–6.
59. Einsiedel J, Thomas C, Hübner H, Gmeiner P. Phenyloxazoles and phenylthiazoles as benzamide bioisosteres: synthesis and dopamine receptor binding profiles. Bioorg Med Chem Lett 2000;10:2041–4.
60. Huang Y, Luedtke RR, Freeman RA, Wu L, Mach RH. Synthesis of 2-(2,3-dimethoxyphenyl)-4-(aminomethyl)imidazole analogues and their binding affinities for dopamine D_2 and D_3 receptors. Bioorg Med Chem 2001;9:3113–22.
61. Mach RH, Huang Y, Freeman RA, Wu L, Blair S, Luedtke RR. Synthesis of 2-(5-bromo-2,3-dimethoxyphenyl)-5-(aminomethyl)-1*H*-pyrrole analogues and their binding affinities for dopamine D_2, D_3, and D_4 receptors. Bioorg Med Chem 2003;11:225–33.
62. Glase SA, Akunne HC, Heffner TG, et al. 4-Bromo-1-methoxy-N-[2-(4-Aryl-1-piperazinyl)ethyl]-2-naphthalencarboxamides: Selective dopamine D_3 receptor partial agonists. Bioorg Med Chem Lett 1996;6:1361–6.

63. Robarge MJ, Husbands SM, Kieltyka A, Brodbeck R, Thurkauf A, Newman AH. Design and synthesis of [(2,3-dichlorophenyl)piperazin-1-yl]alkylfluorenylcarboxamides as novel ligands selective for the dopamine D_3 receptor subtype. J Med Chem 2001;44: 3175–86.
64. Bettinetti L, Schlotter K, Hübner H, Gmeiner P. Interactive SAR studies: Rational discovery of super-potent and highly selective dopamine D_3 receptor antagonists and partial agonists. J Med Chem 2002;45:4594–7.
65. Hocke C, Prante O, Löber S, Hübner H, Gmeiner P, Kuwert T. Synthesis and evaluation of ^{18}F-labeled dopamine D_3 receptor ligands as potential PET imaging agents. Bioorg Med Chem Lett 2005;15:4819–23.
66. Schlotter K, Boeckler F, Hübner H, Gmeiner P. Fancy bioisosteres: Metallocene-derived G-protein-coupled receptor ligands with subnanomolar binding affinity and novel selectivity Profiles. J Med Chem 2005;48:3696–9.
67. Schlotter K, Boeckler F, Hübner H, Gmeiner P. Fancy bioisosteres: novel paracyclophane derivatives as super-affinity dopamine D3 receptor antagonists. J Med Chem 2006;49:3628–35.
68. Leopoldo M, Berardi F, Colabufo NA, et al. Structure-affinity relationship study on *N*-[4-(4-arylpiperazin-1-yl)butyl]arylcarboxamides as potent and selective dopamine D_3 receptor ligands. J Med Chem 2002;45:5727–35.
69. Geneste H, Backfisch G, Braje W, et al. Synthesis and SAR of highly potent and selective dopamine D_3-receptor antagonists: 1 *H*-Pyrimidin-2-one derivatives. Bioorg Med Chem Lett 2006;16:490–4.
70. Geneste H, Backfisch G, Braje W, et al. Synthesis and SAR of highly potent and selective dopamine D3-receptor antagonists: Quinolin(di)one and benzazepin(di)one derivatives. Bioorg Med Chem Lett 2006;16:658–62.
71. Murray PJ, Harrison LA, Johnson MR, et al. A novel series of arylpiperazines with high affinity and selectivity for the dopamine D_3 receptor. Bioorg Med Chem Lett 1995;5:219–22.
72. Newman AH, Cao J, Bennett CJ, Robarge MJ, Freeman RA, Luedtke RR. N-{4-[4-(2,3-Dichlorophenyl)piperazin-1-yl]butyl, butenyl and butynyl}arylcarboxamides as novel dopamine D_3 receptor antagonists. Bioorg Med Chem Lett 2003;13:2179–83.
73. Grundt P, Prevatt KM, Cao J, et al. Heterocyclic analogues of N-(4-(4-(2,3-dichlorophenyl)piperazin-1-yl)butyl)arylcarboxamides with functionalized linking chains as novel dopamine D3 receptor ligands: potential substance abuse therapeutic agents. J Med Chem 2007;50:4135–46.
74. Leopoldo M, Enza L, Colabufo NA, Berardi F, Perrone R. Synthesis and binding profile of constrained analogues of *N*-[4-(4-arylpiperazin-1-yl)butyl]-3-methoxybenzamides, a class of potent dopamine D_3 receptor ligands. J Pharm Pharmacol 2006;58:209–18.
75. Ding K, Chen J, Ji M, et al. Enantiomerically pure hexahydropyrazinoquinolines as potent and selective dopamine 3 subtype receptor ligands. J Med Chem 2005;48:3171–81.
76. Hackling A, Ghosh R, Perachon S, et al. N-(ω-(4-(Methoxyphenyl)piperazin-1-yl)alkyl)carboxamides as dopamine D_2 and D_3 receptor ligands. J Med Chem 2003;46: 3883–99.
77. Grundt P, Carlson EE, Cao J, et al. Novel heterocyclic trans olefin analogues of N-{4-[4-(2,3-dichlorophenyl)piperazin-1-yl]butyl}arylcarboxamides as selective probes with high affinity for the dopamine D3 receptor. J Med Chem 2005;48:839–48.
78. Pilla M, Perachon S, Sautel F, et al. Selective inhibition of cocaine-seeking behavior by a partial dopamine D_3 receptor agonist. Nature 1999;400:371–5.
79. Yuan J, Chen X, Brodbeck R, et al. NGB 2904 and NGB 2849: two highly selective dopamine D_3 receptor antagonists. Bioorg Med Chem Lett 1998;8:2715–8.
80. Boeckler F, Gmeiner P. Dopamine D3 receptor ligands – recent advances in the control of subtype selectivity and intrinsic activity. Biochim Biophys Acta 2007;1768:871–87.
81. Chu W, Tu Z, McElveen E, et al. Synthesis and in vitro binding of *N*-phenyl piperazine analogs as potential dopamine D_3 receptor ligands. Bioorg Med Chem 2005;13:77–87.

82. Dutta AK, Venkataraman SK, Fei X-S, Kolhatkar R, Zhang S, Reith MEA. Synthesis and biological characterization of novel hybrid 7-{[2-(4-phenyl-piperazin-1-yl)-ethyl]-propyl-amino}-5,6,7,8-tetrahydronaphthalen-2-ol and their heterocyclic bioisosteric analogues for dopamine D2 and D3 receptors. Bioorg Med Chem 2004;12:4361–73.
83. Dutta AK, Fei X-S, Reith MEA. A novel series of hybrid compounds derived by combining 2-aminotetralin and piperazine fragments: Binding activity at D_2 and D_3 receptors. Bioorg Med Chem Lett 2002;12:619–22.
84. Bouthenet ML, Souil E, Martres MP, Sokoloff P, Giros B, Schwartz JC. Localization of dopamine D_3 receptor mRNA in the rat brain using in situ hybridization histochemistry: comparison with dopamine D_2 receptor mRNA. Brain Res 1991;564:203–19.
85. Diaz J, Levesque D, Lammers CH, et al. Phenotypical characterization of neurons expressing the dopamine D3 receptor in the rat brain. Neuroscience 1995;65:731–45.
86. Landwehrmeyer B, Mengod G, Palacios JM. Differential visualization of dopamine D2 and D3 receptor sites in rat brain. A comparative study using in situ hybridization histochemistry and ligand binding autoradiography. Eur J Neurosci 1993;5:145–53.
87. Levesque D, Diaz J, Pilon C, et al. Identification, characterization, and localization of the dopamine D3 receptor in rat brain using 7-[^{3}H]hydroxy-N,N-di-n-propyl-2-aminotetralin. Proc Natl Acad Sci U S A 1992;89:8155–9.
88. Akunne HC, Towers P, Ellis GJ, et al. Characterization of binding of [^{3}H]PD 128907, a selective dopamine D_3 receptor agonist ligand, to CHO-K1 cells. Life Sci 1995;57:1401–10.
89. Hall H, Halldin C, Dijkstra D, et al. Autoradiographic localisation of D_3-dopamine receptors in the human brain using the selective D_3-dopamine receptor agonist (+)-[^{3}H]PD 128907. Psychopharmacology (Berl) 1996;128:240.
90. Pugsley TA, Davis MD, Akunne HC, et al. Neurochemical and functional characterization of the preferentially selective dopamine D3 agonist PD 128907. J Pharmacol Exp Ther 1995;275:1355–66.
91. Langer O, Nagren K, Dollé F, et al. Precursor synthesis and radiolabelling of the dopamine D_2 receptor ligand [^{11}C]raclopride from [^{11}C]methyl triflate. J Label Compds Radiopharm 1999;42:1183–93.
92. Mukherjee J, Christian BT, Dunigan KA, et al. Brain imaging of ^{18}F-fallypride in normal volunteers: blood analysis, distribution, test-retest studies, and preliminary assessment of sensitivity to aging effects on dopamine D-2/D-3 receptors. Synapse 2002;46:170–88.
93. Mukherjee J, Shi B, Christian BT, Chattopadhyay S, Narayanan TK. ^{11}C-Fallypride: radiosynthesis and preliminary evaluation of a novel dopamine D_2/D_3 receptor PET radiotracer in non-human primate brain. Bioorg Med Chem 2004;12:95–102.
94. Stark D, Piel M, Hübner H, Gmeiner P, Grunder G, Rösch F. In vitro affinities of various halogenated benzamide derivatives as potential radioligands for non-invasive quantification of D2-like dopamine receptors. Bioorg Med Chem 2007;15:6819–29.
95. de Vries EF, Kortekaas R, van Waarde A, Dijkstra D, Elsinga PH, Vaalburg W. Synthesis and evaluation of dopamine D3 receptor antagonist ^{11}C-GR218231 as PET tracer for P-glycoprotein. J Nucl Med 2005;46:1384–92.
96. Langer O, Gulyas B, Sandell J, et al. Radiochemical labelling of the dopamine D_3 receptor ligand RGH-1756. J Label Compds Radiopharm 2000;43:1069–74.
97. Sovago J, Farde L, Halldin C, et al. Positron emission tomographic evaluation of the putative dopamine-D3 receptor ligand, [^{11}C]RGH-1756 in the monkey brain. Neurochem Int 2004;45:609–17.
98. Hocke C, Prante O, Löber S, Hübner H, Gmeiner P, Kuwert T. Synthesis and radioiodination of selective ligands for the dopamine D_3 receptor subtype. Bioorg Med Chem Lett 2004;14:3963–6.
99. Kuhnast B, Valette H, Besret L, et al. Synthesis and radiolabeling of N-[4-[4-(2-[^{11}C]methoxyphenyl)piperazin-1-yl]butyl]benzo[b]thiophene-2-car boxamide – a potential radiotracer for D_3 receptor imaging with PET. Nucl Med Biol 2006;33:785–95.

100. Hocke C, Prante O, Salama I, et al. ^{18}F-Labeled FAUC 346 and BP 897 derivatives as subtype-selective potential PET radioligands for the dopamine D_3 receptor. Chem Med Chem 2008;3:788–93.
101. Salama I, Hocke C, Utz W, et al. Structure-selectivity investigations of D2-like receptor ligands by CoMFA and CoMSIA guiding the discovery of D_3 selective PET radioligands. J Med Chem 2007;50:489–500.
102. Seeman P. Dopamine receptor sequences. Therapeutic levels of neuroleptics occupy D2 receptors, clozapine occupies D4. Neuropsychopharmacology 1992;7:261–84.
103. Van Tol HH, Bunzow JR, Guan HC, et al. Cloning of the gene for a human dopamine D4 receptor with high affinity for the antipsychotic clozapine. Nature 1991;350:610–4.
104. Elsinga PH, Hatano K, Ishiwata K. PET tracers for imaging of the dopaminergic system. Curr Med Chem 2006;13:2139–53.
105. Enguehard-Gueiffier C, Gueiffier A. Recent progress in medicinal chemistry of D4 agonists. Curr Med Chem 2006;13:2981–93.
106. Zhang A, Neumeyer JL, Baldessarini RJ. Recent progress in development of dopamine receptor subtype-selective agents: potential therapeutics for neurological and psychiatric disorders. Chem Rev 2007;107:274–302.
107. Boström J, Bohm M, Gundertofte K, Klebe G. A 3D QSAR study on a set of dopamine D4 receptor antagonists. J Chem Inf Comput Sci 2003;43:1020–7.
108. Boström J, Gundertofte K, Liljefors T. A pharmacophore model for dopamine D4 receptor antagonists. J Comput Aided Mol Des 2000;14:769–86.
109. Lanig H, Utz W, Gmeiner P. Comparative molecular field analysis of dopamine D_4 receptor antagonists including 3- 4-(4-chlorophenyl)piperazin-1-ylmethyl pyrazolo[1,5-*a*]pyridine (FAUC 113), 3- 4-(4-chlorophenyl)piperazin-1-ylmethyl-1H-pyrrolo[2,3-*b*]pyridine (L-745,870), and clozapine. J Med Chem 2001;44:1151–7.
110. Glase SA, Akunne HC, Georgic LM, et al. Substituted [(4-phenylpiperazinyl)-methyl]benzamides: selective dopamine D4 agonists. J Med Chem 1997;40:1771–2.
111. Hsieh GC, Hollingsworth PR, Martino B, et al. Central mechanisms regulating penile erection in conscious rats: the dopaminergic systems related to the proerectile effect of apomorphine. J Pharmacol Exp Ther 2004;308:330–8.
112. Bernaerts P, Tirelli E. Facilitatory effect of the dopamine D_4 receptor agonist PD168,077 on memory consolidation of an inhibitory avoidance learned response in C57BL/6 J mice. Behav Brain Res 2003;142:41–52.
113. Clifford JJ, Waddington JL. Topographically based search for an "Ethogram" among a series of novel D_4 dopamine receptor agonists and antagonists. Neuropsychopharmacology 2000;22:538–44.
114. Melis MR, Succu S, Mascia MS, Argiolas A. PD-168077, a selective dopamine D_4 receptor agonist, induces penile erection when injected into the paraventricular nucleus of male rats. Neurosci Lett 2005;379:59–62.
115. Matulenko MA, Hakeem AA, Kolasa T, et al. Synthesis and functional activity of (2-aryl-1-piperazinyl)-N-(3-methylphenyl)acetamides: selective dopamine D4 receptor agonists. Bioorg Med Chem 2004;12:3471–83.
116. Kolasa T, Matulenko MA, Hakeem AA, et al. 1-aryl-3-(4-pyridine-2-ylpiperazin-1-yl)propan-1-one oximes as potent dopamine D4 receptor agonists for the treatment of erectile dysfunction. J Med Chem 2006;49:5093–109.
117. Zorn SH, Jackson E, Johnson C, Lewis J, Fliri A. CP-226,269 is a selective dopamine D_4 receptor agonist. Soc Neurosci Abstr 1997;23:685.
118. Moreland RB, Patel M, Hsieh GC, Wetter JM, Marsh K, Brioni JD. A-412997 is a selective dopamine D4 receptor agonist in rats. Pharmacol Biochem Behav 2005;82:140–7.
119. Stewart AO, Cowart MD, Moreland RB, et al. Dopamine D4 ligands and models of receptor activation: 2-(4-pyridin-2-ylpiperazin-1-ylmethyl)-1H-benzimidazole and related heteroarylmethylarylpiperazines exhibit a substituent effect responsible for additional efficacy tuning. J Med Chem 2004;47:2348–55.

120. Cowart M, Latshaw SP, Bhatia P, et al. Discovery of 2-(4-pyridin-2-ylpiperazin-1-ylmethyl)-1H-benzimidazole (ABT-724), a dopaminergic agent with a novel mode of action for the potential treatment of erectile dysfunction. J Med Chem 2004;47:3853–64.
121. Brioni JD, Moreland RB, Cowart M, et al. Activation of dopamine D_4 receptors by ABT-724 induces penile erection in rats. Proc Natl Acad Sci U S A 2004;101:6758–63.
122. Patel MV, Kolasa T, Mortell K, et al. Discovery of 3-methyl-N-(1-oxy-3′,4′,5′,6′-tetrahydro-2′H-[2,4′-bipyridine]-1′-ylmethyl)benzamide (ABT-670), an orally bioavailable dopamine D4 agonist for the treatment of erectile dysfunction. J Med Chem 2006;49:7450–65.
123. Browman KE, Curzon P, Pan JB, et al. A-412997, a selective dopamine D4 agonist, improves cognitive performance in rats. Pharmacol Biochem Behav 2005;82:148–55.
124. Hübner H, Kraxner J, Gmeiner P. Cyanoindole derivatives as highly selective dopamine D_4 receptor partial agonists: Solid-phase synthesis, binding assays, and functional experiments. J Med Chem 2000;43:4563–9.
125. Löber S, Aboul-Fadl T, Hübner H, Gmeiner P. Di- and trisubstituted pyrazolo[1,5-*a*]pyridine derivatives: Synthesis, dopamine receptor binding and ligand efficacy. Bioorg Med Chem Lett 2002;12:633–6.
126. Löber S, Hübner H, Gmeiner P. Azaindole derivatives with high affinity for the dopamine D_4 receptor: synthesis, ligand binding studies and comparison of molecular electrostatic potential maps. Bioorg Med Chem Lett 1999;9:97–102.
127. Moll A, Hubner H, Gmeiner P, Troschutz R. Phenylpiperazinylmethylindolecarboxylates and derivatives as selective D_4-ligands. Bioorg Med Chem 2002;10:1671–9.
128. Einsiedel J, Hübner H, Gmeiner P. Benzamide bioisosteres incorporating dihydroheteroazole substructures: EPC synthesis and SAR leading to a selective dopamine D4 receptor partial agonist (FAUC 179). Bioorg Med Chem Lett 2001;11:2533–6.
129. Bergauer M, Hubner H, Gmeiner P. 2,4-Disubstituted pyrroles: synthesis, traceless linking and pharmacological investigations leading to the dopamine D_4 receptor partial agonist FAUC 356. Bioorg Med Chem Lett 2002;12:1937–40.
130. Einsiedel J, Hübner H, Gmeiner P. Cyclic amidines as benzamide bioisosteres: EPC synthesis and SAR studies leading to the selective dopamine D4 receptor agonist FAUC 312. Bioorg Med Chem Lett 2003;13:851–4.
131. Prante O, Löber S, Hübner H, Gmeiner P, Kuwert T. Synthesis and in vitro evaluation of iodine labelled pyrazolo[1,5-*a*]pyridines as highly selective dopamine D_4 receptor ligands. J Label Compds Radiopharm 2001;44:849–58.
132. Löber S, Ortner B, Bettinetti L, Hübner H, Gmeiner P. Analogs of the dopamine D_4 receptor ligand FAUC 113 with planar- and central-chirality. Tetrahedron Asymmetry 2002;13:2303–10.
133. Enguehard-Gueiffier C, Hübner H, El Hakmaoui A, et al. 2-[(4-Phenylpiperazin-1-yl)methyl]imidazo(di)azines as selective D_4-ligands. Induction of penile erection by 2-[4-(2-methoxyphenyl)piperazin-1-ylmethyl]imidazo[1,2-*a*]pyridine (PIP3EA), a potent and selective D_4 partial agonist. J Med Chem 2006;49:3938–47.
134. Melis MR, Succu S, Sanna F, et al. PIP3EA and PD-168077, two selective dopamine D4 receptor agonists, induce penile erection in male rats: site and mechanism of action in the brain. Eur J Neurosci 2006;24:2021–30.
135. Succu S, Sanna F, Melis T, Boi A, Argiolas A, Melis MR. Stimulation of dopamine receptors in the paraventricular nucleus of the hypothalamus of male rats induces penile erection and increases extra-cellular dopamine in the nucleus accumbens: involvement of central oxytocin. Neuropharmacology 2007;52:1034–43.
136. Wang X, Bhatia PA, Daanen JF, et al. Synthesis and evaluation of 3-aryl piperidine analogs as potent and efficacious dopamine D_4 receptor agonists. Bioorg Med Chem 2005;13:4667–78.
137. Powell SB, Paulus MP, Hartman DS, Godel T, Geyer MA. RO-10-5824 is a selective dopamine D_4 receptor agonist that increases novel object exploration in C57 mice. Neuropharmacology 2003;44:473–81.

138. Rostovtsev VV, Green LG, Fokin VV, Sharpless KB. A stepwise Huisgen cycloaddition process: Copper(I)-catalyzed regioselective "ligation" of azides and terminal alkynes. Angew Chem Int Ed Engl 2002;41:2596–9.
139. Löber S, Hübner H, Gmeiner P. Synthesis and biological investigations of dopaminergic partial agonists preferentially recognizing the D_4 receptor subtype. Bioorg Med Chem Lett 2006;16:2955–9.
140. Gazi L, Bobirnac I, Danzeisen M, et al. The agonist activities of the putative antipsychotic agents, L-745,870 and U-101958 in HEK293 cells expressing the human dopamine $D_{4.4}$ receptor. Br J Pharmacol 1998;124:889–96.
141. Gazi L, Sommer B, Nozulak J, Schoeffter P. NGD 94-1 as an agonist at human recombinant dopamine $D_{4.4}$ receptors expressed in HEK293 cells. Eur J Pharmacol 1999;372:R9–10.
142. Kulagowski JJ, Broughton HB, Curtis NR, et al. 3-((4-(4-Chlorophenyl)piperazin-1-yl)-methyl)-1H-pyrrolo[2,3-*b*]pyridine: an antagonist with high affinity and selectivity for the human dopamine D_4 receptor. J Med Chem 1996;39:1941–2.
143. Patel S, Freedman S, Chapman KL, et al. Biological profile of L-745,870, a selective antagonist with high affinity for the dopamine D4 receptor. J Pharmacol Exp Ther 1997;283:636–47.
144. Schlachter SK, Poel TJ, Lawson CF, et al. Substituted 4-aminopiperidines having high in vitro affinity and selectivity for the cloned human dopamine D4 receptor. Eur J Pharmacol 1997;322:283–6.
145. Tallman JF, Primus RJ, Brodbeck R, et al. NGD 94-1: identification of a novel, high-affinity antagonist at the human dopamine D4 receptor. J Pharmacol Exp Ther 1997;282: 1011–9.
146. Gazi L, Bobirnac I, Danzeisen M, et al. Receptor density as a factor governing the efficacy of the dopamine D_4 receptor ligands, L-745,870 and U-101958 at human recombinant $D_{4.4}$ receptors expressed in CHO cells. Br J Pharmacol 1999;128:613–20.
147. Seeman P, Guan HC, Van Tol HH. Dopamine D4 receptors elevated in schizophrenia. Nature 1993;365:441–5.
148. Bristow LJ, Collinson N, Cook GP, et al. L-745,870, a subtype selective dopamine D4 receptor antagonist, does not exhibit a neuroleptic-like profile in rodent behavioral tests. J Pharmacol Exp Ther 1997;283:1256–63.
149. Bristow LJ, Kramer MS, Kulagowski J, Patel S, Ragan CI, Seabrook GR. Schizophrenia and L-745,870, a novel dopamine D4 receptor antagonist. Trends Pharmacol Sci 1997;18:186–8.
150. Löber S, Hübner H, Utz W, Gmeiner P. Rationally based efficacy tuning of selective dopamine D_4 receptor ligands leading to the complete antagonist 2-[4-(4-chlorophenyl)piperazin-1-yl]methylpyrazolo[1,5-*a*]pyridine (FAUC 213). J Med Chem 2001;44:2691–4.
151. Boeckler F, Russig H, Zhang W, et al. FAUC 213, a highly selective dopamine D4 receptor full antagonist, exhibits atypical antipsychotic properties in behavioural and neurochemical models of schizophrenia. Psychopharmacology (Berl) 2004;175:7–17.
152. Huang Y, Kegeles LS, Bae S, et al. Synthesis of potent and selective dopamine D_4 antagonists as candidate radioligands. Bioorg Med Chem Lett 2001;11:1375–7.
153. Sanner MA, Chappie TA, Dunaiskis AR, et al. Synthesis, sar and pharmacology of CP-293,019: a potent, selective dopamine D_4 receptor antagonist. Bioorg Med Chem Lett 1998;8:725–30.
154. Merchant KM, Gill GS, Harris DW, et al. Pharmacological characterization of U-101387, a dopamine D4 receptor selective antagonist. J Pharmacol Exp Ther 1996;279:1392–403.
155. TenBrink RE, Bergh CL, Duncan JN, et al. (S)-(-)-4-[4-[2-(isochroman-1-yl)ethyl]-piperazin-1-yl] benzenesulfonamide, a selective dopamine D4 antagonist. J Med Chem 1996;39:2435–7.
156. Corrigan MH, Gallen CC, Bonura ML, Merchant KM. Effectiveness of the selective D4 antagonist sonepiprazole in schizophrenia: a placebo-controlled trial. Biol Psychiatry 2004;55:445–51.

157. Wedemeyer C, Goutman JD, Avale ME, Franchini LF, Rubinstein M, Calvo DJ. Functional activation by central monoamines of human dopamine D_4 receptor polymorphic variants coupled to GIRK channels in Xenopus oocytes. Eur J Pharmacol 2007;562:165–73.
158. Thurkauf A, Chen X, Zhang S, et al. 1H-Pyrazolo-[3,4-c]cyclophepta[1,2-*c*]thiophenes: a unique structural class of dopamine D_4 selective ligands. Bioorg Med Chem Lett 2003;13:2921–4.
159. Nakane M, Cowart MD, Hsieh GC, et al. 2-[4-(3,4-Dimethylphenyl)piperazin-1-ylmethyl]-1H benzoimidazole (A-381393), a selective dopamine D_4 receptor antagonist. Neuropharmacology 2005;49:112–21.
160. Bitner RS, Nikkel AL, Otte S, et al. Dopamine D_4 receptor signaling in the rat paraventricular hypothalamic nucleus: evidence of natural coupling involving immediate early gene induction and mitogen activated protein kinase phosphorylation. Neuropharmacology 2006;50:521–31.
161. Audouze K, Nielsen EO, Peters D. New series of morpholine and 1,4-oxazepane derivatives as dopamine D_4 receptor ligands: synthesis and 3D-QSAR model. J Med Chem 2004;47:3089–104.
162. Oh SJ, Lee KC, Lee SY, et al. Synthesis and evaluation of fluorine-substituted 1H-pyrrolo[2,3-*b*]pyridine derivatives for dopamine D4 receptor imaging. Bioorg Med Chem 2004;12:5505–13.
163. Egle I, Barriault N, Bordeleau M, et al. N-(1-Benzylpyrrolidin-3-yl)arylbenzamides as potent and selective human dopamine D4 antagonists. Bioorg Med Chem Lett 2004;14:4847–50.
164. Heindl C, Hübner H, Gmeiner P. Ex-chiral pool synthesis and receptor binding studies of 4-substituted prolinol derivatives. Tetrahedron Asymmetry 2003;14:3141–52.
165. Nishimura T, Igarashi J, Sunagawa M. Conformational analysis of tandospirone in aqueous solution: lead evolution of potent dopamine D_4 receptor ligands. Bioorg Med Chem Lett 2001;11:1141–4.
166. Awadallah FM, Muller F, Lehmann J, Abadi AH. Synthesis of novel lactam derivatives and their evaluation as ligands for the dopamine receptors, leading to a D_4-selective ligand. Bioorg Med Chem 2007;15:5811–8.
167. Defagot MC, Antonelli MC. Autoradiographic localization of the putative D4 dopamine receptor in rat brain. Neurochem Res 1997;22:401–7.
168. Lahti RA, Roberts RC, Tamminga CA. D2-family receptor distribution in human postmortem tissue: an autoradiographic study. Neuroreport 1995;6:2505–12.
169. Murray AM, Hyde TM, Knable MB, et al. Distribution of putative D_4 dopamine receptors in postmortem striatum from patients with schizophrenia. J Neurosci 1995;15:2186–91.
170. Defagot MC, Falzone TL, Low MJ, Grandy DK, Rubinstein M, Antonelli MC. Quantitative analysis of the dopamine D4 receptor in the mouse brain. J Neurosci Res 2000;59:202–8.
171. De La Garza R, Madras BK. [^{3}H]PNU-101958, a D_4 dopamine receptor probe, accumulates in prefrontal cortex and hippocampus of non-human primate brain. Synapse 2000;37:232–44.
172. Primus RJ, Thurkauf A, Xu J, et al. II. Localization and characterization of dopamine D4 binding sites in rat and human brain by use of the novel, D_4 receptor-selective ligand [3H]NGD 94-1. J Pharmacol Exp Ther 1997;282:1020–7.
173. Matulenko MA, Surber B, Fan L, et al. Synthesis and activity of 2-[4-(4-[^{3}H]-2-cyanophenyl)piperazinyl]-N-(2,4,6-[^{3}H]3-3-methylphenyl)acetamide: a selective dopamine D_4 receptor agonist and radioligand. Bioorg Med Chem Lett 2004;14:5095–8.
174. Moreland RB, Terranova MA, Chang R, et al. [^{3}H] A-369508 ([2-[4-(2-cyanophenyl)-1-piperazinyl]-N-(3-methylphenyl) acetamide): an agonist radioligand selective for the dopamine D_4 receptor. Eur J Pharmacol 2004;497:147–54.
175. Farde L, Wiesel FA, Hall H, Halldin C, Stone-Elander S, Sedvall G. No D_2 receptor increase in PET study of schizophrenia. Arch Gen Psychiatry 1987;44:671–2.

176. Wong DF, Wagner HN, Jr., Tune LE, et al. Positron emission tomography reveals elevated D_2 dopamine receptors in drug-naive schizophrenics. Science 1986;234:1558–63.
177. Bender D, Holschbach M, Stöcklin G. Synthesis of n.c.a. carbon-11 labelled clozapine and its major metabolite clozapine-N-oxide and comparison of their biodistribution in mice. Nucl Med Biol 1994;21:921–5.
178. Boy C, Klimke A, Holschbach M, et al. Imaging dopamine D_4 receptors in the living primate brain: a positron emission tomography study using the novel D_1/D_4 antagonist C-11 SDZ GLC 756. Synapse 1998;30:341–50.
179. Hidaka K, Tada S, Matsumoto M, Ohmori J, Maeno K, Yamaguchi T. YM-50001: a novel, potent and selective dopamine D_4 receptor antagonist. Neuroreport 1996;7:2543–6.
180. Perrone R, Berardi F, Colabufo NA, Leopoldo M, Tortorella V. N-[2-[4-(4-Chlorophenyl)piperazin-1-yl]ethyl]-3-methoxybenzamide: a potent and selective dopamine D_4 ligand. J Med Chem 1998;41:4903–9.
181. Langer O, Halldin C, Chou YH, et al. Carbon-11 PB-12: An attempt to visualize the dopamine D_4 receptor in the primate brain with positron emission tomography. Nucl Med Biol 2000;27:707–14.
182. Zhang MR, Haradahira T, Maeda J, et al. Syntheses and pharmacological evaluation of two potent antagonists for dopamine D_4 receptors: [^{11}C]YM-50001 and N-[2-[4-(4-chlorophenyl)-piperizin-1-yl]ethyl]-3-[^{11}C]methoxybenzamide. Nucl Med Biol 2002;29:233–41.
183. Unangst PC, Capiris T, Connor DT, et al. Chromeno[3,4-*c*]pyridin-5-ones: selective human dopamine D_4 receptor antagonists as potential antipsychotic agents. J Med Chem 1997;40:2688–93.
184. Zhang M-R, Haradahira T, Maeda J, et al. Synthesis and evaluation of 3-(4-chlorobenzyl)-8-[^{11}C]methoxy-1,2,3,4-tetrahydrochromeno[3,4-*c*]pyridin-5-one: a PET tracer for imaging sigma1 receptors. Nucl Med Biol 2002;29:469–76.
185. Tian H-B, Yin D-Z, Zhang L, et al. Dopamine D4 receptor antagonist 3-(4-[^{18}F]fluorobenzyl)-8-methoxy-1,2,3,4-tetrahydrochromeno[3,4-*c*]pyridin-5-one([^{18}F]FMTP): Radiosynthesis and in vivo characterization in rats. Appl Radiat Isot 2005;63:333–42.
186. Li G-C, Yin D-Z, Wang M-W, Cheng D-F, Wang Y-X. Syntheses of two potential dopamine D_4 receptor radioligands: ^{18}F-labeled chromeno[3,4-*c*]pyridin-5-ones. Radiochim Acta 2006;94:119–22.
187. Eskola O, Bergman J, Lehikoinen P, et al. Synthesis of 3-[[4-(4-[^{18}F]fluorophenyl)piperazin-1-yl]methyl]-1H-pyrrolo[2,3-*b*]pyridine. J Label Compds Radiopharm 2002;45:687–96.
188. Kung M-P, Stevenson DA, Zhuang Z-P, et al. Characterization of a novel iodinated ligand, IPMPP, for human dopamine D4 receptors expressed in CHO cells. Life Sci 1996;60:91–100.
189. Patel S, Patel S, Marwood R, et al. Identification and pharmacological characterization of [^{125}I]L750,667, a novel radioligand for the dopamine D_4 receptor. Mol Pharmacol 1996;50:1658–64.
190. Staley JK, Tamagnan G, Baldwin RM, et al. SPECT imaging with the D-4 receptor antagonist L-750,667 in nonhuman primate brain. Nucl Med Biol 2000;27:547–56.
191. Boyfield I, Brown TH, Coldwell MC, et al. Design and synthesis of 2-naphthoate esters as selective dopamine D_4 antagonists. J Med Chem 1996;39:1946–8.
192. Matarrese M, Soloviev D, Moresco RM, et al. Synthesis and in vivo evaluation of 3-[^{11}C]methyl-(3-methoxy-naphthalen)-2-yl-(1-benzyl-piperidin)-4-yl-acetate (SB-235753), as a putative dopamine D_4 receptors antagonist for PET. J Label Compds Radiopharm 2000;43:359–74.
193. Prante O, Hocke C, Löber S, Hübner H, Gmeiner P, Kuwert T. Tissue distribution of radioiodinated FAUC113 – Assessment of a pyrazolo [1,5-*a*] pyridine based dopamine D4 receptor radioligand candidate. Nuklearmed-Nucl Med 2006;45:41–8.

194. Tietze R, Hocke C, Löber S, et al. Syntheses and radiofluorination of two derivatives of 5-cyano-indole as selective ligands for the dopamine subtype-4 receptor. J Label Compds Radiopharm 2006;49:55–70.
195. Tietze R, Löber S, Hübner H, Gmeiner P, Kuwert T, Prante O. Discovery of a dopamine D_4 selective PET ligand candidate taking advantage of a click chemistry based REM linker. Bioorg Med Chem Lett 2008;18:982–7.
196. Prante O, Tietze R, Hocke C, et al. Synthesis, radiofluorination, and in vitro evaluation of pyrazolo[1,5-*a*]pyridine-based dopamine D_4 receptor ligands: Discovery of an inverse agonist radioligand for PET. J Med Chem 2008;51:1800–10.
197. Tietze R, Hocke C, Löber S, et al. In-vitro characterization, ^{18}F-synthesis and biodistribution of a pyrazolo[1,5-*a*]pyridine based dopamine D_4 receptor radioligand candidate. J Label Compds Radiopharm 2007;50:S74.

Chapter 6
Dopamine Receptor Signaling: Intracellular Pathways to Behavior

Robert J. Romanelli, John T. Williams, and Kim A. Neve

Abstract Dopamine receptors belong to the large family of heptahelical transmembrane spanning G protein-coupled receptors (GPCRs). Five mammalian dopamine receptor subtypes have been identified and are classified into two major groups, the D1-like (D_1 and D_5) and D2-like (D_2, D_3, and D_4) receptors. Two splice variants of the D_2 receptor exist, $D_{2\text{-Long}}$ (D_{2L}) and $D_{2\text{-Short}}$ (D_{2S}), which differ by an insertion of 29 amino acids in the third intracellular loop of D_{2L}. In this chapter, we discuss canonical and non-canonical signaling pathways regulated by individual dopamine receptor subtypes and the contribution of these pathways to dopamine-induced behaviors. Particular focus is given to the behavioral effects of drugs of abuse, including the psychostimulants cocaine, amphetamine, and methamphetamine.

Keywords Dopamine · Dopamine receptor · Adenylate cyclase · G protein · Arrestin · Akt · Phospholipase C · Protein kinase A · Cyclic AMP

6.1 Dopamine Receptor Overview

6.1.1 Introduction

Dopamine receptors belong to the large family of heptahelical transmembrane spanning G protein-coupled receptors (GPCRs). Five mammalian dopamine receptor subtypes have been identified and are classified into two major groups, the D1- (D_1 and D_5) and D2- (D_2, D_3, D_4) like receptors. Two splice variants of the D_2 receptor exist, $D_{2\text{-Long}}$ (D_{2L}) and $D_{2\text{-Short}}$ (D_{2S}), which differ by an insertion of 29 amino acids in the third intracellular loop of D_{2L} [1, 2].

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Although dopamine receptors are similar in structure, receptor subtypes differ by their affinity for dopamine and coupling to downstream effectors, including heterotrimeric guanine nucleotide-binding proteins (G proteins) [3]. These factors, in addition to the differential distribution of individual receptor subtypes, contribute to the complexity of dopaminergic signaling in the brain.

Signaling through dopamine receptors regulates neural processes such as motor activity, motivation and reward (including drug-seeking behavior), and higher cognition (including working memory) [4]. Dysfunction of dopamine signaling contributes to the pathophysiology of various neurological and psychiatric illnesses, including Parkinson's disease, Tourette's syndrome, schizophrenia, mood disorders, and drug addiction. The signaling pathways through which dopamine receptors mediate such behaviors in normal and disease states remain enigmatic. Our challenge is to understand how dopamine-regulated signaling pathways are integrated downstream of the multiple receptor subtypes to produce diverse behavioral outcomes.

In this chapter, we discuss the signaling pathways regulated by individual dopamine receptor subtypes and the contribution of these pathways to dopamine-induced behaviors. Particular focus is given to the behavioral effects of drugs of abuse, including the psychostimulants cocaine, amphetamine, and methamphetamine, which act as non-selective, indirect agonists of dopamine receptors by elevating extracellular levels of dopamine. These drugs have been well studied in rodent models of addiction, in which endogenous dopamine receptors can be modified genetically or pharmacologically. Accordingly, these studies provide a valuable tool for parsing the role of dopamine receptor signaling pathways and their behavioral correlates.

6.1.2 Expression

Dopamine receptor subtypes are expressed differentially throughout the brain. Of the D1-like receptors, D_1 receptors are the most abundant, with mRNA transcripts found in the neostriatum, nucleus accumbens, and olfactory tubercle. Lower levels of D_1 receptor mRNA are found in the cerebral cortex, hypothalamus, and thalamus [5]. D_5 receptor mRNA is found mainly in the hippocampus and hypothalamus [6].

Of the D2-like receptors, D_2 receptors are the most abundant, with mRNA transcripts found in the neostriatum, nucleus accumbens, and olfactory tubercle, as well as the midbrain, including the substantia nigra and ventral tegmental area [5]. D_2 mRNA transcripts also are found in the pituitary [7]. Khan and colleagues, using specific antibodies directed against the D_2 splice variants, report that D_{2S} is located predominantly in cell bodies and axons of dopaminergic neurons of the primate midbrain, whereas D_{2L} is more strongly expressed by neurons of the striatum and nucleus accumbens that are targeted by dopaminergic neurons [8]. Accordingly, in the primate brain, D_{2S} and D_{2L} are primarily localized to pre- and postsynaptic

membranes, respectively. The physiological role for pre- or postsynaptic dopamine receptors is discussed at length later in Section 6.7.

D_3 and D_4 receptors are less abundant and less widely distributed compared to D_2 receptors. D_3 receptor mRNA transcripts are found in the nucleus accumbens, olfactory tubercle, and hippocampus [9]. Lower levels of D_3 receptor mRNA are found in the hypothalamus, neostriatum, and midbrain. Finally, D_4 receptor mRNA is found primarily in areas of the cerebral cortex, hippocampus, cerebellum, and amygdala [10].

6.2 Dopamine Receptor Coupling to G Proteins

Dopamine receptor signaling is mediated chiefly through heterotrimic G proteins, which are comprised of an α, β, and γ subunit complex [11]. The G protein α subunit (Gα) binds guanine nucleotides and cycles between an *inactive* GDP-bound state and an *active* GTP-bound state [12]. Upon agonist binding, receptors undergo a conformation change that activates Gα through the exchange of GDP for GTP, resulting in the dissociation of the constitutive dimeric G protein β and γ subunits (Gβγ). Gα-GTP and Gβγ directly modulate downstream targets of dopamine receptors, including second messengers and ion channels [13]. Finally, G protein activity is terminated by GTP hydrolysis and the re-formation of the G protein heterotrimer [12]. It is important to keep in mind that, although we speak of "dissociation" and "re-formation" of the heterotrimer to describe what is happening functionally, the extent to which there is physical dissociation, as opposed to protein conformational changes within a stable multi-protein signalplex, is still being determined [14, 15].

A distinguishing feature of D1- and D2-like receptors is their differential coupling to heterotrimeric G proteins. In many cell types D1-like receptors couple to $G\alpha_s$, which activates adenylate cyclase [16, 17]; however, recent evidence suggests that $G\alpha_{olf}$, a G protein originally identified as a mediator of olfaction, also couples to D1-like receptors for the stimulation of adenylate cyclase. In the rodent neostriatum, where expression of $G\alpha_{olf}$ is abundant and $G\alpha_s$ is relatively low [18], D_1 receptor activation of adenylate cyclase is mediated by $G\alpha_{olf}$ [19, 20]. In contrast, D2-like receptors couple to the pertussis toxin-sensitive $G\alpha_i$ or $G\alpha_o$, which inhibit adenylate cyclase [21]. Numerous studies have reported that D_2 receptors are capable of coupling to and activating multiple pertussis toxin-sensitive G proteins, and it seems likely that different G protein subtypes mediate distinct signaling responses [22, 23], but evidence from a variety of experimental approaches suggests that $G\alpha_o$ is particularly important for signaling by D_2 receptors [22–28] and by D_3 receptors [29, 30], particularly in the rodent brain [31]. Characterization of $G\alpha_z$ null mutant mice suggests that this member of the $G\alpha_i$ family is also important for D2-like receptor function [32].

The coupling of dopamine receptors to specific Gβγ proteins is less characterized. In mice lacking $G\gamma_7$, a subunit enriched in the dopamine receptor-expressing medium spiny neurons of the neostriatum [33], D1-like receptor-induced adenylate

cyclase activity is reduced in this brain region [34]. Notably, these animals also display a significant and selective reduction in $G\alpha_{olf}$ expression in the striatum. Thus, striatal D1-like receptors likely regulate adenylate cyclase through $G\alpha_{olf}/G\beta\gamma_7$. In HEK293 cells, reduced expression of $G\gamma_7$ also causes a loss of expression of $G\beta_1$; the loss of these two subunits is associated with decreased stimulation of adenylate cyclase by D_1 receptors, but not D_5 receptors [35].

In addition to the aforementioned G proteins, which regulate adenylate cyclase activity, increasing evidence suggests that dopamine receptors couple to G proteins that modulate alternative effectors. For example, D1- and D2-like receptor coupling to $G\alpha_q$, which stimulates phospholipases and the consequent hydrolysis of phosphoinositides, has been described [13]. In what follows, we discuss the coupling of dopamine receptor subtypes to G proteins in the context of specific signaling and behavioral responses.

6.3 Regulation of Adenylate Cyclase

6.3.1 D1-Like Receptor Regulation of Adenylate Cyclase

D1-like receptor stimulation of $G\alpha_s$ or $G\alpha_{olf}$ induces the activation of adenylate cyclase, an enzyme that catalyzes the conversion of ATP to cyclic AMP, and consequently the disinhibition of the catalytic subunit of cyclic AMP-dependent protein kinase (PKA). In the striatum, D1-like receptors likely activate PKA through adenylate cyclase 5, which is highly expressed in this brain region [36], since genetic ablation of this isoform almost eliminates D1-like agonist-stimulated adenylate cyclase activity [37]. PKA in turn phosphorylates multiple downstream targets, including the cyclic AMP regulatory element-binding protein (CREB), the dopamine and cyclic AMP-regulated phosphoprotein, 32 kDa (DARPP-32), and various ion channels (Fig. 6.1).

D1-like receptor-mediated phosphorylation of CREB at Ser133 by PKA leads to the translocation of CREB to the nucleus and CREB-dependent transcription of numerous genes, including the immediate early gene *c-fos* [38]. CREB-induced gene transcription is associated with synaptic plasticity and memory formation [39] and with long-term changes in gene expression and synaptic function associated with drug addiction [40].

PKA-dependent phosphorylation of DARPP-32, a striatal-enriched phosphoprotein, at Thr34 leads to DARPP-32-dependent inhibition of protein phosphatase 1 (PP1) [41, 42]. PP1 inhibition, however, is negatively regulated through cyclin-dependent kinase 5-induced phosphorylation of DARPP-32 at Thr75, resulting in DARPP-32-mediated inhibition of PKA [43]. In contrast, PP1 inhibition is augmented by PKA-dependent activation of protein phosphatase 2A, which dephosphorylates DARPP-32 at Thr75 [44]. Therefore, DARPP-32 is regulated by phosphorylation at multiple residues through a complex positive and negative feedback system. DARPP-32 also activates the transcription factor ΔFosB, which regulates

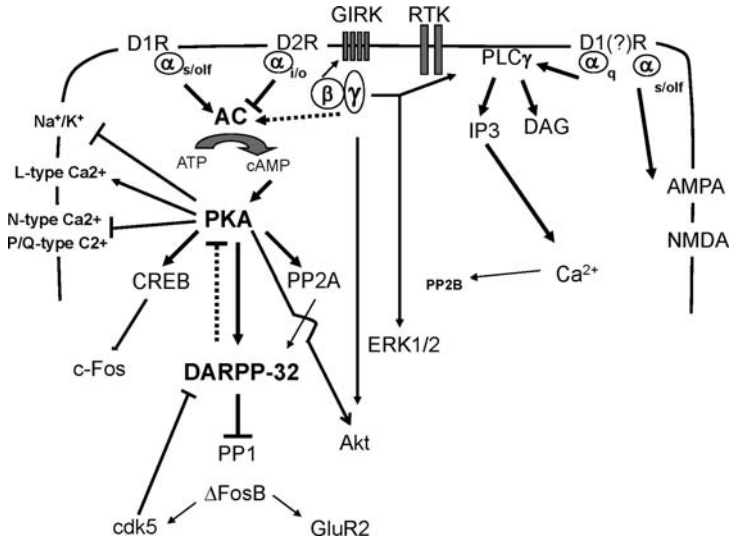

Fig. 6.1 Dopamine receptor G protein-mediated signaling. D1- and D2-like receptors activate multiple signaling pathways through G proteins, including the canonical adenylate cyclase-PKA-DARPP-32 and PLC pathways, as well as the non-canonical Akt and ERK pathways. Although not depicted in the figure, many PKA-mediated responses involve both direct PKA-catalyzed phosphorylation and inhibition of dephosphorylation via DARPP-32-mediated inhibition of PP1. The D_1 receptor, in particular, influences the localization and function of AMPA- and NMDA-type glutamate receptors by both PKA-mediated phosphorylation and protein:protein interactions. The D1-like receptor that activates PLC might be a D_1 receptor, a D_5 receptor, or a D_1/D_2 heteromer. AC, adenylate cyclase; CaMK, calcium-, calmodulin-dependent kinase; Cdk5, cyclic-dependent kinase 5; CREB, cyclic AMP response element-binding protein; DAG, diacylglycerol; GIRK, G protein-regulated inwardly rectifying potassium channel; IP3, inositol trisphosphate; PKA, cyclic AMP-dependent protein kinase; PLC, phospholipase C; PP1, 2A, 2B, protein phosphatase-1,- 2A, -2B; RTK, receptor tyrosine kinase

the expression of the GluR2 AMPA–glutamate receptor subunit, cyclin-dependent kinase 5, and dynorphin [45, 46].

Several lines of evidence suggest that the activity of DARPP-32 is regulated by drugs of abuse. For example, amphetamine increases DARPP-32 phosphorylation at Thr34 and Ser130 in the frontal cortex and neostriatum [47]. Acute methamphetamine administration also increases DARPP-32 phosphorylation at Thr34 in the nucleus accumbens, but chronic administration of methamphetamine decreases Thr34 phosphorylation [48]. DARPP-32 is required for cocaine-induced expression of ΔFosB [49], which persists for days to weeks following administration of various drugs of abuse and may serve as a molecular switch for long-term changes in gene expression associated with addiction [46, 50].

PKA directly phosphorylates voltage- and ligand-gated ion channels or indirectly promotes phosphorylation of these channels through DARPP-32-mediated inhibition of PP1 [13]. Multiple putative PKA phosphorylation sites have been identified in voltage-gated Na^+ channels [51, 52]. D1-like receptor-mediated PKA activation

decreases Na^+ and K^+ inward rectifying channels [53, 54] but increases L-type and decreases N and P/Q-type Ca^{2+} channel activity [55]. Direct interactions between the D_1 receptor and Ca^{2+} channels may also contribute to regulation of channel distribution and function [56].

Additionally, D1-like receptors modulate glutamatergic and GABAergic neurotransmission through protein–protein interactions [57–59] and via the phosphorylation of NMDA, AMPA, and GABA receptor subunits [60, 61], thereby altering receptor currents. D1-like receptor-induced phosphorylation of the GluR1 AMPA receptor subunit is observed in mice after treatment with cocaine and methamphetamine, and requires DARPP-32 [62]. The regulation of ion channels by dopamine receptors is discussed at greater length in Chapters 7, 11, and 14.

6.3.2 D2-Like Receptor Regulation of Adenylate Cyclase

D2-like receptors inhibit adenylate cyclase through coupling to the pertussis toxin-sensitive G proteins $G\alpha_i$ and $G\alpha_o$, thereby decreasing or preventing stimulation of cyclic AMP production [63–66]. Accordingly, D2-like receptors act oppositely to D1-like receptors in the regulation of substrates downstream of cyclic AMP, including PKA and DARPP-32. D_2 receptor stimulation decreases DARPP-32 phosphorylation at Thr34 and increases DARPP-32 phosphorylation at Thr75, inhibiting this phosphoprotein [44, 67]. It would be expected that D2-like receptor stimulation also decreases CREB phosphorylation. However, in sagittal brain slices (including the neocortex, neostriatum, hippocampus, thalamus, and substantia nigra) the D2-like agonist quinpirole stimulates CREB phosphorylation, presumably through a cyclic AMP-independent mechanism [68].

The nature of functional differences between D_2 receptor splice variants remains controversial; however, a few studies have reported differences in their pharmacological properties, such that D_{2S} receptors have a two to threefold higher affinity for some substituted benzamide drugs compared to D_{2L} receptors [69, 70]. D_{2S} and D_{2L} receptors are also thought to couple differently to G proteins, since the alternatively spliced exon lies in a region that mediates G protein interactions. This is supported by evidence that heterologously expressed D_{2S} receptors in cell lines have a higher affinity for dopamine in the absence of GTP compared to D_{2L} receptors [69, 71, 72]. Furthermore, D_{2S} receptors more potently inhibit adenylate cyclase than D_{2L} receptors [73, 74]. Taken together, D_{2S} receptors may be more efficiently coupled to or interact with distinct subtypes of G proteins.

6.3.3 Cyclic AMP-Dependent Signaling and Behavior

Considerable evidence suggests that cyclic AMP-dependent signaling is important for modulating dopamine-induced behaviors. A null mutation of $G\alpha_{olf}$ or adenylate cyclase 5 in mice increases spontaneous locomotor activity [19, 37], whereas when

stimulated with psychostimulants, animals deficient in either $G\alpha_{olf}$ or DARPP-32 display an attenuated locomotor response [19, 49, 75], consistent with the phenotype of D_1 receptor KO animals [76, 77]. Notably, adenylate cyclase 5 KO mice retain D_1 agonist-induced locomotor behaviors, but haloperidol-induced catalepsy and the ability of sulpiride and haloperidol to suppress locomotor behavior (D_2 receptor-mediated effects) are markedly impaired in these animals [37].

Although PKA-deficient mice display no change in spontaneous activity [78], psychostimulant-induced expression of *c-fos* in the dorsal medial striatum and D_2 antagonist-induced catalepsy are attenuated in PKA KO mice [79, 80]. PKA KO mice display no changes in locomotor behavior after acute administration of amphetamine or cocaine but exhibit a heightened sensitization to repeated administration of amphetamine [80]. Direct infusion of cyclic AMP analogs into the nucleus accumbens, resulting in the inhibition or activation of PKA, respectively, reduces or enhances cocaine-seeking behavior [81, 82]. Thus, PKA activity is associated with psychostimulant-seeking behavior and sensitization but may not be required for acute responses to these drugs.

DARPP-32 KO mice are also unresponsive to inhibition of locomotor activity via D_2 antagonists [42], and lose D_2 receptor-mediated inhibition of glutamatergic neurotransmission and enhancement of intercellular coupling in nucleus accumbens spiny neurons [83]. Together, these data suggest that D1-like receptors can regulate locomotor activity independent of cyclic AMP signaling but that normal regulation of this behavior by D2-like receptors requires cyclic AMP.

6.4 Regulation of Phospholipase C

6.4.1 D1-Like Receptor Regulation of Phospholipase C

Stimulation of phospholipase C (PLC) induces phosphoinositide hydrolysis, leading to the production of inositol trisphosphate (IP3) and the concomitant mobilization of calcium from intracellular stores [84, 85]. Calcium release, in turn, regulates calcium-dependent proteins such as calcium/calmodulin-dependent kinase II (CaMKII) and protein phosphatase 2B (calcineurin).

A putative D1-like receptor that stimulates PLC has been proposed. Most D_1 receptor agonists stimulate accumulation of inositol phosphate in several brain regions; in addition, a D_1 agonist, SKF83959, with behavioral effects similar to other D_1 agonists but that antagonizes D_1 receptor stimulation of adenylate cyclase, stimulates PLC rather than adenylate cyclase [86–91]. Interestingly, another ligand, SKF83822, has been identified that activates D1-like receptor-mediated cyclic AMP accumulation but not stimulation of PLC [92]; this compound has behavioral effects that are distinct from those of most other D1-like receptor agonists [93]. In particular, SKF83822 induces seizures that are abolished in D1 KO mice and decreased in DARPP-32 KO mice, indicating a significant contribution of this signaling pathway to the behavior [94]. As mentioned in the previous section, the existence of D1-like

receptors that signal independently of $G\alpha_{olf}$/adenylate cyclase is underscored in both $G\alpha_{olf}$ and adenylate cyclase 5 KO mice, in which D_1 agonist-induced locomotor activity is preserved [37, 95].

D1-like receptors stimulate PLC through coupling to the G protein $G\alpha_q$ [89, 96–98]. The distribution of $G\alpha_q$ overlaps with D_1 agonist-induced PLC stimulation in brain regions in which expression of $G\alpha_{olf}$ is low and adenylate cyclase activation is weak, namely the amygdala and hippocampus [86, 96, 99]. However, several studies report that D_1 receptor agonists stimulate PLC in neurons from other brain regions, such as the neostriatum and prefrontal cortex, and regulate neuronal excitability [100, 101].

In D_1 receptor KO mice SKF83959-mediated production of inositol phosphate and the coupling of [^{3}H]SCH23390-binding sites to $G\alpha_q$ have been reported to be preserved, indicating that the PLC-linked D1-like receptor is not a product of the *drd1* gene [102]. Notably, the D_1 antagonist SCH23390 attenuates D_1 agonist-induced inositol phosphate production in these animals, confirming that even in the D_1 receptor KO animals the response is mediated by a D1-like receptor. This is, however, contradicted by a more recent finding that activation of $G\alpha_q$ by dopamine receptor agonists was abolished in D_1 and in D_2 receptor KO mice [103]. It has also been suggested that the PLC-linked receptor is possibly of the D_5 receptor subtype since genetic deletion of this receptor attenuates SFK83959-induced grooming behavior [104]. Indeed, Undie and colleagues recently reported that D1-like receptor activation of PLC is reduced or ablated in D_5 receptor KO mice [105]. On the other hand, D_5 receptor abundance is low in the mouse amygdala [106], a brain region which in the rat is rich in D_1-linked PLC [86].

6.4.2 D2-Like Receptor Regulation of PLC

In contrast to D1-like receptors, activation of D2-like receptors stimulates PLC and calcium mobilization through mechanisms including $G\beta\gamma$ and receptor tyrosine kinase transactivation, depending on the receptor subtype and brain region. For example, in acutely dissociated neostriatal medium spiny neurons, D_2 receptor stimulation produces $G\beta\gamma$-mediated activation of PLC, resulting in dephosphorylation and inhibition of L-type Ca^{2+} channels [107]. In prefrontal cortical neuronal cultures, D_4 but not D_{2L} receptors stimulate a rapid translocation of CaMKII to postsynaptic membranes and consequent GluR1 AMPA receptor subunit phosphorylation via the stimulation of PLC and calcium mobilization [108]. D_2 and D_3 receptor-induced transactivation of the platelet-derived growth factor receptor (PDGFR) inhibits NDMA-evoked currents in a PLCγ-dependent manner in prefrontal cortical neurons [109]. In cultured hippocampal neurons, D_4 receptor-induced transactivation of the PDGFR inhibits NMDA-evoked currents in a $G\alpha_{i/o}$-, PLCγ-dependent manner [110]. These pathways may contribute to the cyclic AMP-independent regulation of CREB observed by Yan and colleagues [68].

6.4.3 Regulation of PLC Through D_1 and D_2 Receptor Heteromerization

Recent evidence suggests that pharmacologically unique D_1 and D_2 receptor heteromers stimulate PLC and induce the release of calcium from intracellular stores through $G\alpha_q$ coupling [103, 111]. In HEK293 cells co-expressing D_1 and D_{2L} receptors, co-application of the D_1 agonist SKF81297 and the D_2 agonist quinpirole, or application of dopamine, stimulates calcium mobilization in a PLC- and IP3-dependent manner. The application of D_1 agonist alone produces only a small rise in intracellular calcium; this, however, is not observed in cells individually expressing the D_1 or D_{2L} receptor, where agonist stimulation has no effect on calcium mobilization, suggesting that an interaction between the D_1 and D_2 receptor is required. A role for activation of $G\alpha_q$ in the response is indicated by its insensitivity to pertussis toxin and sensitivity to a $G\alpha_q$ inhibitor, and by the demonstration of heteromer-mediated activation of $G\alpha_q$. Interestingly, unlike most adenylate cyclase-stimulating D_1 agonists, the adenylate cyclase-specific agonist SKF83822 does not increase Ca^{2+} mobilization even in the presence of quinpirole, and the PLC-specific D_1 agonist SKF83959 activates the putative heteromer and Ca^{2+} mobilization in the absence of a D_2 agonist [103].

Heteromerization of D_1 and D_{2L} receptors is supported by studies in which both receptor subtypes have been co-immunoprecipitated from the rat neostriatum and from heterologous expression systems [111, 112]. Notably, the abundance of the putative D_1/D_{2L} receptor complex increases in the rat neurostriatum during brain development, becoming most abundant in late adulthood [103].

The aforementioned studies suggest that the formation of dopamine receptor heteromers mediates coupling to $G\alpha_q$ and consequently increases dopamine-regulated calcium signaling. The implications of this developmental "switch" for dopamine receptor signaling in the context of neural development and neurological diseases remain undefined, and significant questions remain about the extent of co-localization of D_1 and D_2 receptors in striatal neurons [113].

It is tempting to conclude that the $G\alpha_q$-coupled D_1/D_2 heteromer and the PLC-linked D_1 receptor are the same molecular entity, particularly considering the correspondence between the effects of adenylate cyclase-selective SKF83822 and PLC-selective SKF83959 in the two systems, but as alluded to above there are two significant discrepancies. First, the PLC-linked D_1 receptor has not been reported to require co-activation of D_2 receptors, whereas both receptors in the D_1/D_2 heteromer must be activated. It has been argued that the high concentrations of D_1 agonists used to characterize the PLC-linked D_1 receptor would likely activate D_2 receptors, as well [114]; however, in earlier reports D_2 receptor antagonists did not prevent activation of $G\alpha_q$ by dopamine [96, 99], formation of IP3 by SKF83959 [89], or SKF83959-induced Ca^{2+} mobilization in hippocampal neurons [115], indicating that activation of D_2 receptors was not involved in these responses. Second, as mentioned above, Friedman et al. reported that dopamine- and SKF38393-induced inositol phosphate accumulation in the cortical slices and coupling of [^{3}H]SCH23390-binding sites to $G\alpha_q$ were unaltered in D_1 receptor KO

mice [102] and Sahu et al. reported that D1-like receptor-induced inositol phosphate accumulation is lost in D_5 receptor KO mice [105], whereas Rashid et al. reported that activation of $G\alpha_q$ in neostriatal tissue by co-activation of D_1 and D_2 receptors was abolished in either D_1 or D_2 receptor KO mice [103].

6.4.4 PLC and Behavior

Compared to dopamine-induced cyclic AMP signaling, less is known about the behavioral implications of dopamine-induced PLC signaling. However, that disruption of cyclic AMP-dependent signaling results in modest effects on many D_1 receptor-mediated behaviors suggests that alternative pathways such as PLC might regulate those behaviors. Indeed, D_1 agonists that stimulate both adenylate cyclase and PLC and the atypical D_1 agonist SKF83959 that stimulates only PLC elicit similar behaviors when administered to rodents and primates [87, 116–118]. In addition, like other D_1 agonists, SKF83939 induces contralateral rotations in the unilateral 6-hydroxydopamine-lesioned rodent model of Parkinson's disease [119, 120]. In contrast, the adenylate cyclase-specific D_1 agonist SFK83822 induces sniffing and seizures in mice [93]. These observations suggest that many behaviors induced by D_1 receptor agonists require activation of PLC.

Deletion of PLCβ-1 in mice enhances locomotor hyperactivity but impairs sensorimotor gating, socialization, and cognitive skills, behaviors generally associated with schizophrenia in humans. The D_2 antagonists and antipsychotic drugs clozapine and haloperidol rescue some of these impairments [121, 122] suggesting a role for dopaminergic signaling in this phenotype. Furthermore, an increase and decrease of PLCβ-1 in the prefrontal cortex and superior temporal cortex, respectively, has been reported in patients with schizophrenia [123].

6.5 Arrestin-Dependent Signaling

6.5.1 Overview

Originally identified as a protein involved in receptor desensitization [124] and then resensitization [125], there is increasing evidence for a role of arrestin in GPCR signaling [126, 127], particularly for signaling pathways other than the canonical heterotrimeric G protein-regulated pathways (adenylate cyclase, phospholipase C, Gβγ-regulated ion channels). It ought to be noted that although in some cases arrestin-mediated signaling has been shown to be independent of G proteins because it can be induced by ligands that are antagonists for receptor activation of G proteins [128, 129] or mediated by mutant receptors that are incapable of coupling to G proteins [130], in other cases it seems possible that stimulation of G proteins is *required* for receptor phosphorylation and arrestin binding prior to arrestin-dependent signaling.

Arrestin-dependent signaling may involve the spatial redistribution of dopamine receptors from the plasma membrane into endosomal vesicles, facilitating the interaction of receptors with distinct cytosolic signaling proteins not available at the cell surface, a mechanism that has been demonstrated for other receptor families such as receptor tyrosine kinases [131]. In addition, arrestin may serve as a scaffold for specific signaling proteins, such as Akt, during desensitization of G protein-mediated signaling [132].

Arrestin2 KO mice display normal spontaneous locomotor behavior but reduced apomorphine-induced climbing [133]. Arrestin3 KO mice also exhibit reduced apomorphine-induced climbing, along with reduced amphetamine-induced locomotor activity [133, 134]. (Arrestin2 and 3 are also referred to as βarrestin1 and 2, respectively.) These data suggest roles for arrestin2 and, in particular, arrestin3, that are independent of desensitization, because responses would be elevated in the arrestin KO mice if the primary function of arrestin were to reduce dopamine receptor responsiveness.

In the following sections we discuss the regulation of non-canonical dopamine receptor-mediated signaling pathways that have been often described as arrestin dependent, namely the MAP kinase and Akt/GSK-3β pathways. However, in many studies these pathways also are activated in a G protein-dependent manner. Regardless, these non-canonical pathways are important for the regulation of dopamine-induced behaviors and are discussed in the context of activation by psychostimulants and through individual dopamine receptor subtypes.

6.5.2 Regulation of MAP Kinases

6.5.2.1 Overview of MAP Kinases

Mitogen-activated protein (MAP) kinases regulate a variety of cellular processes including cell growth, proliferation, differentiation, and survival [135]. MAP kinases are divided into three major groups: the extracellular-regulated kinases (ERKs), the c-jun N-terminal kinases (JNKs), and the p38 kinase [135, 136]. In the brain, signaling through the ERKs, particularly ERKs 1 and 2 (ERK1/2), is involved in neuronal plasticity, memory formation, and locomotor behavior, whereas the JNK and p38 signaling pathways are involved in neuronal stress responses [137–141]. Most studies of psychostimulant regulation of MAP kinase pathways in the brain have focused on ERK1/2.

The ERK1/2 pathway is regulated by drugs of abuse through enhanced dopaminergic signaling in rodents, although there are discrepancies among studies that may reflect time- and brain region-dependent changes in ERK1/2 phosphorylation. Repeated but not acute cocaine administration induced ERK1/2 phosphorylation in the ventral tegmental area [142]. In a study by Valjent and colleagues, however, acute injection of cocaine induced phosphorylation of ERK1/2 in medium spiny neurons of the nucleus accumbens and neostriatum [143]. In other studies, both

acute and chronic administration of cocaine induced ERK1/2 phosphorylation in the prefrontal cortex, neostriatum, and amygdala [144–146].

Activation of the ERK1/2 pathway, in turn, is required for the expression of many psychostimulant-induced behaviors. In mice, the blockade of the ERK1/2 pathway with MAP kinase and ERK kinase (MEK) inhibitors attenuates the rewarding properties of cocaine and amphetamine [143, 147, 148]. Furthermore, ERK1/2 inhibition prevents cocaine-induced sensitization and expression of the immediate-early gene *c-fos* [149–151]. Interestingly, the genetic deletion of ERK1 in mice results in an increased cocaine-induced sensitization and an enhanced response to the rewarding properties of morphine due to potentiated ERK2 signaling [152, 153]. These data are consistent with other studies in vivo and in vitro demonstrating that ERK1 is a less efficient kinase and consequently attenuates ERK2 signaling [154–156]. Thus, ERK1 and ERK2 may negatively and positively regulate psychostimulant-induced behaviors, respectively. The above data strongly implicate ERK1/2 activation in dopamine receptor-mediated behavioral responses to psychostimulant drugs. Evidence for the direct regulation of the MAP kinase pathways through specific dopamine receptor subtypes is discussed below.

6.5.2.2 D1-Like Receptor Regulation of MAP Kinases

The regulation of MAP kinases through the D_1 receptor appears to be agonist-, cell-, and time-dependent. In primary striatal cultures, acute stimulation (2–20 min) with the selective D1-like receptor agonist SKF38393 induces ERK1/2 activation [157, 158]. Co-activation of D1-like receptors and metabotropic glutamate receptors also causes a PKC-dependent activation of ERK that is greater than the activation for either receptor subtype alone [158]. In contrast, acute stimulation of endogenous D_1 receptors with SKF38393 activates JNK and p38, but not ERK1/2 in a neuroblastoma cell line [159]. However, more prolonged (60–120 min) stimulation with dopamine or with the D_1 agonist SKF38393 in these cells results in activation of JNK, p38, and ERK1/2 in addition to ERK1/2-mediated oxidative stress and cytotoxicity [160]. This pathway requires an interaction between the D_1 receptor, phosphorylated ERK1/2, and arrestin3, which retains activated ERKs in the cytosol (Fig. 6.2).

Recent evidence suggests that ERKs activate distinct substrates in cytosol compared to the nucleus. For example, ERKs localized in the nucleus induce gene transcription leading to mitogenesis and survival, whereas ERKs localized in the cytosol are reported to induce cytotoxicity [161]. D_1 receptor-mediated cytotoxicity as described by Chen and colleagues [160] provides a novel mechanism by which dysfunction of dopaminergic neurotransmission, in cases such as chronic psychostimulant drug use, may cause striatal neurodegeneration [162, 163].

In vivo, stimulation of D1-like receptors after dopamine depletion in the neostriatum induces ERK1/2 activation [164]. Furthermore, D_1 receptors stimulate NDMA receptor-dependent activation of the ERK1/2, JNK, and p38 kinase pathways in the neostriatum [151, 165]. Selective antagonists to D1- but not D2-like receptors attenuate ERK1/2 phosphorylation in the neostriatum [143, 146]. Valjent and colleagues also show diminished cocaine-induced ERK1/2 phosphorylation in DARPP-32 KO

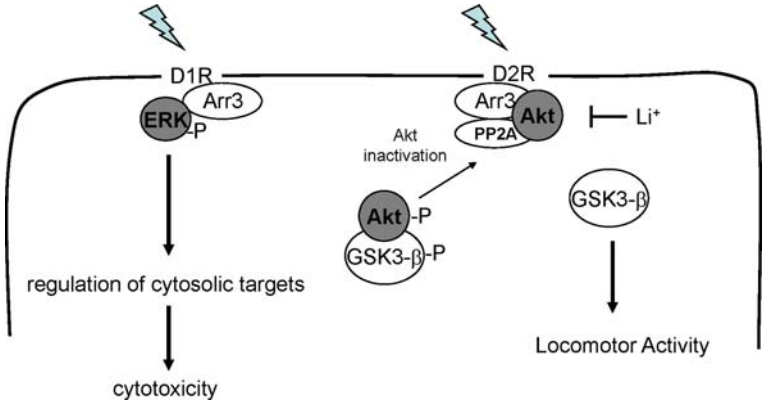

Fig. 6.2 Dopamine receptor arrestin-mediated signaling. D1- and D2-like receptors can mediate non-canonical signaling pathways (ERK and Akt/GSK-3β) through the formation of arrestin-dependent signaling complexes. Receptor-activated arrestin3 is a scaffold for both PP2A and Akt, promoting PP2A-mediated dephosphorylation and inactivation of Akt. GSK3-β is activated by the reduced Akt-mediated inhibitory phosphorylation. Lithium disrupts the arrestin3/Akt/PP2A signaling complex and thus prevents the activation of GSK3-β. Arr3, arrestin3 (βarrestin2); GSK3-β, glycogen synthase kinase 3-β; PP2A, protein phosphatase-2A

mice, further suggesting a role of D1-like receptors in the activation of this pathway [150]. Similarly, direct administration of the selective D1-like receptor antagonist SCH-23390 into the prefrontal cortex of rodents attenuates ERK activation [166]. Anatomical evidence also supports a role for the D_1 receptor, but not the D_2 receptor, in psychostimulant-induced activation of ERK1/2 [167]. Thus, considerable evidence suggests that combined stimulation of ERK2 by D_1 and NMDA receptors mediates many psychostimulant-induced behaviors [153].

6.5.2.3 D2-Like Receptor Regulation of MAP Kinases

Various studies report that D2-like receptors activate MAP kinase pathways; however, the mechanism of activation appears to differ depending on D2-like receptor subtype and cell type. Such mechanisms include the pertussis toxin-sensitive $G\alpha_{i/o}$ proteins, $G\beta\gamma$ proteins, phosphotidylinositol-3 kinase (PI-3 K), MEK, receptor tyrosine kinase transactivation, and through arrestin binding and arrestin-mediated endocytosis.

The first evidence of D_{2L} receptor-mediated activation of ERK1/2 was from the work of Faure et al. [168], who demonstrated a requirement for $G\beta\gamma$, but not activated $G\alpha_i$, in HEK293 cells. Subsequently, Luo et al. [169] showed that the D_{2L} receptor expressed in C6 glioma cells activates JNK in addition to ERK1/2, and that the activation of ERK1/2 requires Ras and MEK, two upstream protein kinases in the ERK1/2 signaling cascade. In Chinese hamster ovary (CHO) cells expressing the D_{2S} or D_{2L} receptor, dopamine induces a rapid and transient activation of ERK1/2 in a pertussis toxin-sensitive and $G\beta\gamma$-dependent manner [170]. However,

D_{2S} but not D_{2L} receptor-stimulated ERK1/2 activation requires PKC [170]. In another study, Kim et al. [171] showed that overexpression of arrestin2 or 3 in CHO cells increases D_{2S} but not D_{2L} receptor-induced activation of ERK1/2. Additional evidence of a selective requirement for arrestin-dependent internalization for activation of ERK1/2 by D_{2S} receptors is that expression of a dominant negative dynamin1 and treatment of cells with pharmacological inhibitors of receptor internalization decreases D_{2S} but not D_{2L} receptor-induced ERK activation. On the other hand, D_{2L} receptor-induced ERK1/2 activation is dependent upon PDGFR transactivation [171], presumably at the cell surface, and work with an arrestin-insensitive mutant D_{2L} receptor suggests that most D_{2L} receptor-mediated activation of ERK1/2 in HEK293 cells is mediated by pertussis toxin-sensitive G proteins, and not by arrestin [172]. Together, these studies point to mechanistic differences in signaling through D_2 receptor splice variants and support the notion that D_{2L} and D_{2S} receptors are not functionally redundant.

Activation of D_{2L} or D_4 receptors in CHO cells results in a transient, dose-dependent increase in ERK1/2 activation in a $G\alpha_{i/o}$-, PI-3 K-, PKC-, MEK-, and PDGFR-dependent manner [173]. Studies from our laboratory corroborate the notion that D_{2L} receptors activate ERK through transactivation of receptor tyrosine kinases. In HEK293 cells, D_{2L} receptor-stimulated ERK1/2 phosphorylation is dependent upon the PDGFR, whereas in a neuroblastoma cell line and primary neuronal cultures D_{2L} receptor-stimulated ERK1/2 phosphorylation is dependent upon the epidermal growth factor receptor (EGFR) [174]. Beom and colleagues, on the other hand, report that D_3 receptor but not D_{2L} receptor-stimulated ERK1/2 phosphorylation requires EGFR transactivation in several non-neuronal cells [175].

Activation of D_2 receptors induces mitogenesis in various cell types in a PKA/cyclic AMP-independent manner [176]. D_2 receptor-mediated activation of ERK1/2 induces mitogenesis in C6 glioma cells [169] and an increase in the number of dopaminergic neurons in mid-brain cultures [177].

The aforementioned in vitro studies implicate D2-like receptors in the activation of the MAP kinase pathways, leading to mitogenesis in non-neuronal cells. Additionally, one study suggests that D_2 receptors activate ERK1/2 in the dopamine-denervated neostriatum, and that activation of the pathway is required for behavioral expression of D_2 receptor supersensitivity [178]. On the other hand, contrary evidence suggests that D2-like receptors suppress tonic activity of MAP kinases. In vivo, administration of D_2 antagonists such as haloperidol or clozapine increases ERK phosphorylation in the mouse prefrontal cortex and neostriatum [146, 179] and in D_2 receptor-expressing neostriatal medium spiny neurons [167]. Jiao et al. [165] demonstrated that D_3 receptors inhibit NMDA-induced ERK, JNK, and p38 MAP kinase pathways in the neostriatum. In primary striatal cultures, the D2-like receptor agonist quinpirole inhibits potassium-stimulated ERK1/2 phosphorylation [180], and the selective D2-like receptor antagonists haloperidol and risperidone increase the phosphorylation of ERK in primary hippocampal neurons [181].

D_2 receptor-mediated inhibition of ERK1/2 has also been demonstrated in neuroendocrine pituitary cells. D_{2S} but not D_{2L} receptors inhibited basal and thyrotropin-releasing hormone-stimulated ERK1/2 phosphorylation in pituitary cell

lines [180, 182, 183]. On the other hand, Iaccarino et al. obtained results that were similar with regard to differences between the alternatively spliced receptor isoforms, but different with regard to the effect of $D2_S$ on ERK1/2; overexpression of D_{2S} caused activation of ERK1/2 that was associated with inhibition of lactotroph proliferation, whereas expression of D_{2L} had little effect on phosphorylation of ERK1/2 or lactotroph proliferation [184].

6.5.3 Regulation of the Akt/GSK-3β Pathway

6.5.3.1 Akt/GSK-3β Pathway Overview

Protein kinase B (Akt) is a well conserved serine/threonine kinase involved in the survival of most cells [185]. Classically, Akt is activated by receptor tyrosine kinases via phosphatidylinositol-3 kinase (PI-3K), although PI-3K-independent mechanisms of Akt activation have been described [186]. Akt is activated by phosphorylation at two distinct residues, Ser473 and Thr308, and in turn phosphorylates various downstream targets, leading to the inhibition of apoptosis and the regulation of gene transcription and cellular metabolism [187]. One such target of Akt is the glycogen synthase kinase (GSK)-3β, which is constitutively active until phosphorylated at Ser9 by Akt [186].

In recent years, the Akt/GSK-3β pathway has become a particularly interesting line of investigation concerning dopaminergic signaling in the brain. The Akt/GSK-3β pathway is regulated by drugs of abuse through enhanced dopaminergic signaling. Cocaine and amphetamine induce a rapid phosphorylation (within 10–20 min) of Akt and GSK-3β, respectively, in the mouse striatum [47, 157]; but other studies demonstrate that amphetamine induces dephosphorylation of Akt at later time points (30–60 min) [134, 188, 189]. Similarly, rats treated with methamphetamine display an initial increase (15 min) and subsequent decrease (120 min) in Akt phosphorylation [190]. These data suggest that enhanced dopaminergic signaling in the brain activates or inhibits the Akt/GSK-3β pathway depending on the duration of stimulation.

The Akt/GSK-3β pathway is also implicated in drug-induced behaviors and psychiatric disorders associated with dopaminergic dysfunction. Partial deletion of GSK-3β in mice or inhibition of GSK-3β with lithium – a drug commonly used to treat bipolar and other mood disorders – attenuates amphetamine-induced locomotor activity [191]. In patients with schizophrenia, total levels of Akt and phosphorylated levels of GSK-3β are decreased [192]. Interestingly, four studies report an association between schizophrenia and *akt1* genetic variants [192–195]. Moreover, Akt1 KO mice display impaired sensory/motor gating [192], which is also a characteristic of patients with schizophrenia [196].

The aforementioned studies strongly suggest that regulation of the Akt/GSK-3β pathway by dopamine receptors is a behaviorally relevant signaling response. The results of these studies are corroborated by evidence for the regulation of

the Akt/GSK-3β pathway through individual dopamine receptor subtypes. This evidence is discussed below.

6.5.3.2 D1-Like Receptor Regulation of the Akt/GSK-3β Pathway

In primary striatal neurons, the selective D1-like receptor agonist SKF38393 induces Akt phosphorylation at Thr308, but not Ser473, in a PI-3K-independent, MEK-dependent manner [157]. Moreover, an Akt mutant defective in kinase activity expressed in these cells blocks agonist-stimulated phosphorylation of CREB. Together, these data suggest cross-talk between the PKA, MAP kinase, and Akt signaling pathways through D1-like receptor stimulation. Interestingly, deletion of the D_1 receptor in mice has no effect on basal Akt or GSK-3β phosphorylation or on Akt phosphorylation in response to apomorphine or amphetamine in the striatum [188]. Therefore, although D1-like receptors induce Akt phosphorylation when directly stimulated with a selective agonist in striatal neurons and may be involved in psychostimulant-induced rapid activation of Akt, these receptors likely are not involved in the tonic regulation and psychostimulant-induced inhibition of the Akt/GSK-3β pathway.

6.5.3.3 D2-Like Receptor Regulation of the Akt/GSK-3β Pathway

In primary striatal neurons the selective D2-like receptor agonist quinpirole induces Akt phosphorylation at Thr308, but not Ser473, in a PI-3K-independent and MEK-dependent manner [157]. Additionally, a kinase-defective Akt mutant expressed in these cells blocks D_2 receptor-stimulated CREB phosphorylation. Thus, activation of D1- and D2-like receptors has similar effects on this pathway in neostriatal neurons. In PC12 cells heterologously expressing the D_{2L} receptor, in a nigral cell line that endogenously expresses D_2 receptors, and in nigral dopaminergic neurons, the D2-like receptor agonist bromocriptine stimulates Akt (Ser473) phosphorylation through the transactivation of the epidermal growth factor receptor (EGFR) and c-src and activation of PI-3K. The D_{2L} receptor co-immunoprecipitates with the EGFR and c-src from PC12-D_{2L} cells, indicating that these proteins form a complex. The resulting inhibition of GSK-3β protects the cells against oxidative stress [197–199]. Additionally, a D_4-selective agonist increases Akt kinase activity in D4-MN9D cells [200].

That Akt phosphorylation is observed at distinct residues in primary striatal neurons and cell lines suggests different mechanisms of action of endogenous and heterologous expressed dopamine receptors in the regulation of this kinase. Interestingly and in contrast to bromocriptine and ropinirole, the D2-like receptor agonist pramipexole fails to phosphorylate Akt [197–199]. Thus, certain D_2 receptor agonists may confer distinct conformations of the receptor, resulting in the activation of different downstream signaling pathways.

The above studies demonstrate that D2-like receptors stimulate Akt phosphorylation. Other evidence, however, implicates D2-like receptors in Akt dephosphorylation/inhibition. As noted above, this discrepancy may be in part due to

time-dependent effects of D2-like receptor stimulation on the activation state of Akt. In dopamine transporter (DAT) KO mice, which have increased dopaminergic tone due to loss of the primary mechanism for inactivation of dopaminergic neurotransmission, decreases in basal Akt (Thr308) and GSK-3β phosphorylation are reversed by administration of the D2-like receptor antagonist raclopride but not the D1-like receptor antagonist SCH23390 [191]. Moreover, rodents treated with D2-like receptor antagonists and commonly used antipsychotics, haloperidol, risperidone, and clozapine, display increases in Akt and GSK-3β phosphorylation [192, 201].

The role of D2-like receptors in regulating the Akt/GSK3β pathway is further confirmed by deletion studies of individual D2-like receptor subtypes [188]. D_{2L} and D_3 receptor KO mice display an increase in the basal phosphorylation of Akt at Thr308; however, D_{2L} receptor KO mice also display a decrease in the basal phosphorylation of Akt at Ser473. These data imply that D_{2L} and D_3 receptors modify Akt differently. Whereas both D_{2L} and D_3 receptor KO mice display an increase in basal phosphorylation of GSK-3β, this increase is more pronounced in D_3 receptor KO animals, implying that of the D2-like receptor subtypes, the D_3 receptor is more salient in the tonic disinhibition of GSK-3β. To date, Akt/GSK-3β phosphorylation has not been examined in D_4 KO mice, but mice treated with a selective antagonist to the D_4 receptor display no change in basal Akt or GSk-3β phosphorylation, suggesting that the D_4 receptor is not involved in the tonic regulation of this pathway in vivo [188].

Beaulieu and colleagues report that arrestin3 is necessary for the regulation of the Akt/GSK-3β pathway by D2-like receptors, serving as a scaffold for protein phosphatase 2A-mediated Akt dephosphorylation [134] (Fig. 6.2). Genetic deletion of arrestin3 in mice results in disinhibition of Akt, phosphorylation-induced inactivation of GSK-3β, and consequently an attenuated response to amphetamine-induced locomotor activity [134, 202]. The role of arrestin3 in dopamine-induced locomotor activity is underscored by the fact that arrestin3-DAT double KO mice do not display the hyperactive phenotype of DAT KO animals. Interestingly, a recent report shows that the *arrestin3* gene is associated with methamphetamine addiction in Japanese patients [203], suggesting a role for this gene in the etiology of psychostimulant-induced addiction in distinct populations.

6.6 D1-/D2-Like Receptor Cooperativity

6.6.1 Overview

D1- and D2-like receptors are thought to act cooperatively, or synergistically, to modulate dopamine-mediated behaviors, including psychiatric and motor disorders, and behavioral responses to drugs of abuse [204, 205]. Behaviorally, the requirement for activation of both receptors is reflected in the observation that administration of either D_1 or D_2 antagonists can prevent many dopamine-dependent responses [206].

Depending on the efficacy of the agonist and the endogenous dopamine tone, co-administration of D1- and D2-like agonists may be required for the full expression of some responses [207].

As reviewed by Marshall and colleagues [205], a variety of models have been proposed for D_1/D_2 receptor synergism. The models that are most relevant for this chapter involve signal transduction pathways that require co-expression of the receptors in striatal neurons which is problematic because functionally D_1 and D_2 receptors are fairly strictly segregated in distinct populations of neostriatal neurons (e.g., [167]; see also Chapter 11). Although the two receptor subtypes are clearly antagonistic for some signaling pathways (e.g., $G\alpha_s$- and $G\alpha_i$-dependent regulation of adenylate cyclase), there are other pathways where stimulation of D_1 and D_2 receptors has cooperative effects. These include cyclic AMP- and $G\beta\gamma$-dependent modulation of ion channels [208, 209], hypothesized to involve stimulation of adenylate cyclase by both $G\alpha_s$ (D_1) and $G\beta\gamma$ (D_2) subunits [210], inhibition of Na^+,K^+-ATPase [211], and activation of PLC by D_1/D_2 receptor heteromers [103, 111]. In addition, prolonged stimulation of D_2 receptors can lead to enhanced D_1 receptor stimulation of adenylate cyclase by heterologous sensitization [212], a phenomenon discussed in more detail below.

Of particular interest is the role of individual D2-like receptor subtypes and splice variants in dopamine-induced behavior. The co-localization of D_1 and D_3 receptors in the islands of Calleja in the rat ventral forebrain raises the possibility that the D_3 receptor mediates synergism between D1-like and D2-like receptors [213], and although there is some evidence to support that hypothesis [213, 214], studies with dopamine receptor KO mice suggest that genetic deletion of the D_3 receptor has little effect on the function of D1-like receptors [77, 215]. The use of receptor subtype-selective antagonists indicates that it is the D_2 receptor that synergizes with the D_1 receptor in the neostriatum [216]. Evidence from D_2 and D_{2L} receptor KO mice also suggests that D_2 splice variants modulate behaviors differently. For example, some behaviors induced by amphetamine, the D1/D2 agonist apomorphine, or combined administration of D1-like and D2-like agonists, are attenuated in D_{2L} KO mice [217]. Responsiveness to the selective D1-like agonists, SKF82958 and SKF81297, was also significantly attenuated in D_{2L} receptor KO mice but not in mice lacking both the splice variants (D_2 KO mice) [218], consistent with the hypothesis that D_{2L} receptors promote D_1 receptor function and D_{2S} receptors inhibit D_1 receptor function, but this effect on D_1 receptor-mediated behavior was not observed in the D_{2L} KO mice created by Wang and colleagues [217].

6.6.2 Heterologous Sensitization

Whereas acute activation of $G\alpha_i/G\alpha_o$-coupled D_2 receptors inhibits adenylate cyclase, prolonged activation leads to enhanced adenylate cyclase activity (i.e., heterologous sensitization) [212]. In HEK293 and C6 glioma cells, prolonged dopamine stimulation (2 h) of $G\alpha_i$-coupled D_2 and D_4 receptors but not D_3

receptors leads to sensitization of adenylate cyclase to subsequent activation by forskolin or $G\alpha_s$-coupled adrenergic receptors [219]. In vivo, repeated administration of the D2-like receptor agonist quinpirole selectively increases cyclic AMP signaling in the nucleus accumbens of rats, as measured by PKA activity and CREB phosphorylation [220]. Similarly, female hamsters that receive repeated quinpirole administration display an enhanced D_1 receptor-stimulated cyclic AMP accumulation in the neostriatum [221].

The dimeric $G\beta\gamma$, once released from the $G\alpha_i/G\alpha_o$, plays a central role in heterologous sensitization. $G\beta\gamma$ regulates the activity or subcellular localization of numerous effectors, including adenylate cyclases type II, IV, and VII [222]. Sequestration of $G\beta\gamma$ attenuates sensitization of adenylate cyclase by $G\alpha_i/G\alpha_o$-coupled receptors [212, 223–225].

Long-term (78 h) stimulation of D_2 receptors expressed in cultured cells enhances subsequent basal and forskolin-stimulated adenylate cyclase activity, which is accompanied by a decreased expression of $G\alpha_i$ and increased expression levels of $G\alpha_s$ [226–229]. However, sensitization cannot be explained simply by desensitization of $G\alpha_i$-coupled receptors or by the downregulation of $G\alpha_i$, since other reports have shown that heterologous sensitization occurs in the absence of these contributing factors and very rapidly [212, 230].

As previously mentioned, enhanced cyclic AMP-dependent signaling is observed in rodents sensitized to environmental stimuli. For example, repeated psychostimulant and morphine administration enhances D_1 receptor cyclic AMP-dependent signaling in nucleus accumbens and striatal neurons [231, 232]. In female Syrian hamsters, repeated sexual experience, which leads to a marked increase in extracellular dopamine levels and behavioral sensitization [233], enhances D_1 receptor-stimulated cyclic AMP accumulation in the nucleus accumbens [234]. Notably, the rewarding consequences of sexual experience require D2-like dopamine receptors [235].

6.7 Autoreceptors

Distinct pre-synaptic dopamine receptor subtypes, termed autoreceptors, regulate the tone of dopamine neurotransmission. Autoreceptors are expressed on the cell body and dendrites of dopaminergic midbrain neurons and modulate the rate of cell firing and impulse activity [236, 237]. It is well established that autoreceptors are D2-like receptors, as both agonists (such as quinpirole) and antagonists (such as sulpiride or eticlopride) that are selective for D2-like receptors act on dopamine neurons to cause inhibition and block dopamine-induced inhibition, respectively [238, 239]. Autoreceptors are also located on dopaminergic nerve terminals where they decrease dopamine synthesis, through inhibition of the rate-limiting enzyme tyrosine hydroxylase, and dopamine release [240, 241]. D2-like autoreceptors also decrease dopaminergic activity by enhancing dopamine re-uptake via the dopamine

transporter [242–244]. Enhanced dopamine transporter activity results at least in part from increased cell surface expression of the transporter [245].

There has been considerable investigation of which D2-like receptors are capable of functioning as autoreceptors. D_2 and D_3 receptors have been commonly implicated in dopamine autoreceptor function. In a mouse mesenchymal cell line (MN9D cells), that both synthesizes and releases dopamine, heterologously expressed D_2 and D_3 receptors but not D_4 receptors inhibit tyrosine hydroxylase activity [246] and dopamine release [247]. Notably, D_2 receptors inhibit tyrosine hydroxylase activity for longer periods than D_3 receptors, suggesting that D_2 receptors have a more potent autoreceptor function. In cell expression systems, both D_2 and D_3 receptors regulate dopamine transporter function [245, 248].

Although both D_2 and D_3 receptors can function as autoreceptors in model systems, the question of which subtypes *are* autoreceptors in vivo has been more controversial. Initially many studies with D_3-selective agonists suggested a dominant role for the D_3 receptor [249], but a different picture has resulted from the combined use of pharmacological and genetic tools. Genetic deletion of the D_2 receptor in mice abolishes all dopamine or D2-like agonist-induced inhibition of dopamine release [250–253], activation of G protein-regulated inwardly rectifying potassium (GIRK) channels [254, 255], inhibition of dopaminergic neuron firing [256], and enhancement of dopamine transporter function ([257]; but see also [252]). Pharmacological studies with D_2-selective and D_3-selective antagonists suggest that the autoreceptor in the guinea pig retina is also the D_2 receptor [258]. Mice with a genetic deletion of the D_3 receptor have enhanced basal dopamine levels [259, 260], but agonist-induced inhibition of dopamine synthesis and release, inhibition of GIRK channels and dopamine neuron firing, and enhancement of dopamine transporter activity are either unaltered or only modestly reduced in these mice [254, 255, 259–261]. These results indicate that the D_2 receptor is the predominant D_2 autoreceptor in the rodent brain, with the D_3 receptor possibly contributing to regulation of dopamine release at low concentrations of dopamine.

Surprisingly, given the similarity between D_{2L} and D_{2S} in heterologous expression systems, two groups have used mice in which the alternatively spliced exon 6 is deleted to identify differences in the roles that the splice variants play in vivo [218, 262]. These D_{2L} receptor KO mice are deficient in behavioral and biochemical measures of postsynaptic D_2 receptor activation; that is, responses that are thought to be mediated by D_2 receptors expressed on medium spiny neurons that are postsynaptic to dopamine terminals in the basal forebrain. It is important to note that these mice express D_{2S} receptors at approximately the levels at which wild-type mice express D_{2S} and D_{2L} receptors in all the neurons that normally express either variant. Even though medium spiny neurons in D_{2L} receptor KO mice express D_{2S} receptors, and the ability of quinpirole to stimulate binding of [^{35}S]GTPγS is unimpaired [263], the D_{2S} receptor does not appear to be functioning in the same way that the D_{2L} receptor functions. Of most relevance for the topic of autoreceptors is that D_2 autoreceptor function is spared in D_{2L} receptor KO mice. Thus, the ability of D_2-like agonists to suppress the firing rate of midbrain neurons, to decrease the phosphorylation and activation of tyrosine hydroxylase, and, at low doses, to inhibit

locomotor activity is absent in D_2 receptor KO mice but spared in mice that express only the D_{2S} receptor [218, 262–264]. Although this is often interpreted to mean that the D_{2S} receptor is *the* D_2 autoreceptor, it is more accurate to interpret these data as demonstrating that the D_{2S} receptor has the capability to function as the D_2 autoreceptor. Studies with D_{2S} or D_{2L} receptors expressed in cultured mesencephalic neurons suggest that D_{2L} receptors can also mediate autoreceptor activity [265]. The conclusion that the D_{2S} receptor is the autoreceptor in vivo is supported by one study with antibodies selective for D_{2S} and D_{2L} receptors reporting that, in the rhesus monkey brain, tyrosine hydroxylase-immunoreactive neurons in the substantia nigra express mainly D_{2S} receptors. In contrast, in the striatum D_{2S} receptor immunoreactivity is associated mainly with axons and D_{2L} receptor immunoreactivity is in the GABAergic medium spiny neurons [8]. There is also one report that D_{2S} receptor mRNA is more abundant than D_{2L} receptor mRNA in the murine brainstem [266].

Several signaling pathways modulated by D_2 receptors might be predicted to mediate autoreceptor activity, including inhibition of adenylate cyclase activity, inhibition of Ca^{2+} channels, and activation of K^+ channels. Stimulation of D_2 autoreceptors activates a potassium conductance as shown in brain slices [239], acutely isolated neurons [267], and cultured dopamine cells [268]. The properties of the potassium conductance indicate that the subtype is a GIRK (Kir3) channel, activated by association with the $\beta\gamma$ subunits of the $G\alpha_{i/o}$ heterotrimeric G protein [269]. GIRK2 and GIRK3 subunits are abundantly expressed by midbrain dopamine neurons [254]; experiments using GIRK KO animals indicate that channels containing GIRK2, but not GIRK3, carry most of the D_2 receptor-regulated current [255]. Thus, the primary conductance underlying the D_2 receptor inhibition of dopamine neuron firing results from activation of the G protein dependent inwardly rectifying potassium channel [269]. Activation of potassium channels also contributes to autoreceptor regulation of dopamine release [270, 271].

Inhibition of Ca^{2+} channels could also decrease cell firing or directly inhibit the secretory process. The high threshold P/Q calcium conductance is also regulated by activation of D2-autoreceptors [272] and regulates the intrinsic firing rate of dopaminergic neurons [273]. It is not known if the inhibition of calcium conductance resulting from the activation of D_2 autoreceptors is an additional mechanism that regulates the firing rate of dopamine neurons. Inhibition of calcium current does, however, decrease dendritic release of dopamine, resulting in a decrease in the inhibitory postsynaptic current that is regulated by dopamine in the cell body region [255].

Synthesis-modulating autoreceptors reduce the activity of the rate-limiting enzyme for dopamine synthesis, the tyrosine hydroxylase. Tyrosine hydroxylase contains four residues phosphorylated by PKA in its amino-terminal regulatory domain. Phosphorylation of Ser40, in particular, mediates forskolin-stimulated activation of tyrosine hydroxylase [274]. D_2 autoreceptor inhibition of adenylate cyclase decreases dopamine synthesis by decreasing PKA-dependent phosphorylation of Ser40 [263, 275, 276], decreasing the availability of dopamine to fill synaptic vesicles [277]. Because ERK1/2 activate tyrosine hydroxylase by phosphorylation

on Ser31 [278, 279], inhibition of ERK1/2 by D_{2S} receptors [180] could in theory inhibit dopamine synthesis, but the existing data most solidly support the conclusion that the effect of synthesis-modulating autoreceptors is mediated predominantly by inhibition of adenylate cyclase activity.

The mechanism by which D_2 autoreceptor stimulation enhances dopamine transporter function and localization at the membrane is less clear. ERK1/2 constitutively increase cell surface localization of the transporter in striatal synaptosomes and in HEK 293 cells heterologously expressing the transporter [280], and also mediate recruitment of the transporter to the cell surface by D_{2S} in HEK 293 cells [281]. Although it is tempting to attribute autoreceptor regulation of the dopamine transporter to the ERK1/2 signaling pathway, this conclusion seems premature in view of the lack of evidence for activation of ERK by D_2 receptors in the intact (i.e., nondenervated) neostriatum. A second mechanism by which D_2 autoreceptors can alter dopamine transporter localization and enhance re-uptake of dopamine is by a direct interaction between the third cytoplasmic loop of the receptor and the amino terminus of the transporter; this interaction, however, is constitutive and not influenced by D_2 receptor activation [282].

Not all dopamine neurons have D_2 autoreceptors and GIRKs [283, 284]. In fact, accumulating evidence indicates that mid-brain dopaminergic cells are a highly heterogeneous population of neurons when the expression of various ion channels and receptors is taken into consideration. The demonstration that neurons that project to the medial prefrontal cortex lack both GIRK and autoreceptors sets them apart from other dopamine neurons in the midbrain and indicates that the regulation of activity of these neurons specifically is vastly different from other dopamine neurons [269, 283].

6.8 Summary

Dopamine receptors regulate a variety of signal transduction pathways through which complex behaviors are expressed. In this chapter, we have focused on signaling pathways for which there is evidence of their contribution to behavior, including the PKA, PLC, ERK1/2, and Akt/GSK-3β pathways. Not discussed in this chapter are some signaling responses that are downstream of the adenylate cyclase-PKA-DARPP-32 pathway, such as regulation of renal Na^+–H^+ exchange and Na^+,K^+-ATPase activity [13, 285], or signaling pathways, such as phospholipases other than PLC, whose contributions to dopamine-dependent behaviors have not been extensively investigated. In addition, to achieve a comprehensive understanding of the mechanisms of dopamine receptor signaling we must also consider receptor modulation of ion channels (Chapters 7 and 11), and the effects of novel receptor-interacting proteins (Chapter 9) and receptor oligomerization (Chapter 10) on function. The past decade has seen great advances on all of these fronts, producing a much richer picture of receptor function than was previously available. In

particular, the genetic deletion of individual dopamine receptors has greatly contributed to our understanding of their specific roles in regulating signaling pathways and their behavioral correlates (see also Chapter 12 on dopamine receptor KO mice and Chapter 13 on the contribution of individual receptor subtypes to behavior). Focusing the role of particular signaling pathways in behavior, our goal was to produce a chapter that would complement these other behavior-focused chapters to provide a comprehensive review of our current understanding of how the binding of dopamine to its receptors alters cellular function and behavior.

References

1. Giros B, Sokoloff P, Martres M-P, Riou J-F, Emorine LJ, Schwartz J-C. Alternative splicing directs the expression of two D_2 dopamine receptor isoforms. Nature 1989;342:923–6.
2. Neve KA, Neve RL. Molecular biology of dopamine receptors. In: Neve KA, Neve RL, eds. The dopamine receptors. Totawa, NJ: Humana Press, 1997:27–76.
3. Callier S, Snapyan M, Le Crom S, Prou D, Vincent JD, Vernier P. Evolution and cell biology of dopamine receptors in vertebrates. Biol Cell 2003;95:489–502.
4. Kienast T, Heinz A. Dopamine and the diseased brain. CNS Neurol Disord Drug Targets 2006;5:109–31.
5. Meador-Woodruff JH, Mansour A, Healy DJ et al. Comparison of the distributions of D1 and D2 dopamine receptor mRNAs in rat brain. Neuropsychopharmacology 1991;5:231–42.
6. Meador-Woodruff JH, Mansour A, Grandy DK, Damask SP, Civelli O, Watson SJ. Distribution of D_5 dopamine receptor messenger RNA in rat brain. Neurosci Lett 1992;145:209–12.
7. Chronwall BM, Dickerson DS, Huerter BS, Sibley DR, Millington WR. Regulation of heterogeneity in D_2 dopamine receptor gene expression among individual melanotropes in the rat pituitary intermediate lobe. Mol Cell Neurosci 1994;5:35–45.
8. Khan ZU, Mrzljak L, Gutierrez A, De la Calle A, Goldman-Rakic PS. Prominence of the dopamine D2 short isoform in dopaminergic pathways. Proc Natl Acad Sci USA 1998;95:7731–6.
9. Bouthenet M-L, Souil E, Martres M-P, Sokoloff P, Giros B, Schwartz J-C. Localization of dopamine D_3 receptor mRNA in the rat brain using in situ hybridization histochemistry: comparison with dopamine D_2 receptor mRNA. Brain Res 1991;564:203–19.
10. Mulcrone J, Kerwin RW. The regional pattern of D4 gene expression in human brain. Neurosci Lett 1997;234:147–50.
11. McCudden CR, Hains MD, Kimple RJ, Siderovski DP, Willard FS. G-protein signaling: back to the future. Cell Mol Life Sci 2005;62:551–77.
12. Oldham WM, Hamm HE. Heterotrimeric G protein activation by G-protein-coupled receptors. Nat Rev Mol Cell Biol 2008;9:60–71.
13. Neve KA, Seamans JK, Trantham-Davidson H. Dopamine receptor signaling. J Recept Signal Transduct Res 2004;24:165–205.
14. Rebois RV, Hebert TE. Protein complexes involved in heptahelical receptor-mediated signal transduction. Receptors Channels 2003;9:169–94.
15. Galés C, Rebois RV, Hogue M et al. Real-time monitoring of receptor and G-protein interactions in living cells. Nat Methods 2005;2:177–84.
16. Brown JH, Makman MH. Stimulation by dopamine of adenylate cyclase in retinal homogenates and of adenosine-3′:5′-cyclic monophosphate formation in intact retina. Proc Natl Acad Sci USA 1972;69:539–43.
17. Kebabian JW, Petzold GL, Greengard P. Dopamine-sensitive adenylate cyclase in caudate nucleus of rat brain, and its similarity to the “dopamine receptor”. Proc Natl Acad Sci USA 1972;69:2145–9.

18. Drinnan SL, Hope BT, Snutch TP, Vincent SR. Golf in the basal ganglia. Mol Cell Neurosci 1991;2:66–70.
19. Zhuang X, Belluscio L, Hen R. $G_{olf\alpha}$ mediates dopamine D_1 receptor signaling. J Neurosci 2000;20:NIL1–L5.
20. Corvol JC, Studler JM, Schonn JS, Girault JA, Hervé D. $G\alpha_{olf}$ is necessary for coupling D1 and A2a receptors to adenylyl cyclase in the striatum. J Neurochem 2001;76:1585–8.
21. Ohara K, Haga K, Berstein G, Haga T, Ichiyama A. The interaction between D-2 dopamine receptors and GTP-binding proteins. Mol Pharmacol 1988;33:290–6.
22. Lledo PM, Homburger V, Bockaert J, Vincent J-D. Differential G protein-mediated coupling of D_2 dopamine receptors to K^+ and Ca^{2+} currents in rat anterior pituitary cells. Neuron 1992;8:455–63.
23. Liu YF, Jakobs KH, Rasenick MM, Albert PR. G protein specificity in receptor-effector coupling: analysis of the roles of G_o and G_{i2} in GH4C1 pituitary cells. J Biol Chem 1994;269:13880–6.
24. Painson J-C, Wenger T, Lagacé G, Masson ND, Collu R. Cellular distribution of G protein Goα in pituitary lactotrophs: effects of dopamine. J Neuroendocrinol 1994;6:447–55.
25. Watts VJ, Wiens BL, Cumbay MG, Vu MN, Neve RL, Neve KA. Selective activation of $G\alpha_o$ by D_{2L} dopamine receptors in NS20Y neuroblastoma cells. J Neurosci 1998;18:8692–9.
26. Leaney JL, Tinker A. The role of members of the pertussis toxin-sensitive family of G proteins in coupling receptors to the activation of the G protein-gated inwardly rectifying potassium channel. Proc Natl Acad Sci USA 2000;97:5651–6.
27. Cordeaux Y, Nickolls SA, Flood LA, Graber SG, Strange PG. Agonist regulation of D_2 dopamine receptor/G protein interaction – Evidence for agonist selection of G protein subtype. J Biol Chem 2001;276:28667–75.
28. Nickolls SA, Strange PG. Interaction of the D_{2short} dopamine receptor with G proteins: analysis of receptor G protein selectivity. Biochem Pharmacol 2003;65:1139–50.
29. Zaworski PG, Alberts GL, Pregenzer JF, Bin Im W, Slightom JL, Gill GS. Efficient functional coupling of the human D3 dopamine receptor to G_o subtype of G proteins in SH-SY5Y cells. Br J Pharmacol 1999;128:1181–8.
30. Lane JR, Powney B, Wise A, Rees S, Milligan G. G protein coupling and ligand selectivity of the D_{2L} and D_3 dopamine receptors. J Pharmacol Exp Ther 2008;325:319–30.
31. Jiang MS, Spicher K, Boulay G, Wang Y, Birnbaumer L. Most central nervous system D2 dopamine receptors are coupled to their effecters by Go. Proc Natl Acad Sci USA 2001;98:3577–82.
32. Leck KJ, Blaha CD, Matthaei KI, Forster GL, Holgate J, Hendry IA. G_z proteins are functionally coupled to dopamine D2-like receptors in vivo. Neuropharmacology 2006;51:597–605.
33. Watson JB, Coulter II PM, Margulies JE et al. G-protein γ7 subunit is selectively expressed in medium-sized neurons and dendrites of the rat neostriatum. J Neurosci Res 1994;39: 108–16.
34. Schwindinger WF, Betz KS, Giger KE, Sabol A, Bronson SK, Robishaw JD. Loss of G protein γ_7 alters behavior and reduces striatal α_{olf} level and cAMP production. J Biol Chem 2003;278:6575–9.
35. Wang Q, Jolly JP, Surmeier JD et al. Differential dependence of the D_1 and D_5 dopamine receptors on the G protein γ7 subunit for activation of adenylyl cyclase. J Biol Chem 2001;276:39386–93.
36. Mons N, Cooper DM. Selective expression of one Ca^{2+}-inhibitable adenylyl cyclase in dopaminergically innervated rat brain regions. Brain Res Mol Brain Res 1994;22: 236–44.
37. Lee KW, Hong JH, Choi IY et al. Impaired D2 dopamine receptor function in mice lacking type 5 adenylyl cyclase. J Neurosci 2002;22:7931–40.
38. Konradi C, Cole RL, Heckers S, Hyman SE. Amphetamine regulates gene expression in rat striatum via transcription factor CREB. J Neurosci 1994;14:5623–34.

39. Josselyn SA, Nguyen PV. CREB, synapses and memory disorders: past progress and future challenges. Curr Drug Targets CNS Neurol Disord 2005;4:481–97.
40. Berke JD, Hyman SE. Addiction, dopamine, and the molecular mechanisms of memory. Neuron 2000;25:515–32.
41. Hemmings HC, Jr., Greengard P, Tung HY, Cohen P. DARPP-32, a dopamine-regulated neuronal phosphoprotein, is a potent inhibitor of protein phosphatase-1. Nature 1984;310: 503–5.
42. Greengard P, Allen PB, Nairn AC. Beyond the dopamine receptor: the DARPP-32/Protein phosphatase-1 cascade. Neuron 1999;23:435–47.
43. Bibb JA, Snyder GL, Nishi A et al. Phosphorylation of DARPP-32 by Cdk5 modulates dopamine signalling in neurons. Nature 1999;402:669–71.
44. Nishi A, Bibb JA, Snyder GL, Higashi H, Nairn AC, Greengard P. Amplification of dopaminergic signaling by a positive feedback loop. Proc Natl Acad Sci USA 2000;97:12840–5.
45. Bibb JA, Chen J, Taylor JR et al. Effects of chronic exposure to cocaine are regulated by the neuronal protein Cdk5. Nature 2001;410:376–80.
46. Nestler EJ. Molecular basis of long-term plasticity underlying addiction. Nat Rev Neurosci 2001;2:119–28.
47. Svenningsson P, Tzavara ET, Carruthers R et al. Diverse psychotomimetics act through a common signaling pathway. Science 2003;302:1412–5.
48. Chen PC, Chen JC. Enhanced Cdk5 activity and p35 translocation in the ventral striatum of acute and chronic methamphetamine-treated rats. Neuropsychopharmacology 2005;30: 538–49.
49. Fienberg AA, Hiroi N, Mermelstein PG et al. DARPP-32: regulator of the efficacy of dopaminergic neurotransmission. Science 1998;281:838–9.
50. Kelz MB, Chen J, Carlezon WA, Jr. et al. Expression of the transcription factor ΔFosB in the brain controls sensitivity to cocaine. Nature 1999;401:272–6.
51. Murphy BJ, Rossie S, De Jongh KS, Catterall WA. Identification of the sites of selective phosphorylation and dephosphorylation of the rat brain Na^+ channel α subunit by cAMP-dependent protein kinase and phosphoprotein phosphatases. J Biol Chem 1993;268: 27355–62.
52. Cantrell AR, Smith RD, Goldin AL, Scheuer T, Catterall WA. Dopaminergic modulation of sodium current in hippocampal neurons via cAMP-dependent phosphorylation of specific sites in the sodium channel α subunit. J Neurosci 1997;17:7330–8.
53. Li M, West JW, Lai Y, Scheuer T, Catterall WA. Functional modulation of brain sodium channels by cAMP-dependent phosphorylation. Neuron 1992;8:1151–9.
54. Smith RD, Goldin AL. Phosphorylation at a single site in the rat brain sodium channel is necessary and sufficient for current reduction by protein kinase A. J Neurosci 1997;17: 6086–93.
55. Arias-Montaño JA, Floran B, Floran L, Aceves J, Young JM. Dopamine D_1 receptor facilitation of depolarization-induced release of γ-amino-butyric acid in rat striatum is mediated by the cAMP/PKA pathway and involves P/Q-type calcium channels. Synapse 2007;61: 310–9.
56. Kisilevsky AE, Mulligan SJ, Altier C et al. D1 receptors physically interact with N-type calcium channels to regulate channel distribution and dendritic calcium entry. Neuron 2008;58:557–70.
57. Liu F, Wan Q, Pristupa ZB, Yu XM, Wang YT, Niznik HB. Direct protein-protein coupling enables cross-talk between dopamine D5 and γ-aminobutyric acid A receptors. Nature 2000;403:274–80.
58. Lee FJ, Xue S, Pei L et al. Dual regulation of NMDA receptor functions by direct protein-protein interactions with the dopamine D1 receptor. Cell 2002;111:219–30.
59. Fiorentini C, Gardoni F, Spano PF, Di Luca M, Missale C. Regulation of dopamine D_1 receptor trafficking and desensitization by oligomerization with glutamate *N*-methyl-D-aspartate receptors. J Biol Chem 2003;278:20196–202.

60. Chao SZ, Lu WX, Lee HK, Huganir RL, Wolf ME. D $_1$ dopamine receptor stimulation increases GluR1 phosphorylation in postnatal nucleus accumbens cultures. J Neurochem 2002;81:984–92.
61. Surmeier DJ, Ding J, Day M, Wang Z, Shen W. D1 and D2 dopamine-receptor modulation of striatal glutamatergic signaling in striatal medium spiny neurons. Trends Neurosci 2007;30:228–35.
62. Snyder GL, Allen PB, Fienberg AA et al. Regulation of phosphorylation of the GluR1 AMPA receptor in the neostriatum by dopamine and psychostimulants *in vivo*. J Neurosci 2000;20:4480–8.
63. De Camilli P, Macconi D, Spada A. Dopamine inhibits adenylate cyclase in human prolactin-secreting pituitary adenomas. Nature 1979;278:252–4.
64. Stoof JC, Kebabian JW. Opposing roles for D-1 and D-2 dopamine receptors in efflux of cyclic AMP from rat neostriatum. Nature 1981;294:366–8.
65. Chio CL, Lajiness ME, Huff RM. Activation of heterologously expressed D3 dopamine receptors: comparison with D2 dopamine receptors. Mol Pharmacol 1994;45:51–60.
66. Chio CL, Drong RF, Riley DT, Gill GS, Slightom JL, Huff RM. D4 dopamine receptor-mediated signaling events determined in transfected Chinese hamster ovary cells. J Biol Chem 1994;269:11813–9.
67. Nishi A, Snyder GL, Greengard P. Bidirectional regulation of DARPP-32 phosphorylation by dopamine. J Neurosci 1997;17:8147–55.
68. Yan Z, Feng J, Fienberg AA, Greengard P. D_2 dopamine receptors induce mitogen-activated protein kinase and cAMP response element-binding protein phosphorylation in neurons. Proc Natl Acad Sci USA 1999;96:11607–12.
69. Castro SW, Strange PG. Differences in the ligand binding properties of the short and long versions of the D2 dopamine receptor. J Neurochem 1993;60:372–5.
70. 70.Malmberg Å, Jackson DM, Eriksson A, Mohell N. Unique binding characteristics of antipsychotic agents interacting with human dopamine D_{2A}, D_{2B}, and D_3 receptors. Mol Pharmacol 1993;43:749–54.
71. Montmayeur JP, Guiramand J, Borrelli E. Preferential coupling between dopamine D2 receptors and G- proteins. Mol Endocrinol 1993;7:161–70.
72. Falardeau P. Functional distinctions of dopamine D_2long and D_2 short receptors. In: Niznik HB, ed. Dopamine receptors and transporters. New York: Marcel Dekker, 1994:323–42.
73. Montmayeur J-P, Borrelli E. Transcription mediated by a cAMP-responsive promoter element is reduced upon activation of dopamine D_2 receptors. Proc Natl Acad Sci USA 1991;88:3135–9.
74. Hayes G, Biden TJ, Selbie LA, Shine J. Structural subtypes of the dopamine D2 receptor are functionally distinct: expression of the cloned $D2_A$ and $D2_B$ subtypes in a heterologous cell line. Mol Endocrinol 1992;6:920–6.
75. Fienberg AA, Greengard P. The DARPP-32 knockout mouse. Brain Res Brain Res Rev 2000;31:313–9.
76. Tran AH, Tamura R, Uwano T, Kobayashi T, Katsuki M, Ono T. Dopamine D1 receptors involved in locomotor activity and accumbens neural responses to prediction of reward associated with place. Proc Natl Acad Sci USA 2005;102:2117–22.
77. Karasinska JM, George SR, Cheng R, O'Dowd BF. Deletion of dopamine D1 and D3 receptors differentially affects spontaneous behaviour and cocaine-induced locomotor activity, reward and CREB phosphorylation. Eur J Neurosci 2005; 22:1750.
78. Howe DG, Wiley JC, McKnight GS. Molecular and behavioral effects of a null mutation in all PKA Cβ isoforms. Mol Cell Neurosci 2002;20:515–24.
79. Adams MR, Brandon EP, Chartoff EH, Idzerda RL, Dorsa DM, McKnight GS. Loss of haloperidol induced gene expression and catalepsy in protein kinase A-deficient mice. Proc Natl Acad Sci U S A 1997;94:12157–61.
80. Brandon EP, Logue SF, Adams MR et al. Defective motor behavior and neural gene expression in RIIβ-protein kinase a mutant mice. J Neurosci 1998;18:3639–49.

81. Lynch WJ, Taylor JR. Persistent changes in motivation to self-administer cocaine following modulation of cyclic AMP-dependent protein kinase A (PKA) activity in the nucleus accumbens. Eur J Neurosci 2005;22:1214–20.
82. Self DW, Genova LM, Hope BT, Barnhart WJ, Spencer JJ, Nestler EJ. Involvement of cAMP-dependent protein kinase in the nucleus accumbens in cocaine self-administration and relapse of cocaine-seeking behavior. J Neurosci 1998;18:1848–59.
83. Onn SP, Lin M, Liu JJ, Grace AA. Dopamine and cyclic-AMP regulated phosphoprotein-32-dependent modulation of prefrontal cortical input and intercellular coupling in mouse accumbens spiny and aspiny neurons. Neuroscience 2008;151:802–16.
84. Burgess GM, Godfrey PP, McKinney JS, Berridge MJ, Irvine RF, Putney JW, Jr. The second messenger linking receptor activation to internal Ca release in liver. Nature 1984;309:63–6.
85. Kirk CJ, Bone EA, Palmer S, Michell RH. The role of phosphatidylinositol 4,5 bisphosphate breakdown in cell-surface receptor activation. J Recept Res 1984;4:489–504.
86. Undie AS, Friedman E. Stimulation of a dopamine D1 receptor enhances inositol phosphates formation in rat brain. J Pharmacol Exp Ther 1990;253:987–92.
87. Arnt J, Hyttel J, Sánchez C. Partial and full dopamine D_1 receptor agonists in mice and rats: relation between behavioural effects and stimulation of adenylate cyclase activity in vitro. Eur J Pharmacol 1992;213:259–67.
88. Panchalingam S, Undie AS. SKF83959 exhibits biochemical agonism by stimulating [(35)S]GTP gamma S binding and phosphoinositide hydrolysis in rat and monkey brain. Neuropharmacology 2001;40:826–37.
89. Jin LQ, Goswami S, Cai GP, Zhen XC, Friedman E. SKF83959 selectively regulates phosphatidylinositol-linked D_1 dopamine receptors in rat brain. J Neurochem 2003;85: 378–86.
90. Tang TS, Bezprozvanny I. Dopamine receptor-mediated Ca^{2+} signaling in striatal medium spiny neurons. J Biol Chem 2004;279:42082–94.
91. Noriyama Y, Ogawa Y, Yoshino H, Yamashita M, Kishimoto T. Dopamine profoundly suppresses excitatory transmission in neonatal rat hippocampus via phosphatidylinositol-linked D1-like receptor. Neuroscience 2006;138:475–85.
92. Undie AS, Weinstock J, Sarau HM, Friedman E. Evidence for a distinct D_1-like dopamine receptor that couples to activation of phosphoinositide metabolism in brain. J Neurochem 1994;62:2045–8.
93. O'Sullivan GJ, Roth BL, Kinsella A, Waddington JL. SK&F 83822 distinguishes adenylyl cyclase from phospholipase C-coupled dopamine D1-like receptors: behavioural topography. Eur J Pharmacol 2004;486:273–80.
94. O'Sullivan GJ, Dunleavy M, Hakansson K et al. Dopamine D_1 vs D_5 receptor-dependent induction of seizures in relation to DARPP-32, ERK1/2 and GluR1-AMPA signalling. Neuropharmacology 2008;54:1051–61.
95. Iwamoto T, Okumura S, Iwatsubo K et al. Motor dysfunction in type 5 adenylyl cyclase-null mice. J Biol Chem 2003;278:16936–40.
96. Wang HY, Undie AS, Friedman E. Evidence for the coupling of G_q protein to D_1-like dopamine sites in rat striatum: possible role in dopamine-mediated inositol phosphate formation. Mol Pharmacol 1995;48:988–94.
97. Pacheco MA, Jope RS. Comparison of [^{3}H]phosphatidylinositol and [^{3}H]phosphatidylinositol 4,5-bisphosphate hydrolysis in postmortem human brain membranes and characterization of stimulation by dopamine D1 receptors. J Neurochem 1997;69:639–44.
98. Mannoury la Cour C, Vidal S, Pasteau V, Cussac D, Millan MJ. Dopamine D_1 receptor coupling to Gs/olf and Gq in rat striatum and cortex: a scintillation proximity assay (SPA)/antibody-capture characterization of benzazepine agonists. Neuropharmacology 2007;52:1003–14.
99. Jin L-Q, Wang H-Y, Friedman E. Stimulated D_1 dopamine receptors couple to multiple Gα proteins in different brain regions. J Neurochem 2001;78:981–90.

100. Lezcano N, Bergson C. D1/D5 dopamine receptors stimulate intracellular calcium release in primary cultures of neocortical and hippocampal neurons. J Neurophysiol 2002;87:2167–75.
101. Chen L, Bohanick JD, Nishihara M, Seamans JK, Yang CR. Dopamine D1/5 receptor-mediated long-term potentiation of intrinsic excitability in rat prefrontal cortical neurons: Ca^{2+}-dependent intracellular signaling. J Neurophysiol 2007;97:2448–64.
102. Friedman E, Jin LQ, Cai GP et al. D_1-like dopaminergic activation of phosphoinositide hydrolysis is independent of D_{1A} dopamine receptors: evidence from D_{1A} knockout mice. Mol Pharmacol 1997;51:6–11.
103. Rashid AJ, So CH, Kong MM et al. D1-D2 dopamine receptor heterooligomers with unique pharmacology are coupled to rapid activation of G_q/11 in the striatum. Proc Natl Acad Sci U S A 2007;104:654–9.
104. O'Sullivan GJ, Clifford JJ, Tomiyama K et al. D1-like dopamine receptor-mediated function in congenic mutants with D1 vs. D5 receptor "knockout". J Recept Signal Transduct Res 2004;24:107–16.
105. Sahu A, Tyeryar KR, Vongtau HO, Sibley DR, Undieh AS. D5 dopamine receptors are required for dopaminergic activation of phospholipase C. Mol Pharmacol 2009;75:In Press.
106. Montague DM, Striplin CD, Overcash JS, Drago J, Lawler CP, Mailman RB. Quantification of D_{1B} (D_5) receptors in dopamine D_{1A} receptor-deficient mice. Synapse 2001;39:319–22.
107. Hernández-López S, Tkatch T, Perez-Garci E et al. D_2 dopamine receptors in striatal medium spiny neurons reduce L-type Ca^{2+} currents and excitability via a novel PLCβ1-IP_3-calcineurin-signaling cascade. J Neurosci 2000;20:8987–95.
108. Gu Z, Jiang Q, Yuen EY, Yan Z. Activation of dopamine D_4 receptors induces synaptic translocation of Ca^{2+}/calmodulin-dependent protein kinase II in cultured prefrontal cortical neurons. Mol Pharmacol 2006;69:813–22.
109. Beazely MA, Tong A, Wei WL, Van Tol H, Sidhu B, MacDonald JF. D_2-class dopamine receptor inhibition of NMDA currents in prefrontal cortical neurons is platelet-derived growth factor receptor-dependent. J Neurochem 2006;98:1657–63.
110. Kotecha SA, Oak JN, Jackson MF et al. A D2 class dopamine receptor transactivates a receptor tyrosine kinase to inhibit NMDA receptor transmission. Neuron 2002;35:1111–22.
111. Lee SP, So CH, Rashid AJ et al. Dopamine D1 and D2 receptor co-activation generates a novel phospholipase C-mediated calcium signal. J Biol Chem 2004;279:35671–8.
112. So CH, Varghese G, Curley KJ et al. D1 and D2 dopamine receptors form heterooligomers and cointernalize after selective activation of either receptor. Mol Pharmacol 2005;68: 568–78.
113. Shuen JA, Chen M, Gloss B, Calakos N. *Drd1a*-tdTomato BAC transgenic mice for simultaneous visualization of medium spiny neurons in the direct and indirect pathways of the basal ganglia. J Neurosci 2008;28:2681–5.
114. Rashid AJ, O'Dowd BF, Verma V, George SR. Neuronal Gq/11-coupled dopamine receptors: an uncharted role for dopamine. TIPS 2007;28:551–5.
115. Ming Y, Zhang H, Long L, Wang F, Chen J, Zhen X. Modulation of Ca^{2+} signals by phosphatidylinositol-linked novel D1 dopamine receptor in hippocampal neurons. J Neurochem 2006;98:1316–23.
116. Deveney AM, Waddington JL. Pharmacological characterization of behavioural responses to SK&F 83959 in relation to $'D_1$-like$'$ dopamine receptors not linked to adenylyl cyclase. Br J Pharmacol 1995;116:2120–6.
117. Clifford JJ, Tighe O, Croke DT et al. Conservation of behavioural topography to dopamine D_1-like receptor agonists in mutant mice lacking the D_{1A} receptor implicates a D_1-like receptor not coupled to adenylyl cyclase. Neuroscience 1999;93:1483–9.
118. Peacock L, Gerlach J. Aberrant behavioral effects of a dopamine D1 receptor antagonist and agonist in monkeys: evidence of uncharted dopamine D1 receptor actions. Biol Psychiatry 2001;50:501–9.
119. Zhen X, Goswami S, Friedman E. The role of the phosphatidyinositol-linked D_1 dopamine receptor in the pharmacology of SKF83959. Pharmacol Biochem Behav 2005;80:597–601.

120. Wirtshafter D, Osborn CV. The atypical dopamine D1 receptor agonist SKF 83959 induces striatal Fos expression in rats. Eur J Pharmacol 2005;528:88–94.
121. McOmish CE, Burrows E, Howard M et al. Phospholipase C-beta1 knockout mice exhibit endophenotypes modeling schizophrenia which are rescued by environmental enrichment and clozapine administration. Mol Psychiatry 2008;13:661–72.
122. Koh HY, Kim D, Lee J, Lee S, Shin HS. Deficits in social behavior and sensorimotor gating in mice lacking phospholipase Cbeta1. Genes Brain Behav 2008;7:120–8.
123. Lin ZC, Wang WF, Kopajtic T, Revay RS, Uhl GR. Dopamine transporter: transmembrane phenylalanine mutations can selectively influence dopamine uptake and cocaine analog recognition. Mol Pharmacol 1999;56:434–47.
124. Lohse MJ, Benovic JL, Codina J, Caron MG, Lefkowitz RJ. β-Arrestin: a protein that regulates β-adrenergic receptor function. Science 1990;248:1547–50.
125. Zhang J, Barak LS, Winkler KE, Caron MG, Ferguson SS. A central role for β-arrestins and clathrin-coated vesicle-mediated endocytosis in β_2-adrenergic receptor resensitization–Differential regulation of receptor resensitization in two distinct cell types. J Biol Chem 1997;272:27005–14.
126. Lefkowitz RJ, Shenoy SK. Transduction of receptor signals by β-arrestins. Science 2005;308:512–7.
127. DeFea K. β-arrestins and heterotrimeric G-proteins: collaborators and competitors in signal transduction. Br J Pharmacol 2008;153(Suppl 1):S298-309. Epub;%2007 Nov 26: S298–S309.
128. Wei H, Ahn S, Shenoy SK et al. Independent β-arrestin 2 and G protein-mediated pathways for angiotensin II activation of extracellular signal-regulated kinases 1 and 2. Proc Natl Acad Sci USA 2003;100:10782–7.
129. Wisler JW, DeWire SM, Whalen EJ et al. A unique mechanism of β-blocker action: carvedilol stimulates β-arrestin signaling. Proc Natl Acad Sci U S A 2007;104: 16657–62.
130. Shenoy SK, Drake MT, Nelson CD et al. β-Arrestin-dependent, G protein-independent ERK1/2 activation by the β2 adrenergic receptor. J Biol Chem 2006;281:1261–73.
131. Romanelli RJ, Wood TL. Directing traffic in neural cells: determinants of receptor tyrosine kinase localization and cellular responses. J Neurochem 2008.
132. Hall FS, Li XF, Sora I et al. Cocaine mechanisms: Enhanced cocaine, fluoxetine and nisoxetine place preferences following monoamine transporter deletions. Neuroscience 2002;115:153–61.
133. Gainetdinov RR, Premont RT, Bohn LM, Lefkowitz RJ, Caron MG. Desensitization of G protein-coupled receptors and neuronal functions. Ann Rev Neurosci 2004;27:107-44.
134. Beaulieu JM, Sotnikova TD, Marion S, Lefkowitz RJ, Gainetdinov RR, Caron MG. An Akt/β-arrestin 2/PP2A signaling complex mediates dopaminergic neurotransmission and behavior. Cell 2005;122:261–73.
135. Sweatt JD. The neuronal MAP kinase cascade: a biochemical signal integration system subserving synaptic plasticity and memory. J Neurochem 2001;76:1–10.
136. Johnson GL, Lapadat R. Mitogen-activated protein kinase pathways mediated by ERK, JNK, and p38 protein kinases. Science 2002;298:1911–2.
137. Mielke K, Herdegen T. JNK and p38 stresskinases–degenerative effectors of signal-transduction-cascades in the nervous system. Prog Neurobiol 2000;61:45–60.
138. Atkins CM, Selcher JC, Petraitis JJ, Trzaskos JM, Sweatt JD. The MAPK cascade is required for mammalian associative learning. Nat Neurosci 1998;1:602–9.
139. Schafe GE, Nadel NV, Sullivan GM, Harris A, LeDoux JE. Memory consolidation for contextual and auditory fear conditioning is dependent on protein synthesis, PKA, and MAP kinase. Learn Mem 1999;6:97–110.
140. Einat H, Manji HK, Gould TD, Du J, Chen G. Possible involvement of the ERK signaling cascade in bipolar disorder: behavioral leads from the study of mutant mice. Drug News Perspect 2003;16:453–63.

141. Chen YL, Law PY, Loh HH. Sustained activation of phosphatidylinositol 3-kinase/Akt/nuclear factor B signaling mediates G protein-coupled -opioid receptor gene expression. J Biol Chem 2006;281:3067–74.
142. Berhow MT, Hiroi N, Nestler EJ. Regulation of ERK (extracellular signal regulated kinase), part of the neurotrophin signal transduction cascade, in the rat mesolimbic dopamine system by chronic exposure to morphine or cocaine. J Neurosci 1996;16:4707–15.
143. Valjent E, Corvol JC, Pagès C, Besson MJ, Maldonado R, Caboche J. Involvement of the extracellular signal-regulated kinase cascade for cocaine-rewarding properties. J Neurosci 2000;20:8701–9.
144. Jenab S, Festa ED, Nazarian A et al. Cocaine induction of ERK proteins in dorsal striatum of Fischer rats. Brain Res Mol Brain Res 2005;142:134–8.
145. Radwanska K, Caboche J, Kaczmarek L. Extracellular signal-regulated kinases (ERKs) modulate cocaine-induced gene expression in the mouse amygdala. Eur J Neurosci 2005;22:939–48.
146. Valjent E, Pages C, Herve D, Girault JA, Caboche J. Addictive and non-addictive drugs induce distinct and specific patterns of ERK activation in mouse brain. Eur J Neurosci 2004;19:1826–36.
147. Salzmann J, Marie-Claire C, Le Guen S, Roques BP, Noble F. Importance of ERK activation in behavioral and biochemical effects induced by MDMA in mice. Br J Pharmacol 2003;140:831–8.
148. Gerdjikov TV, Ross GM, Beninger RJ. Place preference induced by nucleus accumbens amphetamine is impaired by antagonists of ERK or p38 MAP kinases in rats. Behav Neurosci 2004;118:740–50.
149. Pierce RC, Pierce-Bancroft AF, Prasad BM. Neurotrophin-3 contributes to the initiation of behavioral sensitization to cocaine by activating the Ras/Mitogen-activated protein kinase signal transduction cascade. J Neurosci 1999;19:8685–95.
150. Valjent E, Pascoli V, Svenningsson P et al. Regulation of a protein phosphatase cascade allows convergent dopamine and glutamate signals to activate ERK in the striatum. Proc Natl Acad Sci USA 2005;102:491–6.
151. Zhang L, Lou D, Jiao H et al. Cocaine-induced intracellular signaling and gene expression are oppositely regulated by the dopamine D1 and D3 receptors. J Neurosci 2004;24: 3344–54.
152. Ferguson SM, Fasano S, Yang P, Brambilla R, Robinson TE. Knockout of ERK1 enhances cocaine-evoked immediate early gene expression and behavioral plasticity. Neuropsychopharmacology 2006;31:2660–8.
153. Girault JA, Valjent E, Caboche J, Hervé D. ERK2: a logical AND gate critical for drug-induced plasticity? Curr Opin Pharmacol 2007;7:77–85.
154. Vantaggiato C, Formentini I, Bondanza A, Bonini C, Naldini L, Brambilla R. ERK1 and ERK2 mitogen-activated protein kinases affect Ras-dependent cell signaling differentially. J Biol 2006;5:14.
155. Pagès G, Guérin S, Grall D et al. Defective thymocyte maturation in p44 MAP kinase (Erk 1) knockout mice. Science 1999;286:1374–7.
156. Mazzucchelli C, Vantaggiato C, Ciamei A et al. Knockout of ERK1 MAP kinase enhances synaptic plasticity in the striatum and facilitates striatal-mediated learning and memory. Neuron 2002;34:807–20.
157. Brami-Cherrier K, Valjent E, Garcia M, Pagès C, Hipskind RA, Caboche J. Dopamine induces a PI3-kinase-independent activation of Akt in striatal neurons: A new route to cAMP response element-binding protein phosphorylation. J Neurosci 2002;22:8911–21.
158. Voulalas PJ, Holtzclaw L, Wolstenholme J, Russell JT, Hyman SE. Metabotropic glutamate receptors and dopamine receptors cooperate to enhance extracellular signal-regulated kinase phosphorylation in striatal neurons. J Neurosci 2005;25:3763–73.
159. Zhen XC, Uryu K, Wang HY, Friedman E. D_1 dopamine receptor agonists mediate activation of p38 mitogen-activated protein kinase and c-jun amino-terminal kinase by a protein

kinase a dependent mechanism in SK-N-MC human neuroblastoma cells. Mol Pharmacol 1998;54:453–8.
160. Chen J, Rusnak M, Luedtke RR, Sidhu A. D1 dopamine receptor mediates dopamine-induced cytotoxicity via the ERK signal cascade. J Biol Chem 2004;279:39317–30.
161. Zhuang S, Schnellmann RG. A death-promoting role for extracellular signal-regulated kinase. J Pharmacol Exp Ther 2006;319:991–7.
162. Stephans SE, Yamamoto BK. Methamphetamine-induced neurotoxicity: roles for glutamate and dopamine efflux. Synapse 1994;17:203–9.
163. LaVoie MJ, Hastings TG. Dopamine quinone formation and protein modification associated with the striatal neurotoxicity of methamphetamine: evidence against a role for extracellular dopamine. J Neurosci 1999;19:1484–91.
164. Gerfen CR, Miyachi S, Paletzki R, Brown P. D1 dopamine receptor supersensitivity in the dopamine-depleted striatum results from a switch in the regulation of ERK1/2/MAP kinase. J Neurosci 2002;22:5042–54.
165. Jiao H, Zhang L, Gao F, Lou D, Zhang J, Xu M. Dopamine D_1 and D_3 receptors oppositely regulate NMDA- and cocaine-induced MAPK signaling via NMDA receptor phosphorylation. J Neurochem 2007;103:840–8.
166. Runyan JD, Dash PK. Intra-medial prefrontal administration of SCH-23390 attenuates ERK phosphorylation and long-term memory for trace fear conditioning in rats. Neurobiol Learn Mem 2004;82:65–70.
167. Bertran-Gonzalez J, Bosch C, Maroteaux M et al. Opposing patterns of signaling activation in dopamine D_1 and D_2 receptor-expressing striatal neurons in response to cocaine and haloperidol. J Neurosci 2008;28:5671–85.
168. Faure M, Voyno-Yasenetskaya TA, Bourne HR. cAMP and $\beta\ \gamma$ subunits of heterotrimeric G proteins stimulate the mitogen-activated protein kinase pathway in COS-7 cells. J Biol Chem 1994;269:7851–4.
169. Luo YQ, Kokkonen GC, Wang XT, Neve KA, Roth GS. D2 dopamine receptors stimulate mitogenesis through pertussis toxin-sensitive G proteins and ras-involved ERK and SAP/JNK pathways in rat C6-D2L glioma cells. J Neurochem 1998;71:980–90.
170. Choi EY, Jeong DW, Park KW, Baik JH. G protein-mediated mitogen-activated protein kinase activation by two dopamine D2 receptors. Biochem Biophys Res Comm 1999;256:33–40.
171. Kim SJ, Kim MY, Lee EJ, Ahn YS, Baik JH. Distinct regulation of internalization and mitogen-activated protein kinase activation by two isoforms of the dopamine D2 receptor. Mol Endocrinol 2004;18:640–52.
172. Lan H, Liu Y, Bell MI, Gurevich VV, Neve KA. A dopamine D_2 receptor mutant capable of G protein-mediated signaling but deficient in arrestin binding. Mol Pharmacol 2009; 75: 113–23.
173. Oak JN, Lavine N, Van Tol HHM. Dopamine D_4 and D_{2L} receptor stimulation of the mitogen-activated protein kinase pathway is dependent on transactivation of the platelet-derived growth factor receptor. Mol Pharmacol 2001;60:92–103.
174. Wang C, Buck DC, Yang R, Macey TA, Neve KA. Dopamine D2 receptor stimulation of mitogen-activated protein kinases mediated by cell type-dependent transactivation of receptor tyrosine kinases. J Neurochem 2005;93:899–909.
175. Beom S, Cheong D, Torres G, Caron MG, Kim KM. Comparative studies of molecular mechanisms of dopamine D_2 and D_3 receptors for the activation of extracellular signal regulated kinase. J Biol Chem 2004.
176. Lajiness ME, Chio CL, Huff RM. D2 dopamine receptor stimulation of mitogenesis in transfected Chinese hamster ovary cells: relationship to dopamine stimulation of tyrosine phosphorylations. J Pharmacol Exp Ther 1993;267:1573–81.
177. Kim SY, Choi KC, Chang MS et al. The dopamine D_2 receptor regulates the development of dopaminergic neurons via extracellular signal-regulated kinase and Nurr1 activation. J Neurosci 2006;26:4567–76.

178. Cai GP, Zhen XC, Uryu K, Friedman E. Activation of extracellular signal-regulated protein kinases is associated with a sensitized locomotor response to D_2 dopamine receptor stimulation in unilateral 6-hydroxydopamine-lesioned rats. J Neurosci 2000;20: 1849–57.
179. Browning JL, Patel T, Brandt PC, Young KA, Holcomb LA, Hicks PB. Clozapine and the mitogen-activated protein kinase signal transduction pathway: implications for antipsychotic actions. Biol Psychiatry 2005;57:617–23.
180. Van Ham II, Banihashemi B, Wilson AM, Jacobsen KX, Czesak M, Albert PR. Differential signaling of dopamine-D_2S and -D_2L receptors to inhibit ERK1/2 phosphorylation. J Neurochem 2007;102:1796–804.
181. Yang BH, Son H, Kim SH, Nam JH, Choi JH, Lee JS. Phosphorylation of ERK and CREB in cultured hippocampal neurons after haloperidol and risperidone administration. Psychiatry Clin Neurosci 2004;58:262–7.
182. Banihashemi B, Albert PR. Dopamine-D2S receptor inhibition of calcium influx, adenylyl cyclase, and mitogen-activated protein kinase in pituitary cells: distinct Gα and Gβ gamma requirements. Mol Endocrinol 2002;16:2393–404.
183. Liu JC, Baker RE, Sun C, Sundmark VC, Elsholtz HP. Activation of G_o-coupled dopamine D2 receptors inhibits ERK1/ERK2 in pituitary cells – A key step in the transcriptional suppression of the prolactin gene. J Biol Chem 2002;277:35819–25.
184. Iaccarino C, Samad TA, Mathis C, Kercret H, Picetti R, Borrelli E. Control of lactotrop proliferation by dopamine: essential role of signaling through D2 receptors and ERKs. Proc Natl Acad Sci USA 2002;99:14530–5.
185. Song G, Ouyang G, Bao S. The activation of Akt/PKB signaling pathway and cell survival. J Cell Mol Med 2005;9:59–71.
186. Datta SR, Brunet A, Greenberg ME. Cellular survival: a play in three Akts. Genes Dev 1999;13:2905–27.
187. Du K, Tsichlis PN. Regulation of the Akt kinase by interacting proteins. Oncogene 2005;24:7401–9.
188. Beaulieu JM, Tirotta E, Sotnikova TD et al. Regulation of Akt signaling by D_2 and D_3 dopamine receptors *in vivo*. J Neurosci 2007;27:881–5.
189. Wei Y, Williams JM, Dipace C et al. Dopamine transporter activity mediates amphetamine-induced inhibition of Akt through a Ca^{2+}/calmodulin-dependent kinase II-dependent mechanism. Mol Pharmacol 2007;71:835–42.
190. Shi X, McGinty JF. Repeated amphetamine treatment increases phosphorylation of extracellular signal-regulated kinase, protein kinase B, and cyclase response element-binding protein in the rat striatum. J Neurochem 2007;103:706–13.
191. Beaulieu JM, Sotnikova TD, Yao W-D et al. Lithium antagonizes dopamine-dependent behaviors mediated by an AKT/glycogen synthase kinase 3 signaling cascade. Proc Natl Acad Sci USA 2004;101:5099–104.
192. Emamian ES, Hall D, Birnbaum MJ, Karayiorgou M, Gogos JA. Convergent evidence for impaired AKT1-GSK3β signaling in schizophrenia. Nat Genet 2004;36:131–7.
193. Schwab SG, Hoefgen B, Hanses C et al. Further evidence for association of variants in the AKT1 gene with schizophrenia in a sample of European sib-pair families. Biol Psychiatry 2005;58:446–50.
194. Tan HY, Nicodemus KK, Chen Q et al. Genetic variation in *AKT1* is linked to dopamine-associated prefrontal cortical structure and function in humans. J Clin Invest 2008;118: 2200–8.
195. Thiselton DL, Vladimirov VI, Kuo PH et al. AKT1 is associated with schizophrenia across multiple symptom dimensions in the Irish study of high density schizophrenia families. Biol Psychiatry 2008;63:449–57.
196. Braff DL, Geyer MA, Swerdlow NR. Human studies of prepulse inhibition of startle: normal subjects, patient groups, and pharmacological studies. Psychopharmacology (Berl) 2001;156:234–58.

197. Nair VD, Sealfon SC. Agonist-specific transactivation of phosphoinositide 3-kinase signaling pathway mediated by the dopamine D_2 receptor. J Biol Chem 2003;278: 47053–61.
198. Nair VD, Olanow CW, Sealfon SC. Activation of phosphoinositide 3-kinase by D_2 receptor prevents apoptosis in dopaminergic cell lines. Biochem J 2003;373:25–32.
199. Nair VD, Olanow CW. Differential modulation of Akt/glycogen synthase kinase-3β pathway regulates apoptotic and cytoprotective signaling responses. J Biol Chem 2008;283: 15469–78.
200. Zhen XC, Zhang J, Johnson GP, Friedman E. D_4 dopamine receptor differentially regulates Akt/nuclear factor-kappaB and extracellular signal-regulated kinase pathways in D_4MN9D cells. Mol Pharmacol 2001;60:857–64.
201. Alimohamad H, Rajakumar N, Seah YH, Rushlow W. Antipsychotics alter the protein expression levels of beta-catenin and GSK-3 in the rat medial prefrontal cortex and striatum. Biol Psychiatry 2005;57:533–42.
202. Beaulieu JM, Marion S, Rodriguiz RM et al. A βarrestin 2 signaling complex mediates lithium action on behavior. Cell 2008;132:125–36.
203. Ikeda M, Ozaki N, Suzuki T et al. Possible association of β-arrestin 2 gene with methamphetamine use disorder, but not schizophrenia. Genes Brain Behav 2007;6:107–12.
204. Clark D, White FJ. Review: D1 dopamine receptor – the search for a function: a critical evaluation of the D1/D2 dopamine receptor classification and its functional implications. Synapse 1987;1:347–88.
205. Marshall JF, Ruskin DN, Lahoste GJ. D1/D2 dopamine receptor interactions in basal ganglia. In: Neve KA, Neve RL, eds. The dopamine receptors. Totawa, NJ: Humana Press, 1997: 193–219.
206. Schmidt HD, Pierce RC. Cooperative activation of D1-like and D2-like dopamine receptors in the nucleus accumbens shell is required for the reinstatement of cocaine-seeking behavior in the rat. Neuroscience 2006;142:451–61.
207. Braun AR, Chase TN. Obligatory D-1/D-2 receptor interaction in the generation of dopamine agonist related behaviors. Eur J Pharmacol 1986;131:301–6.
208. Hopf FW, Cascini MG, Gordon AS, Diamond I, Bonci A. Cooperative activation of dopamine D_1 and D_2 receptors increases spike firing of nucleus accumbens neurons via G-protein β γ subunits. J Neurosci 2003;23:5079–87.
209. Wu J, Hablitz JJ. Cooperative activation of D $_1$ and D_2 dopamine receptors enhances a hyperpolarization-activated inward current in layer I interneurons. J Neurosci 2005;25:6322–8.
210. Watts VJ, Neve KA. Activation of type II adenylate cyclase by D_2 and D_4 but not D_3 dopamine receptors. Mol Pharmacol 1997;52:181–6.
211. Bertorello AM, Hopfield JF, Aperia A, Greengard P. Inhibition by dopamine of $(Na^+ + K^+)$ATPase activity in neostriatal neurons through D_1 and D_2 dopamine receptor synergism. Nature 1990;347:386–8.
212. Watts VJ, Neve KA. Sensitization of adenylate cyclase by $G\alpha_{i/o}$-coupled receptors. Pharmacol Ther 2005;106:405–21.
213. Schwartz JC, Diaz J, Bordet R et al. Functional implications of multiple dopamine receptor subtypes: the D_1/D_3 receptor coexistence. Brain Res Rev 1998;26:236–42.
214. Karasinska JM, George SR, El Ghundi M, Fletcher PJ, O'Dowd BF. Modification of dopamine D_1 receptor knockout phenotype in mice lacking both dopamine D_1 and D_3 receptors. Eur J Pharmacol 2000;399:171–81.
215. Wong JYF, Clifford JJ, Massalas JS, Kinsella A, Waddington JL, Drago J. Essential conservation of D_1 mutant phenotype at the level of individual topographies of behaviour in mice lacking both D_1 and D_3 dopamine receptors. Psychopharmacology 2003;167:167–73.
216. Lahoste GJ, Henry BL, Marshall JF. Dopamine D_1 receptors synergize with D_2, but not D_3 or D_4, receptors in the striatum without the involvement of action potentials. J Neurosci 2000;20:6666–71.

217. Fetsko LA, Xu R, Wang YY. Alterations in D1/D2 synergism may account for enhanced stereotypy and reduced climbing in mice lacking dopamine D2L receptor. Brain Res 2003;967:191–200.
218. Usiello A, Baik JH, Rouge-Pont F et al. Distinct functions of the two isoforms of dopamine D_2 receptors. Nature 2000;408:199–203.
219. Watts VJ, Neve KA. Sensitization of endogenous and recombinant adenylate cyclase by activation of D_2 dopamine receptors. Mol Pharmacol 1996;50:966–76.
220. Culm KE, Lugo-Escobar N, Hope BT, Hammer RP, Jr. Repeated quinpirole treatment increases cAMP-dependent protein kinase activity and CREB phosphorylation in nucleus accumbens and reverses quinpirole-induced sensorimotor gating deficits in rats. Neuropsychopharmacology 2004;29:1823–30.
221. Chester JA, Mullins AJ, Nguyen CH, Watts VJ, Meisel RL. Repeated quinpirole treatments produce neurochemical sensitization and associated behavioral changes in female hamsters. Psychopharmacology (Berl) 2006;188:53–62.
222. Sunahara RK, Taussig R. Isoforms of mammalian adenylyl cyclase: multiplicities of signaling. Mol Interv 2002;2:168–84.
223. Avidor-Reiss T, Nevo I, Levy R, Pfeuffer T, Vogel Z. Chronic opioid treatment induces adenylyl cyclase V superactivation: involvement of $G\beta\gamma$. J Biol Chem 1996;271:21309–15.
224. Thomas JM, Hoffman BB. Isoform-specific sensitization of adenylyl cyclase activity by prior activation of inhibitory receptors: role of $\beta\gamma$ subunits in transducing enhanced activity of the type VI isoform. Mol Pharmacol 1996;49:907–14.
225. Rhee MH, Nevo I, Avidor-Reiss T, Levy R, Vogel Z. Differential superactivation of adenylyl cyclase isozymes after chronic activation of the CB_1 cannabinoid receptor. Mol Pharmacol 2000;57:746–52.
226. Bates MD, Senogles SE, Bunzow JR, Liggett SB, Civelli O, Caron MG. Regulation of responsiveness at D_2 dopamine receptors by receptor desensitization and adenylyl cyclase sensitization. Mol Pharmacol 1991;39:55–63.
227. Ivins KJ, Luedtke RR, Artymyshyn RP, Molinoff PB. Regulation of dopamine D2 receptors in a novel cell line (SUP1). Mol Pharmacol 1991;39:531–9.
228. Filtz TM, Guan W, Artymyshyn RP, Pacheco M, Ford C, Molinoff PB. Mechanisms of up-regulation of $D2_L$ dopamine receptors by agonists and antagonists in transfected HEK-293 cells. J Pharmacol Exp Ther 1994;271:1574–82.
229. Zhang L-J, Lachowicz JE, Sibley DR. The D_{2S} and D_{2L} dopamine receptor isoforms are differentially regulated in Chinese hamster ovary cells. Mol Pharmacol 1994;45:878–89.
230. Thomas JM, Hoffman BB. Adenylate cyclase supersensitivity: a general means of cellular adaptation to inhibitory agonists? TIPS 1987;8:308–11.
231. Zhang XF, Hu XT, White FJ. Whole-cell plasticity in cocaine withdrawal: reduced sodium currents in nucleus accumbens neurons. J Neurosci 1998;18:488–98.
232. Schoffelmeer AN, Voorn P, Jonker AJ et al. Morphine-induced increase in D-1 receptor regulated signal transduction in rat striatal neurons and its facilitation by glucocorticoid receptor activation: possible role in behavioral sensitization. Neurochem Res 1996;21: 1417–23.
233. Bradley KC, Meisel RL. Sexual behavior induction of c-Fos in the nucleus accumbens and amphetamine-stimulated locomotor activity are sensitized by previous sexual experience in female Syrian hamsters. J Neurosci 2001;21:2123–30.
234. Bradley KC, Mullins AJ, Meisel RL, Watts VJ. Sexual experience alters D1 receptor-mediated cyclic AMP production in the nucleus accumbens of female Syrian hamsters. Synapse 2004;53:20–7.
235. Meisel RL, Joppa MA, Rowe RK. Dopamine receptor antagonists attenuate conditioned place preference following sexual behavior in female Syrian hamsters. Eur J Pharmacol 1996;309:21–4.
236. Bunney BS, Aghajanian GK, Roth RH. Comparison of effects of L-dopa, amphetamine and apomorphine on firing rate of rat dopaminergic neurones. Nat New Biol 1973;245:123–5.

237. Aghajanian GK, Bunney BS. Pharmacological characterization of dopamine "autoreceptors" by microiontophoretic single-cell recording studies. Adv Biochem Psychopharmacol 1977;16:433–8.
238. Pinnock RD. Sensitivity of compacta neurones in the rat substantia nigra slice to dopamine agonists. Eur J Pharmacol 1983;96:269–76.
239. Lacey MG, Mercuri NB, North RA. Dopamine acts on D2 receptors to increase potassium conductance in neurones of rat substantia nigra zona compacta. J Physiol (Lond) 1987;392:397–416.
240. Kehr W, Carlsson A, Lindqvist M, Magnusson T, Atack C. Evidence for a receptor-mediated feedback control of striatal tyrosine hydroxylase activity. J Pharm Pharmacol 1972;24: 744–7.
241. Farnebo LO, Hamberger B. Drug-induced changes in the release of 3 H-monoamines from field stimulated rat brain slices. Acta Physiol Scand Suppl 1971;371:35–44.
242. Meiergerd SM, Patterson TA, Schenk JO. D_2 receptors may modulate the function of the striatal transporter for dopamine: kinetic evidence from studies in vitro and in vivo. J Neurochem 1993;61:764–7.
243. Cass WA, Gerhardt GA. Direct in vivo evidence that D2 dopamine receptors can modulate dopamine uptake. Neurosci Lett 1994;176:259–63.
244. Wu Q, Reith ME, Walker QD, Kuhn CM, Carroll FI, Garris PA. Concurrent autoreceptor-mediated control of dopamine release and uptake during neurotransmission: an in vivo voltammetric study. J Neurosci 2002;22:6272–81.
245. Mayfield RD, Zahniser NR. Dopamine D_2 receptor regulation of the dopamine transporter expressed in *Xenopus laevis* oocytes is voltage-independent. Mol Pharmacol 2001;59: 113–21.
246. O'Hara CM, Uhland-Smith A, O'Malley KL, Todd RD. Inhibition of dopamine synthesis by dopamine D2 and D3 but not D4 receptors. J Pharmacol Exp Ther 1996;277:186–92.
247. Tang L, Todd RD, Heller A, O'Malley KL. Pharmacological and functional characterization of D_2, D_3 and D_4 dopamine receptors in fibroblast and dopaminergic cell lines. J Pharmacol Exp Ther 1994;268:495–502.
248. Zapata A, Kivell B, Han Y et al. Regulation of dopamine transporter function and cell surface expression by D3 dopamine receptors. J Biol Chem 2007;282:35842–54.
249. Levant B. The D_3 dopamine receptor: neurobiology and potential clinical relevance. Pharmacol Rev 1997;49:231–52.
250. L'hirondel M, Chéramy A, Godeheu G et al. Lack of autoreceptor-mediated inhibitory control of dopamine release in striatal synaptosomes of D2 receptor-deficient mice. Brain Res 1998;792:253–62.
251. Benoit-Marand M, Borrelli E, Gonon F. Inhibition of dopamine release via presynaptic D2 receptors: time course and functional characteristics *in vivo*. J Neurosci 2001;21:9134–41.
252. Schmitz Y, Schmauss C, Sulzer D. Altered dopamine release and uptake kinetics in mice lacking D_2 receptors. J Neurosci 2002;22:8002–9.
253. Zapata A, Shippenberg TS. Lack of functional D2 receptors prevents the effects of the D3-preferring agonist (+)-PD 128907 on dialysate dopamine levels. Neuropharmacology 2005;48:43–50.
254. Davila V, Yan Z, Craciun LC, Logothetis D, Sulzer D. D_3 dopamine autoreceptors do not activate G-protein-gated inwardly rectifying potassium channel currents in substantia nigra dopamine neurons. J Neurosci 2003;23:5693–7.
255. Beckstead MJ, Grandy DK, Wickman K, Williams JT. Vesicular dopamine release elicits an inhibitory postsynaptic current in midbrain dopamine neurons. Neuron 2004;42:939–46.
256. Mercuri NB, Saiardi A, Bonci A et al. Loss of autoreceptor function in dopaminergic neurons from dopamine D_2 receptor deficient mice. Neuroscience 1997;79:323–7.
257. Dickinson SD, Sabeti J, Larson GA et al. Dopamine D2 receptor-deficient mice exhibit decreased dopamine transporter function but no changes in dopamine release in dorsal striatum. J Neurochem 1999;72:148–56.

258. Weber B, Schlicker E, Sokoloff P, Stark H. Identification of the dopamine autoreceptor in the guinea-pig retina as D_2 receptor using novel subtype-selective antagonists. Br J Pharmacol 2001;133:1243–8.
259. Koeltzow TE, Xu M, Cooper DC et al. Alterations in dopamine release but not dopamine autoreceptor function in dopamine D_3 receptor mutant mice. J Neurosci 1998;18:2231–8.
260. Joseph JD, Wang YM, Miles PR et al. Dopamine autoreceptor regulation of release and uptake in mouse brain slices in the absence of D_3 receptors. Neuroscience 2002;112:39–49.
261. Zapata A, Witkin JM, Shippenberg TS. Selective D3 receptor agonist effects of (+)-PD 128907 on dialysate dopamine at low doses. Neuropharmacology 2001;41:351–9.
262. Wang Y, Xu R, Sasaoka T, Tonegawa S, Kung MP, Sankoorikal EB. Dopamine D2 long receptor-deficient mice display alterations in striatum-dependent functions. J Neurosci 2000;20:8305–14.
263. Lindgren N, Usiello A, Goiny M et al. Distinct roles of dopamine D2L and D2S receptor isoforms in the regulation of protein phosphorylation at presynaptic and postsynaptic sites. Proc Natl Acad Sci USA 2003;100:4305.
264. Centonze D, Usiello A, Gubellini P et al. Dopamine D2 receptor-mediated inhibition of dopaminergic neurons in mice lacking D2L receptors. Neuropsychopharmacology 2002;27:723–6.
265. Jomphe C, Tiberi M, Trudeau LE. Expression of D2 receptor isoforms in cultured neurons reveals equipotent autoreceptor function. Neuropharmacology 2006;50:595–605.
266. Montmayeur JP, Bausero P, Amlaiky N, Maroteaux L, Hen R, Borrelli E. Differential expression of the mouse D_2 dopamine receptor isoforms. FEBS Lett 1991;278:239–43.
267. Uchida S, Akaike N, Nabekura J. Dopamine activates inward rectifier K^+ channel in acutely dissociated rat substantia nigra neurones. Neuropharmacology 2000;39:191–201.
268. Kim KM, Nakajima Y, Nakajima S. G protein-coupled inward rectifier modulated by dopamine agonists in cultured substantia nigra neurons. Neuroscience 1995;69:1145–58.
269. Liss B, Roeper J. Individual dopamine midbrain neurons: functional diversity and flexibility in health and disease. Brain Res Rev 2008;58:314–21.
270. Cass WA, Zahniser NR. Potassium channel blockers inhibit D_2 dopamine, but not A1 adenosine, receptor-mediated inhibition of striatal dopamine release. J Neurochem 1991;57: 147–52.
271. Congar P, Bergevin A, Trudeau LE. D2 receptors inhibit the secretory process downstream from calcium influx in dopaminergic neurons: implication of K^+ channels. J Neurophysiol 2002;87:1046–56.
272. Cardozo DL, Bean BP. Voltage-dependent calcium channels in rat midbrain dopamine neurons: modulation by dopamine and $GABA_B$ receptors. J Neurophysiol 1995;74: 1137–48.
273. Puopolo M, Raviola E, Bean BP. Roles of subthreshold calcium current and sodium current in spontaneous firing of mouse midbrain dopamine neurons. J Neurosci 2007;27:645–56.
274. Harada, Wu J, Haycock JW, Goldstein M. Regulation of L-DOPA biosynthesis by site-specific phosphorylation of tyrosine hydroxylase in AtT-20 cells expressing wild-type and serine 40-substituted enzyme. J Neurochem 1996;67:629–35.
275. Lew JY, Garcia-Espana A, Lee KY et al. Increased site-specific phosphorylation of tyrosine hydroxylase accompanies stimulation of enzymatic activity induced by cessation of dopamine neuronal activity. Mol Pharmacol 1999;55:202–9.
276. Lindgren N, Xu ZQD, Herrera-Marschitz M, Haycock J, Hökfelt T, Fisone G. Dopamine D_2 receptors regulate tyrosine hydroxylase activity and phosphorylation at Ser40 in rat striatum. Eur J Neurosci 2001;13:773–80.
277. Pothos EN, Przedborski S, Davila V, Schmitz Y, Sulzer D. D_2-like dopamine autoreceptor activation reduces quantal size in PC12 cells. J Neurosci 1998;18:5575–85.
278. Haycock JW, Ahn NG, Cobb MH, Krebs EG. ERK1 and ERK2, two microtubule-associated protein 2 kinases, mediate the phosphorylation of tyrosine hydroxylase at serine-31 in situ. Proc Natl Acad Sci U S A 1992;89:2365–9.

279. Lindgren N, Goiny M, Herrera-Marschitz M, Haycock JW, Hökfelt T, Fisone G. Activation of extracellular signal-regulated kinases 1 and 2 by depolarization stimulates tyrosine hydroxylase phosphorylation and dopamine synthesis in rat brain. Eur J Neurosci 2002;15:769–73.
280. Morón JA, Zakharova I, Ferrer JV et al. Mitogen-activated protein kinase regulates dopamine transporter surface expression and dopamine transport capacity. J Neurosci 2003;23:8480–8.
281. Bolan EA, Kivell B, Jaligam V et al. D_2 receptors regulate dopamine transporter function via an extracellular signal-regulated kinases 1 and 2-dependent and phosphoinositide 3 kinase-independent mechanism. Mol Pharmacol 2007;71:1222–32.
282. Lee FJ, Pei L, Moszczynska A, Vukusic B, Fletcher PJ, Liu F. Dopamine transporter cell surface localization facilitated by a direct interaction with the dopamine D2 receptor. EMBO J 2007;26:2127–36.
283. Lammel S, Hetzel A, Hackel O, Jones I, Liss B, Roeper J. Unique properties of mesoprefrontal neurons within a dual mesocorticolimbic dopamine system. Neuron 2008;57:760–73.
284. Margolis EB, Mitchell JM, Ishikawa J, Hjelmstad GO, Fields HL. Midbrain dopamine neurons: projection target determines action potential duration and dopamine D_2 receptor inhibition. J Neurosci 2008;28:8908–13.
285. Jose PA, Eisner GM, Felder RA. Renal dopamine receptors in health and hypertension. Pharmacol Ther 1998;80:149–82.

Chapter 7
Dopaminergic Modulation of Glutamatergic Signaling in Striatal Medium Spiny Neurons

Weixing Shen and D. James Surmeier

Abstract Dopamine (DA) controls a wide variety of striatal functions, including action selection and associative learning. This is achieved by modulating cortical and thalamic glutamatergic synapses formed on principal medium spiny neurons (MSNs) and the way in which these signals are processed. Accumulating evidence suggests that D_1 receptor signaling enhances dendritic excitability and glutamatergic signaling in striatonigral MSNs, whereas D_2 receptor signaling exerts the opposite effect in striatopallidal MSNs. The functional antagonism between these two major striatal DA receptors extends to the regulation of synaptic plasticity. Using brain slices from DA receptor transgenic mice, recent studies have uncovered important differences between MSNs that shape long-term alterations in glutamatergic signaling with experience. These results are consistent with network models of striatal function, suggesting that DA acts in a push–pull fashion in action selection. Work in models of Parkinson's disease has shown this bidirectionality is lost following DA depletion, pointing to the mechanisms underlying network dysfunction in this disease as well as in others with strong DA determinants like drug abuse.

Keywords Long-term potentiation · Long-term depression · Spike-timing-dependent plasticity · Basal ganglia · Voltage-dependent channels · Dendrites · Parkinson's disease models

7.1 Introduction

Dopamine (DA) plays a crucial role in regulating cortical and thalamic signals carried by glutamatergic synapses on the principal neurons of the striatum – medium spiny neurons (MSNs). The regulation of this circuitry by DA is important to a wide

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array of striatal functions, such as associative learning and action selection [1–4]. In spite of its significance, effort to understand how this modulation is exerted has met with only a modest success. One obstacle is cellular heterogeneity within the striatum; there are at least two major populations of MSN that differ along several dimensions, including their expression of DA receptors [5, 6]. However, these subpopulations are difficult to unequivocally distinguish on the basis of their somatodendritic morphology or electrophysiological properties [7]. Moreover, both cell types are imbedded in a rich neuronal network involving both MSNs and interneurons that is modulated by DA. This has made it extremely difficult to sort out what DA is doing directly and what it is doing indirectly through effects on network properties. The recent development of mouse lines in which neurons "report" their expression of D_1 or D_2 receptors by co-expressing enhanced green fluorescent protein (eGFP) has greatly accelerated our pace of discovery. Electrophysiological interrogation of visually identified MSNs in tissue slices from these mice has revealed an unexpected array of functional differences between these cell types [8–13]. Another obstacle is that DA receptors are primarily found in dendrites that are inaccessible to conventional patch clamp techniques, making direct study of their actions on glutamatergic signaling and dendritic excitability difficult. Optical techniques, like two-photon laser scanning microscopy (2PLSM), are giving us access to these regions and providing new insights into their physiology and modulation by DA.

In this review, we will focus our discussion to (1) DA modulation of postsynaptic properties that influence glutamatergic synaptic events and their integration by MSNs and (2) DA modulation of the induction of plasticity at MSN glutamatergic synapses. Only the actions of the principal DA receptors in this region (D_1, D_2 receptors) will be discussed. Even with this rather narrow focus, it is impossible to faithfully summarize what has become an enormous literature in the last decade. The reader is referred to several other recent reviews [14–17]. Moreover, there are several reviews discussing the impact of glutamate on dopaminergic neurons and DA release that would not be covered [18, 19].

7.2 The "Classical" View of DA Modulation

The now "classical" model of how DA shapes striatal activity was advanced almost two decades ago by Albin, Young, and Penny [20]. In this model, D_1 receptors excite MSNs of the "direct" striatonigral pathway whereas D_2 receptors inhibit MSNs of the "indirect" striatopallidal pathway. These were envisioned as acute, readily reversible effects. The evidence for this model stemmed almost entirely from indirect measures of neuronal activity (e.g., alterations in gene expression, glucose utilization, or receptor binding). Subsequent work has proven to be largely consistent with the general principles of this model, revealing that DA activation of G protein-coupled receptors (GPCRs) "excites" or "inhibits" MSNs by modulating the gating and trafficking of voltage-dependent and ligand-gated (ionotropic)

ion channels, essentially altering cellular excitability. However, as discussed below, there are also longer lasting alterations in synaptic strength induced by DA when there is a conjunction of pre- and postsynaptic activity. These lasting changes, rather than the acute effects of DA, are thought to underlie associative learning and action selection.

7.2.1 Modulation of Intrinsic Excitability and Glutamatergic Signaling by D_1 Receptors

Striatonigral MSNs in the so-called direct pathway express D_1 receptors at high levels [5, 6]. These receptors are positively coupled to adenylyl cyclase (type V) through G_{olf} [21]. Elevation in cytosolic cAMP levels leads to the activation of protein kinase A (PKA). PKA has a variety of intracellular targets that affect cellular excitability and glutamatergic signaling.

Several studies suggest that the D_1/PKA cascade has direct effects on AMPA and NMDA receptor function and trafficking. For example, D_1 receptor activation of PKA enhances surface expression of both AMPA and NMDA receptors through a process that is dependent on the phosphoprotein DARPP-32 [22, 23]. The precise mechanisms underlying the trafficking are still being pursued, but the tyrosine kinase Fyn and the protein phosphatase STEP (striatal-enriched phosphatase) appear to be important regulators of surface expression of glutamate receptors [24]. Trafficking and localization might also be affected by a direct interaction between D_1 and NMDA receptors [25, 26].

What is less clear is whether D_1 receptor stimulation has rapid effects on glutamate receptor gating. Although PKA phosphorylation of the NR1 subunit is capable of enhancing NMDA receptor currents [27], the presence of this modulation in MSNs is still in debate. In neurons in which the engagement of dendritic voltage-dependent ion channels has been minimized by dialyzing the cytoplasm with Cs^+, D_1 receptor agonists have little or no discernible effect on AMPA or NMDA receptor-mediated currents in the dorsal striatum [28]. However, in MSNs in which this has not been done, D_1 receptor stimulation rapidly enhances currents evoked by NMDA receptor stimulation [29]. The difference between these results suggests that the effect of D_1 receptors on NMDA receptor currents is indirect and mediated by voltage-dependent dendritic conductances that are taken out of play by blocking K^+ channels and clamping dendritic voltage. Indeed, blocking L-type Ca^{2+} channels, which open in the same voltage range as NMDA receptors (Mg^{2+} unblock), attenuates the D_1 receptor-mediated enhancement of NMDA receptor currents [30].

This type of interaction between voltage-dependent ion channels and ligand-gated channels appears to be common in neurons. Far from the passive entities envisioned 20 years ago, neuronal dendrites are richly invested with voltage-dependent ion channels that shape synaptic responses and plasticity. Although nearly all the studies of active dendrites to date have been in pyramidal neurons, there is evidence that similar mechanisms govern MSNs dendrites [31–33].

However, unlike pyramidal neurons, the dendrites of MSN are too small to accommodate an electrode, so indirect measures have been used to understand how DA modulates the ion channels that invest MSN dendrites. More recently, the combination of imaging (most notably 2PLSM) and patch clamp has been applied to MSN dendrites in organotypic culture and brain slices [31–33]; this approach offers a powerful alternative to conventional approaches, particularly when applied to tissue in which phenotypically homogenous neuronal populations are fluorescently tagged.

Voltage-dependent Na^+ channels were the first well-characterized targets of the D_1 receptor-signaling pathway in MSNs. Confirming inferences drawn from earlier work in tissue slices [34], voltage clamp work showed that D_1 receptor signaling led to a reduction in Na^+ channel availability without altering the voltage-dependence of fast activation or inactivation [35]. Subsequent work has shown that PKA phosphorylation of the pore-forming subunit of the Na^+ channel promotes activity-dependent entry into a non-conducting, slow inactivated state that can be reversed only by membrane hyperpolarization [36]. It is likely that the D_1 receptor modulation is mediated by phosphorylation of somatic Nav1.1 channels, as Nav1.6 channels are not efficiently phosphorylated by PKA [37]. The coupling of the D_1 receptor cascade to dendritic (as opposed to somatic) Nav1.1/Nav1.6 channels remains uncertain and the subcellular positioning of the scaffolding interactions necessary to bring about efficient phosphorylation of Na^+ channel subunits [37] has not been mapped in MSNs.

When the somatic membrane potential is held for several hundred milliseconds near the up-state (∼ –55 mV; Fig. 7.1) [38], D_1 receptor stimulation has a quite different effect than when it is held at nominal down-state potentials (∼ –80 mV). At this up-state membrane potential, the personality of the MSN is transformed, as the constellation of ion channels governing activity is re-configured. Perhaps the most dramatic change is the closure or inactivation of Kir2, Kv1, and Kv4 K^+ channels that oppose the depolarizing influences of glutamate receptors. In this state, D_1 receptor stimulation elevates (rather than lowers) the response to intrasomatic current injection [39]. The augmented response is attributable in part

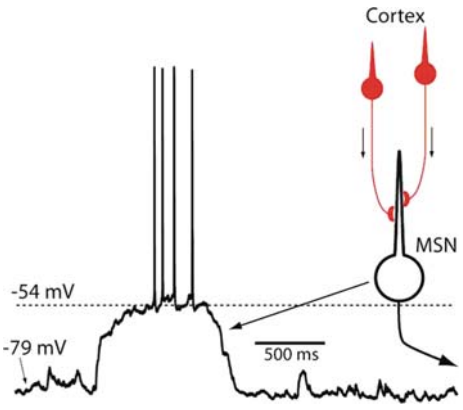

Fig. 7.1 Up- and down-states in MSNs. In the absence of convergent glutamatergic input, the membrane potential of MSNs resides close to the potassium equilibrium potential (∼–80 mV). This is called the down-state. In response to strong glutamatergic input, MSNs depolarize to a second preferred membrane potential around –55 mV, near spike threshold. This is called the up-state. Redrawn, with permission, from [92]

to enhanced opening of L-type Ca^{2+} channels following PKA phosphorylation [40, 41]. L-type channels with a pore-forming Cav1.3 subunit are likely to be major targets of this modulation; these channels have a voltage threshold near –60 mV and are anchored near glutamatergic synapses in spines through a scaffolding interaction with Shank [42]. Enhanced opening of these channels and NMDA receptors [29, 43–45] accounts for the ability of D_1 receptor stimulation to promote synaptically driven plateau potentials of MSNs (resembling up-states in vivo) in corticostriatal slices [46], as in cortical pyramidal neurons [47]. D_1 receptor stimulation also reduces opening of Cav2 Ca^{2+} channels that couple to somatic SK K^+ channels [48], potentially further augmenting dendritic electrogenesis but Cav2 channels appear not to be important in MSN dendrites [31].

Taken together, these results suggest that D_1 receptor signaling through PKA elevates the responsiveness of striatonigral neurons to sustained synaptic release of glutamate that generates up-states but reduces the response to transient or uncoordinated glutamate release that fails to significantly depolarize the dendritic membrane for more than a few tens of milliseconds from the down-state.

7.2.2 Modulation of Intrinsic Excitability and Glutamatergic Signaling by D_2 Receptors

D_2 receptors are expressed at high levels in neurons of the striatopallidal or "indirect" pathway. D_2 receptors couple to $G_{i/o}$ proteins, leading to inhibition of adenylyl cyclase through $G\alpha_i$ subunits [49]. In parallel, released $G\beta\gamma$ subunits are capable of reducing Cav2 Ca^{2+} channel opening and of stimulating phospholipase $C\beta$ isoforms, generating diacylglycerol (DAG) and protein kinase C (PKC) activation as well as inositol trisphosphate (IP3) liberation and the mobilization of intracellular Ca^{2+} stores [50, 51]. D_2 receptors also are capable of transactivating tyrosine kinases [52].

As with D_1 receptor signaling, there are a number of studies showing that D_2 receptor signaling alters glutamate receptor function in dorsal striatal MSNs. Activation of D_2 receptors has been reported to decrease AMPA receptor currents of MSNs recorded in tissue slices [29]. Subsequent work using acutely isolated neurons and voltage clamp techniques supports a direct action on dendritic AMPA receptors [53]. D_2 receptor signaling leads to dephosphorylation of S845 of GluR1 subunit, which should promote trafficking of AMPA receptors out of the synaptic membrane [54]. D_2 receptor stimulation also diminishes presynaptic release of glutamate [55]; however, it is not clear whether this is mediated by presynaptically or postsynaptically positioned D_2 receptors [56].

Studies of voltage-dependent channels are largely consistent with the proposition that D_2 receptors act to reduce the excitability of striatopallidal neurons and their response to glutamatergic synaptic input. D_2 receptor-mediated mobilization of intracellular Ca^{2+} leads to negative modulation of Cav1.3 Ca^{2+} channels through a calcineurin-dependent mechanism [42, 50]. D_2 receptor activation also reduces opening of voltage-dependent Na^+ channels, presumably by a PKC-mediated

enhancement of slow inactivation [35]. This coordinated modulation of ion channels provides a mechanistic foundation for the ability of D_2 receptor agonists to reduce the responsiveness of MSNs in slices at up-state membrane potentials [50].

7.3 Long-Term Depression of Glutamatergic Synaptic Transmission

Although it modulates short-term network activity, DA's role in associative learning and habit formation is commonly thought to be in the regulation of corticostriatal synaptic plasticity. The best-studied form of synaptic plasticity in the striatum is long-term depression (LTD). When postsynaptic depolarization is paired with high-frequency stimulation (HFS) of glutamatergic fibers, a long-lasting reduction in synaptic strength of glutamatergic synapses is seen in most MSNs. Unlike LTD induced by low-frequency stimulation in the ventral striatum [57], LTD induction in the dorsal striatum is not NMDA dependent. This form of LTD (HFS–LTD) is initiated postsynaptically, but expressed through a presynaptic reduction in glutamate release. There is a general agreement that striatal LTD requires activation of Cav1.3 L-type Ca^{2+} channels, G_q-linked mGluR1/5 receptors, and the generation of endocannabinoids (ECs). ECs exert their effect presynaptically by acting at CB1 receptors [58–60]. There is less agreement that activation of D_2 receptors is necessary for LTD induction. Activation of D_2 receptors is a very potent stimulus for EC production [61] and the ability of D_2 receptors to activate PLC [50] certainly is consistent with a direct involvement in EC production. However, attempts to test for the necessity of D_2 receptor expression using bacterial artificial chromosome (BAC) mice have met with mixed results [11, 13]. Kreitzer and Malenka (2007) [11] reported that LTD was inducible only in striatopallidal MSNs using a minimal local stimulation. However, our group and Lovinger's found that HFS–LTD was inducible in both striatonigral and striatopallidal MSNs when using macroelectrode stimulation of the cortex [13], consistent with the high probability of induction seen in previous work [62]. We have reproduced the Kreitzer and Malenka finding using minimal local stimulation, suggesting that the method of induction is important. This result underscores the difficulties inherent in stimulation paradigms that do not activate just glutamatergic fibers, but also a heterogeneous population of dopaminergic, cholinergic, and interneuronal fibers that might influence the induction of plasticity. An example of how we have attempted to sort this out is given below.

One strategy for gaining better control over which fibers are activated in studies of plasticity is to develop in vitro preparations that preserve connectivity between nuclei. Consider the glutamatergic synapses formed on MSNs. Most reviews have focused almost entirely on the cortical innervation of MSNs, leaving the thalamic input to a virtual footnote. Studies using nominal white matter or cortical stimulation of coronal brain slices typically assume that the glutamatergic fibers being stimulated are of cortical origin, but very few of these fibers are left uncut in this preparation [63]. The thalamic innervation of MSNs is similar in magnitude to

that of the cerebral cortex, perhaps constituting as much as 40% of the total glutamatergic input to MSNs, terminating on both shafts and spines [7]. Anatomical studies suggest that the intralaminar nuclei target primarily striatonigral neurons in primate striatum; however, this might not be the case in rodents [64], where "motor" nuclei (ventroanterial (VA) and ventrolateral (VL) nuclei) project primarily to striatopallidal neurons [65, 66]. This apparent dichotomy between motor and "associative" inputs is consistent with recent studies suggesting that input to striatopallidal neurons comes largely from pyramidal neurons contributing to descending motor control circuits, whereas the input to striatonigral neurons comes from neurons whose axons are largely intra-telencephalic [67]. Recently, several studies have shown that parahorizontal slices can preserve both cortical and thalamic connectivity, allowing each to be selectively stimulated [68, 69]. However, these preparations have not been used to date to study the rules governing the induction of plasticity at these two types of synapse.

7.4 Long-Term Potentiation of Glutamatergic Synaptic Transmission

Much less is known about the mechanisms controlling induction and expression of long-term potentiation (LTP) than LTD. Studies in tissue slices have argued that LTP induced by HFS of corticostriatal glutamatergic inputs (HFS–LTP) depends upon co-activation of D_1 and NMDA receptors [70, 71]. Given the apparent requirement to dramatically lower extracellular Mg^{2+} concentration in the slice to induce LTP, there had been some question about the physiological relevance of LTP in MSNs, but this issue has been resolved by the demonstration that it is readily inducible in vivo [72]. The discrepancy presumably stemmed from the difficulty in depolarizing MSN dendrites enough to overcome Mg^{2+} block of NMDA receptors with focal stimulation in a brain slice. How HFS–LTP is expressed has not been carefully examined. As with HFS–LTD, the dependence of a nominally widespread form of synaptic plasticity upon a receptor with restricted distribution is puzzling.

7.5 A Reconciliation of Models of Striatal Synaptic Plasticity

As is apparent from the presentation thus far, there are several obstacles that have slowed progress toward a sound understanding of the dopaminergic modulation of synaptic plasticity in the striatum. Cellular heterogeneity has been the biggest of these in our view. The development of D_1 and D_2 receptor BAC transgenic mice has made this problem tractable. Another issue is the induction protocol. Until very recently, plasticity studies have not attempted to engage the postsynaptic membrane and dendrites in a physiological way during the induction of synaptic plasticity (e.g., Cs^+ loading cells and voltage clamping).

Why is this important? Most learning theories postulate that changes in synaptic strength reflect the precise temporal relationship between presynaptic and postsynaptic activity. Hebb's classic postulate asserts that excitatory, glutamatergic synaptic activity that consistently leads to postsynaptic spiking induces a strengthening or potentiation of the active synapses. An unstated corollary is that synaptic activity that follows postsynaptic activity (and hence cannot be causally linked to spiking) should be weakened or depressed. Dendrites are an integral part of this learning equation, forming the conduit between the axon initial segment where spikes are initiated and synaptic sites where plasticity is induced. DA receptors richly invest dendrites of MSNs [73], putting them in a position to modulate this linkage. The extended Hebbian postulate has been tested in several types of neuron by examining how the temporal relationship between presynaptic and postsynaptic spiking influences lasting changes in synaptic strength [74–76]. Spike-timing-dependent plasticity (STDP) of this sort depends upon back-propagating action potentials (bAPs) that serve to depolarize synaptic regions before, during, or after glutamate release. At most synapses, Hebb's postulate appears to be correct. That is, when presynaptic activity precedes postsynaptic spiking, LTP is induced, whereas reversing the order induces LTD [77–80].

Using perforated patch recordings (to preserve intracellular signaling mechanisms) and minimal local electrical stimulation of glutamatergic afferent fibers in tissue slices from BAC transgenic mice, we have used STDP protocols to examine the rules governing the induction of plasticity at striatonigral and striatopallidal MSN synapses [81]. These studies have revealed a set of rules that are largely consistent with those inferred from studies using conventional induction protocols (see above), but pushed us beyond our current conceptual model by showing that DA controls the induction of Hebbian synaptic plasticity in a receptor- and cell-type-specific manner.

Specifically, D_1 receptor signaling in striatonigral MSNs was necessary for the induction of Hebbian long-term potentiation whereas D_2 receptor signaling in striatopallidal MSNs was necessary for the induction of Hebbian long-term depression. More importantly, our studies demonstrate that DA, in concert with adenosine and glutamate, makes STDP at MSN glutamatergic synapses bidirectional and Hebbian [81].

In striatopallidal MSNs (Fig. 7.2a), repeated pairing of a synaptic stimulation with a postsynaptic spike later (positive timing) resulted in LTP of the synaptic response (Fig. 7.2b). In contrast, preceding synaptic stimulation with a short burst of postsynaptic spikes (negative timing) induced LTD (Fig. 7.2c). The timing-dependent LTP relies upon activation of NMDA and A2a receptors, as blocking them disrupts the potentiation of synaptic response in striatopallidal MSNs (Fig. 7.2d). As with conventional LTD, timing-dependent LTD is disrupted by antagonizing mGluR5, CB1, or D_2 receptors (Fig. 7.2d).

The bidirectionality of STDP appeared to be controlled by a balanced interaction between "opponent" GPCR signaling cascades controlling the induction of LTP and LTD [79, 82, 83]. D_2 and A2a receptor signaling cascades have long been known

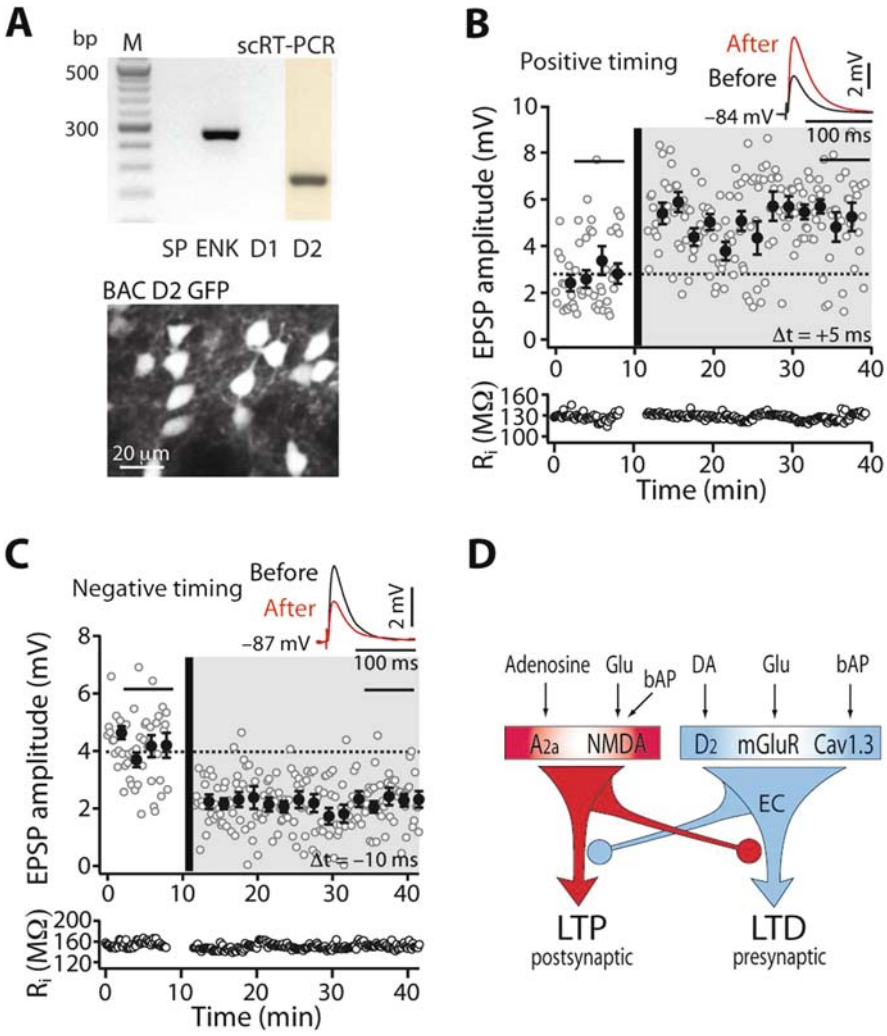

Fig. 7.2 Striatopallidal MSNs displayed bidirectional STDP dependent upon D_2 and A2a receptors. (**A**) *Upper*, single cell RT-PCR (scRT-PCR) amplicons from an individual BAC D_2 eGFP-labeled neuron confirmed co-expression of enkephalin and D_2 receptor mRNA. M, marker; SP, substance P; ENK, enkephalin. *Bottom*, two-photon laser scanning microscopic image of eGFP-labeled MSNs in a slice from a BAC D_2 mouse. (**B**) LTP induced in eGFP-labeled striatopallidal MSN by a positive timing pairing. Plots show EPSP amplitude and input resistance as a function of time in a single cell. The *dashed line* shows the average EPSP amplitude before induction. The induction was performed at the vertical bar. *Filled symbol* shows the averages of 12 trials (± SEM). The averaged EPSP traces before and after induction are showed at the *top*. (**C**) LTD induced by a negative timing pairing. Plots and EPSP traces as in **B**. (**D**) Schematic illustration shows that activation of A2a and NMDA receptors leads to LTP and activation of D_2 and mGluR5 receptors and Cav1.3 channels leads to LTD. Moreover, A2a and D_2 receptor activation oppose each other in inducing plasticity. Glu, glutamate; EC, endocannabinoid. From Shen et al. 2008 [81]

to oppose one another at several levels [84, 85]. In the STDP paradigm, elevating D_2 receptor stimulation by bath application of quinpirole resulted in a robust LTD even when postsynaptic activity followed presynaptic activity, a protocol that would normally induce LTP. In contrast, elevating A2a receptor signaling by bath application of CGS21680 restored LTP, even when presynaptic activity followed postsynaptic activity (Fig. 7.2d).

In striatonigral MSNs (Fig. 7.3a), pairing presynaptic activity with a trailing postsynaptic spike induced robust LTP (Fig. 7.3b). As in striatopallidal MSNs, STDP LTP was dependent upon NMDA receptors (Fig. 7.3d). However, when presynaptic activity followed postsynaptic spiking, EPSP amplitude did not change. In light of the opponent signaling hypothesis, we reasoned that this failure could be due to the activation of the GPCR responsible for LTP induction. To test this hypothesis, D_1 receptors were blocked by SCH23390. In the absence of D_1 receptor activity, pairing postsynaptic spiking with a presynaptic volley led to a robust LTD (Fig. 7.3c). Moreover, the CB1 receptor antagonist AM251 blocked the LTD, establishing a mechanistic parallel to LTD in striatopallidal MSNs. To determine whether attenuating D_1 receptor signaling altered the timing dependence of plasticity, the effects of the positive timing protocol (presynaptic activity followed by postsynaptic activity) were re-examined. In control conditions, this protocol induced a robust LTP (Fig. 7.3b). Blocking D_1 receptors not only prevented LTP induction, it led to the induction of LTD (Fig. 7.3d).

The recognition that DA is not essential for all forms of synaptic plasticity in MSNs resolves the apparent paradox posed by the segregation of DA receptors in the two MSN populations. The finding that STDP plasticity at MSN glutamatergic synapses is Hebbian is consistent with a recent study [80], but conflicts with another [86]. The discrepancy could be attributable to the engagement of GABAergic interneurons in the striatum, confounding modifications in the strength of glutamatergic synapses [87]. Indirect, modulatory influences of other striatal interneurons also have been implicated in the induction of plasticity at glutamatergic synapses when large regions of the striatum are stimulated [13, 62]. Our reliance upon focal stimulation near synaptic sites minimized the involvement of these interneurons and helped to resolve how DA receptors expressed by postsynaptic MSNs shaped the induction process.

These studies suggest that while DA makes STDP in striatal MSNs bidirectional and Hebbian, it is not necessary for the induction of synaptic plasticity. This stands in contrast to previous work asserting that DA is essential for plasticity and that striatal DA depletion in Parkinson's disease models eliminates both LTD and LTP [11, 62]. To test this hypothesis, BAC mice were rendered parkinsonian by unilateral 6-OHDA lesions, sacrificed a week later and slices prepared from their brains. What we found was consistent with the work in unlesioned brains. That is, in striatopallidal MSNs pairing pre- and postsynaptic activity induced LTP, regardless of the order of presentation; in contrast, in striatonigral MSNs, pairing pre- and postsynaptic activity induced LTD, again regardless of order. Thus, synaptic plasticity is not lost in PD models, but it ceases to be bidirectional and dependent upon the timing of pre- and postsynaptic activity [81].

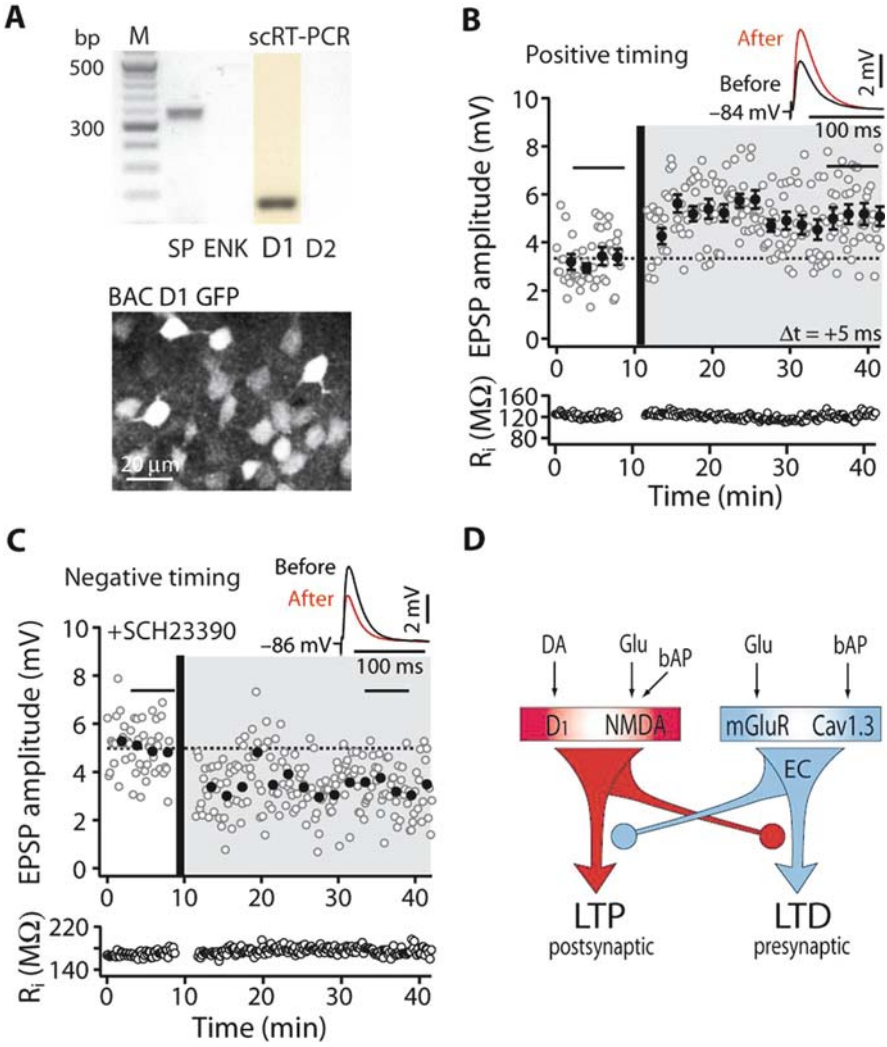

Fig. 7.3 Striatonigral MSNs displayed bidirectional STDP dependent upon D_1 receptors. (**A**) *Upper*, scRT-PCR amplicons from an individual eGFP-labeled neuron from a BAC D_1 mouse confirmed co-expression of substance P and D_1 receptor mRNA. M, marker; SP, substance P; ENK, enkephalin. *Bottom*, two-photon image of eGFP-labeled MSNs in a slice from a BAC D_1 mouse. (**B**) LTP induction in labeled striatonigral neuron by a positive timing pairing protocol (+5 ms) coupled with postsynaptic depolarization to –70 mV. EPSP amplitude and input resistance of the recorded cell were plotted as a function of time. The *dashed line* shows the average of EPSP amplitude before induction. The induction was performed at the vertical bar. *Filled symbol* shows the averages of 12 trials (± SEM). The averaged EPSP traces before and after induction are shown at the *top*. (**C**) In the presence of SCH23390, a negative timing pairing revealed a robust LTD. Plots and EPSP traces are from a single cell as in **B**. (**D**) Schematic drawing shows that activation of D_1 and NMDA receptors evokes LTP and activation of mGluR5 receptor and Cav1.3 channels evokes LTD. Moreover, D_1 and mGluR5 receptor activation oppose each other in inducing plasticity. Glu, glutamate; EC, endocannabinoid. From Shen et al. 2008 [81]

In addition to reconciling a discordant literature on the role of DA in the modulation of glutamatergic synaptic plasticity, these studies establish a parallel between the short-term and long-term effects of DA on MSNs. As reviewed above, the short-term effects of DA are receptor specific, tending to diminish the excitability of striatopallidal MSNs through D_2 receptors and to increase the excitability of striatonigral MSNs through D1 receptors. Now it is clear that the effects of DA on synaptic plasticity are also receptor and cell-type specific. That is, by promoting LTD, D_2 receptors diminish the excitatory synaptic input to striatopallidal MSNs, decreasing their activity; conversely, by promoting LTP, D1 receptors increase the excitatory synaptic input to striatonigral MSNs, enhancing their activity.

7.6 What Might This Mean for Behavior?

What does this mean in the broader functional context of the basal ganglia? One of the most popular theories of the striatum is that it is involved in action selection [3, 88, 89]. When faced with a choice of what to do, the striatum helps us choose the action that will maximize our chances of reward or pleasure and minimize the chances of punishment or aversion. This is where DA comes in. Learning the reward (or punishment) probabilities of particular actions is thought to depend upon DA release in the striatum, where it changes the synaptic weights of cortical inputs associated with particular actions. The two parallel pathways in the striatum have been envisioned to act as a push–pull system with the striatonigral pathway serving to select an action and the striatopallidal pathway serving to inhibit a competing action. Computational models of the striatum suggest that this parallel organization enhances the ability of the system to resolve small differences in reward probability [90].

The recognition that DA affects these two pathways differently, particularly insofar as synaptic plasticity is concerned, is beautifully consistent with this model. In principle, reward-associated elevations in striatal DA promote the strengthening of corticostriatal synapses – that are linked to sensory stimuli that preceded the act and to the internal representation of the act that led to the reward – just on striatonigral MSNs while weakening them on striatopallidal MSNs. When an act leads to a negative consequence, striatal DA levels are thought to fall [91]. Again, in principle, this should promote the strengthening of corticostriatal synapses on striatopallidal MSNs (leading to suppression of the action) and weakening of synapses on striatonigral MSNs (leading to a reduced probability of selection of the action).

Because the induction of synaptic plasticity is controlled by opponent processes, disease states that alter the levels of DA receptor stimulation should distort how experience shapes these connections of these two pathways. So, for example, in Parkinson's disease models the absence of DA should be interpreted by the striatum as equivalent to a situation in which all acts lead to punishment and, hence, all acts should be suppressed. Phenomenologically at least, this is what happens. In contrast, drugs of abuse that dramatically elevate striatal DA should lead to a diminished ability to inhibit action selection. This could help explain some aspects of drug-seeking behavior.

References

1. Balleine BW, Delgado MR, Hikosaka O. The role of the dorsal striatum in reward and decision-making. J Neurosci 2007;27:8161–5.
2. Graybiel AM, Aosaki T, Flaherty AW, Kimura M. The basal ganglia and adaptive motor control. Science 1994;265:1826–31.
3. Schultz W. Predictive reward signal of dopamine neurons. J Neurophysiol 1998;80:1–27.
4. Yin HH, Knowlton BJ. The role of the basal ganglia in habit formation. Nat Rev Neurosci 2006;7:464–76.
5. Gerfen CR. The neostriatal mosaic: multiple levels of compartmental organization in the basal ganglia. Annu Rev Neurosci 1992;15:285–320.
6. Surmeier DJ, Song WJ, Yan Z. Coordinated expression of dopamine receptors in neostriatal medium spiny neurons. J Neurosci 1996;16:6579–91.
7. Wilson CJ. Basal ganglia. In: Shepherd GM, ed. The Synaptic Organization of the Brain. Oxford: Oxford UP; 2004:361–414.
8. Ade KK, Janssen MJ, Ortinski PI, Vicini S. Differential tonic GABA conductances in striatal medium spiny neurons. J Neurosci 2008;28:1185–97.
9. Cepeda C, Andre VM, Yamazaki I, Wu N, Kleiman-Weiner M, Levine MS. Differential electrophysiological properties of dopamine D1 and D2 receptor-containing striatal medium-sized spiny neurons. Eur J Neurosci 2008;27:671–82.
10. Day M, Wang Z, Ding J, et al. Selective elimination of glutamatergic synapses on striatopallidal neurons in Parkinson disease models. Nat Neurosci 2006;9:251–9.
11. Kreitzer AC, Malenka RC. Endocannabinoid-mediated rescue of striatal LTD and motor deficits in Parkinson's disease models. Nature 2007;445:643–7.
12. Shen W, Tian X, Day M, et al. Cholinergic modulation of Kir2 channels selectively elevates dendritic excitability in striatopallidal neurons. Nat Neurosci 2007;10:1458–66.
13. Wang Z, Kai L, Day M, et al. Dopaminergic control of corticostriatal long-term synaptic depression in medium spiny neurons is mediated by cholinergic interneurons. Neuron 2006;50:443–52.
14. Nicola SM, Surmeier J, Malenka RC. Dopaminergic modulation of neuronal excitability in the striatum and nucleus accumbens. Annu Rev Neurosci 2000;23:185–215.
15. Surmeier DJ. Microcircuits in the Striatum: Cell Types, Intrinsic Membrane Properties and Neuromodulation. In: Grillner S, Graybiel AM, eds. Microcircuits: The Interface between Neurons and Global Brain Function. Berlin: MIT Press; 2004:105–26.
16. Arbuthnott GW, Wickens J. Space, time and dopamine. Trends Neurosci 2007;30:62–9.
17. Surmeier DJ, Ding J, Day M, Wang Z, Shen W. D1 and D2 dopamine-receptor modulation of striatal glutamatergic signaling in striatal medium spiny neurons. Trends Neurosci 2007;30:228–35.
18. David HN, Ansseau M, Abraini JH. Dopamine-glutamate reciprocal modulation of release and motor responses in the rat caudate-putamen and nucleus accumbens of "intact" animals. Brain Res Brain Res Rev 2005;50:336–60.
19. Morari M, Marti M, Sbrenna S, Fuxe K, Bianchi C, Beani L. Reciprocal dopamine-glutamate modulation of release in the basal ganglia. Neurochem Int 1998;33:383–97.
20. Albin RL, Young AB, Penney JB. The functional anatomy of basal ganglia disorders. Trends Neurosci 1989;12:366–75.
21. Herve D, Rogard M, Levi-Strauss M. Molecular analysis of the multiple Golf alpha subunit mRNAs in the rat brain. Brain Res Mol Brain Res 1995;32:125–34.
22. Snyder GL, Allen PB, Fienberg AA, et al. Regulation of phosphorylation of the GluR1 AMPA receptor in the neostriatum by dopamine and psychostimulants in vivo. J Neurosci 2000;20:4480–8.
23. Hallett PJ, Spoelgen R, Hyman BT, Standaert DG, Dunah AW. Dopamine D1 activation potentiates striatal NMDA receptors by tyrosine phosphorylation-dependent subunit trafficking. J Neurosci 2006;26:4690–700.

24. Braithwaite SP, Paul S, Nairn AC, Lombroso PJ. Synaptic plasticity: one STEP at a time. Trends Neurosci 2006;29:452–8.
25. Lee FJ, Xue S, Pei L, et al. Dual regulation of NMDA receptor functions by direct protein-protein interactions with the dopamine D1 receptor. Cell 2002;111:219–30.
26. Scott L, Zelenin S, Malmersjo S, et al. Allosteric changes of the NMDA receptor trap diffusible dopamine 1 receptors in spines. Proc Natl Acad Sci USA 2006;103: 762–7.
27. Blank T, Nijholt I, Teichert U, et al. The phosphoprotein DARPP-32 mediates cAMP-dependent potentiation of striatal N-methyl-D-aspartate responses. Proc Natl Acad Sci USA 1997;94:14859–64.
28. Nicola SM, Malenka RC. Modulation of synaptic transmission by dopamine and norepinephrine in ventral but not dorsal striatum. J Neurophysiol 1998;79:1768–76.
29. Cepeda C, Buchwald NA, Levine MS. Neuromodulatory actions of dopamine in the neostriatum are dependent upon the excitatory amino acid receptor subtypes activated. Proc Natl Acad Sci USA 1993;90:9576–80.
30. Liu JC, DeFazio RA, Espinosa-Jeffrey A, Cepeda C, de Vellis J, Levine MS. Calcium modulates dopamine potentiation of N-methyl-D-aspartate responses: electrophysiological and imaging evidence. J Neurosci Res 2004;76:315–22.
31. Carter AG, Sabatini BL. State-dependent calcium signaling in dendritic spines of striatal medium spiny neurons. Neuron 2004;44:483–93.
32. Carter AG, Soler-Llavina GJ, Sabatini BL. Timing and location of synaptic inputs determine modes of subthreshold integration in striatal medium spiny neurons. J Neurosci 2007;27:8967–77.
33. Kerr JN, Plenz D. Action potential timing determines dendritic calcium during striatal up-states. J Neurosci 2004;24:877–85.
34. Calabresi P, Mercuri N, Stanzione P, Stefani A, Bernardi G. Intracellular studies on the dopamine-induced firing inhibition of neostriatal neurons in vitro: evidence for D1 receptor involvement. Neuroscience 1987;20:757–71.
35. Surmeier DJ, Kitai ST. D1 and D2 dopamine receptor modulation of sodium and potassium currents in rat neostriatal neurons. Prog Brain Res 1993;99:309–24.
36. Carr DB, Day M, Cantrell AR, et al. Transmitter modulation of slow, activity-dependent alterations in sodium channel availability endows neurons with a novel form of cellular plasticity. Neuron 2003;39:793–806.
37. Scheuer T, Catterall WA. Control of neuronal excitability by phosphorylation and dephosphorylation of sodium channels. Biochem Soc Trans 2006;34:1299–302.
38. Wickens JR, Wilson CJ. Regulation of action-potential firing in spiny neurons of the rat neostriatum in vivo. J Neurophysiol 1998;79:2358–64.
39. Hernandez-Lopez S, Bargas J, Surmeier DJ, Reyes A, Galarraga E. D1 receptor activation enhances evoked discharge in neostriatal medium spiny neurons by modulating an L-type Ca2+ conductance. J Neurosci 1997;17:3334–42.
40. Gao T, Yatani A, Dell'Acqua ML, et al. cAMP-dependent regulation of cardiac L-type Ca2+ channels requires membrane targeting of PKA and phosphorylation of channel subunits. Neuron 1997;19:185–96.
41. Surmeier DJ, Bargas J, Hemmings HC, Jr., Nairn AC, Greengard P. Modulation of calcium currents by a D1 dopaminergic protein kinase/phosphatase cascade in rat neostriatal neurons. Neuron 1995;14:385–97.
42. Olson PA, Tkatch T, Hernandez-Lopez S, et al. G-protein-coupled receptor modulation of striatal CaV1.3 L-type Ca2+ channels is dependent on a Shank-binding domain. J Neurosci 2005;25:1050–62.
43. Levine MS, Altemus KL, Cepeda C, et al. Modulatory actions of dopamine on NMDA receptor-mediated responses are reduced in D1A-deficient mutant mice. J Neurosci 1996;16:5870–82.

44. Snyder GL, Fienberg AA, Huganir RL, Greengard P. A dopamine/D1 receptor/protein kinase A/dopamine- and cAMP-regulated phosphoprotein (Mr 32 kDa)/protein phosphatase-1 pathway regulates dephosphorylation of the NMDA receptor. J Neurosci 1998;18: 10297–303.
45. Flores-Hernandez J, Cepeda C, Hernandez-Echeagaray E, et al. Dopamine enhancement of NMDA currents in dissociated medium-sized striatal neurons: role of D1 receptors and DARPP-32. J Neurophysiol 2002;88:3010–20.
46. Vergara R, Rick C, Hernandez-Lopez S, et al. Spontaneous voltage oscillations in striatal projection neurons in a rat corticostriatal slice. J Physiol 2003;553:169–82.
47. Tseng KY, O'Donnell P. Dopamine-glutamate interactions controlling prefrontal cortical pyramidal cell excitability involve multiple signaling mechanisms. J Neurosci 2004;24: 5131–9.
48. Vilchis C, Bargas J, Ayala GX, Galvan E, Galarraga E. Ca2+ channels that activate Ca2+-dependent K+ currents in neostriatal neurons. Neuroscience 2000;95:745–52.
49. Stoof JC, Kebabian JW. Two dopamine receptors: biochemistry, physiology and pharmacology. Life Sci 1984;35:2281–96.
50. Hernandez-Lopez S, Tkatch T, Perez-Garci E, et al. D2 dopamine receptors in striatal medium spiny neurons reduce L-type Ca2+ currents and excitability via a novel PLC[beta]1-IP3-calcineurin-signaling cascade. J Neurosci 2000;20:8987–95.
51. Nishi A, Snyder GL, Greengard P. Bidirectional regulation of DARPP-32 phosphorylation by dopamine. J Neurosci 1997;17:8147–55.
52. Kotecha SA, Oak JN, Jackson MF, et al. A D2 class dopamine receptor transactivates a receptor tyrosine kinase to inhibit NMDA receptor transmission. Neuron 2002;35:1111–22.
53. Hernandez-Echeagaray E, Starling AJ, Cepeda C, Levine MS. Modulation of AMPA currents by D2 dopamine receptors in striatal medium-sized spiny neurons: are dendrites necessary? Eur J Neurosci 2004;19:2455–63.
54. Hakansson K, Galdi S, Hendrick J, Snyder G, Greengard P, Fisone G. Regulation of phosphorylation of the GluR1 AMPA receptor by dopamine D2 receptors. J Neurochem 2006;96:482–8.
55. Bamford NS, Zhang H, Schmitz Y, et al. Heterosynaptic dopamine neurotransmission selects sets of corticostriatal terminals. Neuron 2004;42:653–63.
56. Yin HH, Lovinger DM. Frequency-specific and D2 receptor-mediated inhibition of glutamate release by retrograde endocannabinoid signaling. Proc Natl Acad Sci USA 2006;103:8251–6.
57. Brebner K, Wong TP, Liu L, et al. Nucleus accumbens long-term depression and the expression of behavioral sensitization. Science 2005;310:1340–3.
58. Centonze D, Picconi B, Gubellini P, Bernardi G, Calabresi P. Dopaminergic control of synaptic plasticity in the dorsal striatum. Eur J Neurosci 2001;13:1071–7.
59. Kreitzer AC, Malenka RC. Dopamine modulation of state-dependent endocannabinoid release and long-term depression in the striatum. J Neurosci 2005;25:10537–45.
60. Lovinger DM, Tyler EC, Merritt A. Short- and long-term synaptic depression in rat neostriatum. J Neurophysiol 1993;70:1937–49.
61. Giuffrida A, Parsons LH, Kerr TM, Rodriguez de Fonseca F, Navarro M, Piomelli D. Dopamine activation of endogenous cannabinoid signaling in dorsal striatum. Nat Neurosci 1999;2:358–63.
62. Calabresi P, Picconi B, Tozzi A, Di Filippo M. Dopamine-mediated regulation of corticostriatal synaptic plasticity. Trends Neurosci 2007;30:211–9.
63. Kawaguchi Y, Wilson CJ, Emson PC. Intracellular recording of identified neostriatal patch and matrix spiny cells in a slice preparation preserving cortical inputs. J Neurophysiol 1989;62:1052–68.
64. Bacci JJ, Kachidian P, Kerkerian-Le Goff L, Salin P. Intralaminar thalamic nuclei lesions: widespread impact on dopamine denervation-mediated cellular defects in the rat basal ganglia. J Neuropathol Exp Neurol 2004;63:20–31.

65. Smith Y, Raju DV, Pare JF, Sidibe M. The thalamostriatal system: a highly specific network of the basal ganglia circuitry. Trends Neurosci 2004;27:520–7.
66. Hoshi E, Tremblay L, Feger J, Carras PL, Strick PL. The cerebellum communicates with the basal ganglia. Nat Neurosci 2005;8:1491–3.
67. Lei W, Jiao Y, Del Mar N, Reiner A. Evidence for differential cortical input to direct pathway versus indirect pathway striatal projection neurons in rats. J Neurosci 2004;24:8289–99.
68. Ding J, Peterson JD, Surmeier DJ. Corticostriatal and thalamostriatal synapses have distinctive properties. J Neurosci 2008;28:6483–92.
69. Smeal RM, Gaspar RC, Keefe KA, Wilcox KS. A rat brain slice preparation for characterizing both thalamostriatal and corticostriatal afferents. J Neurosci Methods 2007;159:224–35.
70. Centonze D, Grande C, Saulle E, et al. Distinct roles of D1 and D5 dopamine receptors in motor activity and striatal synaptic plasticity. J Neurosci 2003;23:8506–12.
71. Kerr JN, Wickens JR. Dopamine D-1/D-5 receptor activation is required for long-term potentiation in the rat neostriatum in vitro. J Neurophysiol 2001;85:117–24.
72. Mahon S, Deniau JM, Charpier S. Corticostriatal plasticity: life after the depression. Trends Neurosci 2004;27:460–7.
73. Hersch SM, Ciliax BJ, Gutekunst CA, et al. Electron microscopic analysis of D1 and D2 dopamine receptor proteins in the dorsal striatum and their synaptic relationships with motor corticostriatal afferents. J Neurosci 1995;15:5222–37.
74. Dan Y, Poo MM. Spike timing-dependent plasticity of neural circuits. Neuron 2004;44:23–30.
75. Kampa BM, Letzkus JJ, Stuart GJ. Dendritic mechanisms controlling spike-timing-dependent synaptic plasticity. Trends Neurosci 2007;30:456–63.
76. Sjostrom PJ, Rancz EA, Roth A, Hausser M. Dendritic excitability and synaptic plasticity. Physiol Rev 2008;88:769–840.
77. Sjostrom PJ, Nelson SB. Spike timing, calcium signals and synaptic plasticity. Curr Opin Neurobiol 2002;12:305–14.
78. Letzkus JJ, Kampa BM, Stuart GJ. Learning rules for spike timing-dependent plasticity depend on dendritic synapse location. J Neurosci 2006;26:10420–9.
79. Nevian T, Sakmann B. Spine Ca2+ signaling in spike-timing-dependent plasticity. J Neurosci 2006;26:11001–13.
80. Pawlak V, Kerr JN. Dopamine receptor activation is required for corticostriatal spike-timing-dependent plasticity. J Neurosci 2008;28:2435–46.
81. Shen W, Flajolet M, Greengard P, Surmeier DJ. Dichotomous dopaminergic control of striatal synaptic plasticity. Science 2008;321:848–51.
82. Seol GH, Ziburkus J, Huang S, et al. Neuromodulators control the polarity of spike-timing-dependent synaptic plasticity. Neuron 2007;55:919–29.
83. Tzounopoulos T, Rubio ME, Keen JE, Trussell LO. Coactivation of pre- and postsynaptic signaling mechanisms determines cell-specific spike-timing-dependent plasticity. Neuron 2007;54:291–301.
84. Schwarzschild MA, Agnati L, Fuxe K, Chen JF, Morelli M. Targeting adenosine A2a receptors in Parkinson's disease. Trends Neurosci 2006;29:647–54.
85. Fuxe K, Marcellino D, Genedani S, Agnati L. Adenosine A2A receptors, dopamine D2 receptors and their interactions in Parkinson's disease. Mov Disord 2007;22:1990–2017.
86. Fino E, Glowinski J, Venance L. Bidirectional activity-dependent plasticity at corticostriatal synapses. J Neurosci 2005;25:11279–87.
87. Fino E, Deniau JM, Venance L. Cell-specific spike-timing-dependent plasticity in GABAergic and cholinergic interneurons in corticostriatal rat brain slices. J Physiol 2008;586:265–82.
88. Frank MJ, Scheres A, Sherman SJ. Understanding decision-making deficits in neurological conditions: insights from models of natural action selection. Philos Trans R Soc Lond B Biol Sci 2007;362:1641–54.
89. Mink JW. The Basal Ganglia and involuntary movements: impaired inhibition of competing motor patterns. Arch Neurol 2003;60:1365–8.

90. O'Reilly RC, Frank MJ. Making working memory work: a computational model of learning in the prefrontal cortex and basal ganglia. Neural Comput 2006;18:283–328.
91. Mirenowicz J, Schultz W. Preferential activation of midbrain dopamine neurons by appetitive rather than aversive stimuli. Nature 1996;379:449–51.
92. Wilson CJ, Kawaguchi Y. The origins of two-state spontaneous membrane potential fluctuations of neostriatal spiny neurons. J Neurosci 1996;16:2397–410.

Chapter 8
Regulation of Dopamine Receptor Trafficking and Responsiveness

Melissa L. Perreault, Vaneeta Verma, Brian F. O'Dowd, and Susan R. George

Abstract The magnitude of cellular responses resulting from dopamine receptor activation is highly dependent on the balance between exocytic and endocytic trafficking pathways, which together, influence the level of receptor expression at the cell surface. Over the past decade, it has been revealed that the mechanisms involved in dopamine receptor transport are extremely complex, involving numerous protein–protein interactions that assist in targeting the receptors to distinct intracellular compartments. In addition, the importance of oligomerization in dopamine receptor trafficking is becoming increasingly evident, providing new perspectives on the mechanisms of receptor transport. This chapter will review the recent advances that have contributed to the understanding of the molecular mechanisms involved in dopamine receptor trafficking, their role in cellular responsiveness and discuss briefly the significance of receptor trafficking in health and disease.

Keywords Receptor sensitivity · Receptor trafficking · Desensitization · Internalization · Post-translational modifications · Receptor oligomerization

8.1 Introduction

Activation of dopamine receptors induces a cascade of intracellular signaling events, involving numerous effector systems, which mediate the pharmacological and physiological effects of both the receptors' endogenous ligand and exogenous

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therapeutic agents. Dopamine receptor-mediated signaling is a tightly regulated process that is highly dependent on the accessibility of receptors to agonist binding at the cell surface. This availability of functional surface receptors is governed by a strict balance of the various intracellular receptor trafficking pathways that, in association with receptor sensitivity, work in concert to regulate the amplitude of agonist-mediated cellular responsiveness.

In recent years, the focus to uncover the mechanistic processes involved in each step of the trafficking pathway has yielded significant progress. In addition to a number of accessory proteins, molecular chaperones, and receptor motifs that have been identified in the past decade as contributing to dopamine receptor transport (reviewed [1, 2]), the significant role of receptor complexes in signal transduction and trafficking has also been revealed, challenging classical perceptions of receptor stoichiometry and opening up exciting new avenues of research. While traditional thinking has depicted dopamine receptors, and other G-protein-coupled receptors (GPCRs), as monomeric species, an increasing amount of evidence indicates that these receptors are assembled as higher order homo- and heteromers, complexes whose composition contributes to the regulation of receptor trafficking.

8.1.1 GPCRs Traffic as Oligomers

Evidence suggests that the oligomerization of GPCRs occurs early in the biosynthetic process, likely within the endoplasmic reticulum (ER) [3–5], and in some instances, oligomer formation appears to be obligatory to achieve successful export from the ER to the plasma membrane [6–10]. Once the receptor oligomers are correctly assembled, they are then transported through the Golgi, where they receive additional posttranslational modifications, such as glycosylation, for example, to achieve mature status, and are then trafficked to the plasma membrane as functionally active complexes (Fig. 8.1). Upon acute exposure to agonist, these receptors may undergo an agonist-induced decrease in receptor responsiveness over time or desensitization. Agonist-induced desensitization begins with phosphorylation of the receptor by specific kinases, predominantly G-protein receptor kinases (GRKs). In addition, receptor phosphorylation promotes its uncoupling from the G protein and interaction with arrestin proteins, which recruit the receptor complexes for internalization into endosomal compartments (reviewed [11, 12]). Receptors are then recycled back to the plasma membrane, retained in intracellular compartments, or targeted for degradation, processes that are central to the continued maintenance or the resultant termination of the receptor-mediated signal (Fig. 8.1).

This chapter will review in detail the currently known mechanisms that are involved in the regulation of dopamine receptor exocytic and endocytic trafficking and their role in cellular responsiveness. As an abundance of studies have focused on the D_1 and D_2 receptors, this review will focus predominantly on these two receptor

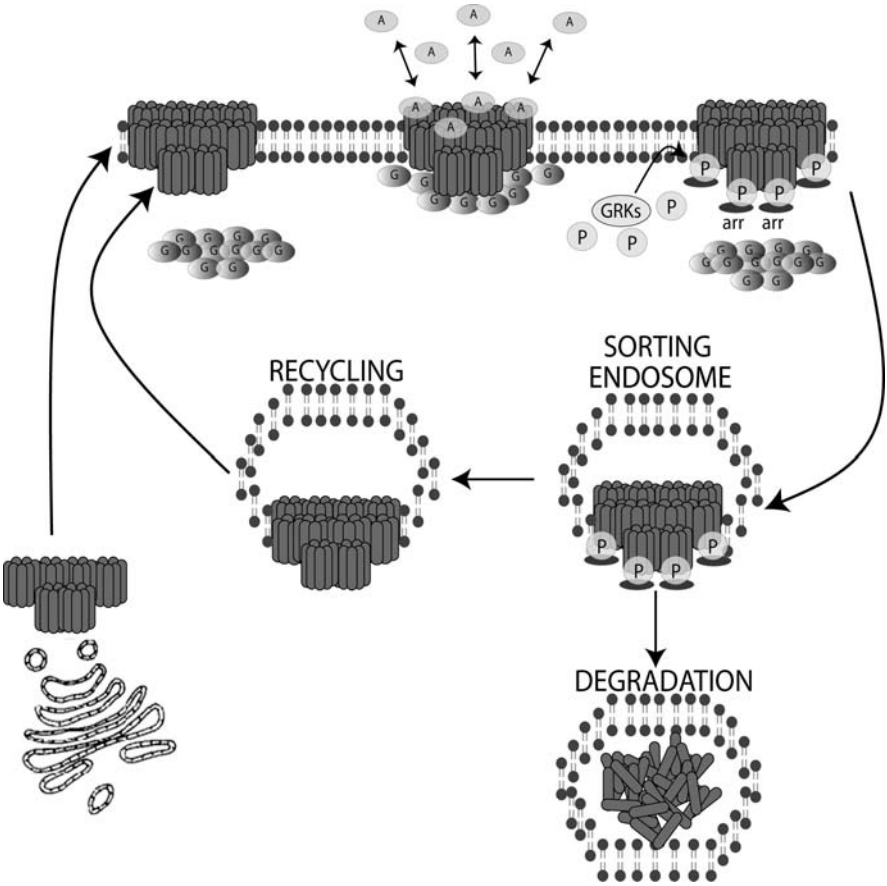

Fig. 8.1 Schematic of a GPCR trafficking as an oligomer. A GPCR oligomer traffics from the Golgi–ER network to the plasma membrane. Exposure to agonist leads to GPCR phosphorylation by G-protein-coupled receptor kinases, which promote its uncoupling from the G protein and its interaction with arrestin proteins, which recruit the receptor complex for internalization into endosomal compartments. The receptor complex is then recycled back to the plasma membrane or targeted for degradation. A, agonist; G, G protein; GRK, G-protein-coupled receptor kinase; P, phosphate; Arr, arrestin

subtypes, both as homomers and as part of the recently identified D_1–D_2 heteromer (reviewed [13]).

8.2 Biosynthesis, Export, and Cell-Surface Stabilization

Dopamine receptors are seven transmembrane receptors that regulate neuronal activity by responding to their endogenous ligand dopamine. The magnitude of the agonist-induced neuronal response is mitigated by the availability of functional

receptors at the cell surface which is contributed to, in part, by the processes of receptor biosynthesis and export trafficking.

8.2.1 Biosynthesis and Cell-Surface Trafficking of Dopamine Receptors

Similar to all GPCRs, the synthesis and folding of dopamine receptors takes place in the ER. At the completion of this process, the receptors are subjected to a stringent ER quality control system that functions to ensure only correctly configured receptors can exit the ER and continue their migration through the endoplasmic compartments toward the Golgi complex. Vital contributors to the ER quality control process are the molecular chaperones, a group of ER resident proteins that have the dual function of assisting in the overall speed and efficiency of glycoprotein folding, and inhibiting the export of misfolded proteins from the ER.

8.2.1.1 Calnexin

Calnexin is a chaperone protein with lectin-like activity that recognizes and binds monoglucosylated N-linked oligosaccharides that have been attached to GPCRs during the translational process. This interaction not only functions to prevent the formation of aggregates and promote receptor folding but also serves to anchor the glycoproteins within the ER until their native conformation is attained or until they are targeted for degradation. An involvement of calnexin in the biosynthesis of D_1 and D_2 receptors has recently been reported [14]. The association of calnexin with the receptors is mediated, at least in part, by glycosylation as the inhibition of glycosylation, through mutation or with the inhibitor tunicamycin, diminished calnexin interactions with the receptors. However, the finding that their association was not completely abolished in the absence of glycosylation is indicative of a direct interaction also with the receptor protein [14]. It has been postulated that these glycan-dependent and glycan-independent actions of calnexin on dopamine receptors may mediate, respectively, the chaperone versus ER retention functions of the protein. This hypothesis was supported by the finding that while the inhibition of glycosylation restricted the cell-surface expression of the D_1 receptor, the increase in calnexin binding that was observed to a trafficking-impaired D_1 receptor was insensitive to glycosylation inhibitors [14].

Although calnexin has been demonstrated to be involved in the biosynthesis of GPCRs [15, 16], a role for this chaperone in oligomerization has not been identified. Indeed, it has been shown that calnexin may not even associate with D_1 and D_2 receptor oligomeric complexes, but only bind to monomeric species [14]. However, while these findings may appear to exclude a role for calnexin in oligomer assembly, it has been suggested that the ability of calnexin to retain individual receptor monomers may function to facilitate the formation of oligomeric complexes [14].

While the mechanisms underlying this hypothesis require further investigation, one possibility is that calnexin may simply restrain each subunit in a spatial orientation that makes the monomer more readily accessible for receptor–receptor interactions. Evidence for self-oligomerization of calnexin [17] suggests a mechanism by which this chaperone may facilitate these interactions by assisting monomers into close proximity so as to promote oligomer assembly.

8.2.1.2 The Triple Phenylalanine Export Motif and DRiP78

Export from the ER has been shown to be a critical rate-limiting step in the trafficking of GPCRs to the cell surface [18]. For many GPCRs the selection for ER export appears to be dependent on the proximal portion of their carboxyl terminus [19–23] and through the use of site-directed mutagenesis, several motifs within the carboxyl terminus have been identified that may serve as ER export signals [19, 24–26]. One such motif has been reported for the D_1 receptor [24]. It was established that the highly conserved triple phenylalanine motif, FxxxFxxxF, was integral for the cell-surface expression of the D_1 receptor as substitution mutations within the motif resulted in ER retention and loss of ligand binding. Moreover, fusion of the motif to an intracellularly trapped receptor protein restored its ER export properties and conferred normal protein transport to the cell surface. Evidence suggests that the motif may promote vesicular transport from the ER or downstream from the ER, by interacting with the vesicular coat protein complex COPI [27], a complex that has been implicated in transport pathways throughout the ER-Golgi network (reviewed [28]) including exit from the ER [29]. Precipitation assays revealed that the D_1 receptor could associate specifically with the γ-subunit of COPI. Although such an interaction between carboxyl terminal motifs and coat protein complexes has not yet been reported for other GPCRs, it has been exhibited for a number of non-GPCR proteins [30–32].

The export capabilities of the FxxxFxxxF motif can be regulated by the molecular chaperone dopamine receptor interacting protein 78 (DRiP78), a membrane-associated ER resident protein [24]. Transport of the D_1 receptor appeared to be highly sensitive to the intracellular levels of DRiP78 as overexpression of the protein led to ER retention. It has been suggested that DRiP78 may function to mask the ER export signal thereby preventing the interaction of the motif with another complex associated with vesicular transport, such as the coatomer complexes, for example. However, given that DRiP78 sequestration similarly resulted in reduced D_1 receptor cell-surface expression, these results suggested that discrete levels of the protein may be required for sufficient export trafficking of the D_1 receptor from the ER [24]. It is possible that, in addition to its role in ER retention through motif binding, DRiP78 may function to assist in protein folding and/or oligomer assembly. Such a role has been recently demonstrated for DRiP78 in the assembly of G-protein $\beta\gamma$ heterodimers [33]. If such was the case for D_1 receptors, insufficient levels of DRiP78 could result in misfolded or unformed oligomer complexes leading to ER retention and reduced cell-surface expression.

8.2.1.3 Role of Glycosylation in Receptor Cell-Surface Targeting

Studies have shown that glycosylation is requisite for the optimal expression of certain dopamine receptor subtypes at the plasma membrane. It has been previously reported that the loss of N-linked glycosylation by the inhibitor tunicamycin, or the mutation of a single N-linked glycosylation site (Asp^4), did not attenuate the cell-surface expression of the D_1 receptor [34]. Yet it has been more recently reported that tunicamycin, under similar treatment conditions, inhibited the cell-surface expression of the D_1 receptor by approximately 27% [14]. Furthermore, this study demonstrated that a D_1 receptor mutant missing both N-linked glycosylation sites (Asp^4 and Asp^{174}) exhibited diminished D_1 receptor cell-surface expression that was comparable to that observed with tunicamycin [14]. The findings from the receptor mutants indicate that mutation of both glycosylation sites, and hence a complete loss of glycosylation, may be required to attenuate D_1 receptor trafficking. However, given that the sole mutation of the Asp^{174} residue was not performed, an important role for this individual site in the cell-surface transport of the D_1 receptor cannot be excluded.

Unlike the D_1 receptor, the inhibitor tunicamycin has been shown to completely abolish the cell-surface localization of the D_5 receptor in cells [34]. Mutation of the three individual N-linked glycosylation sites revealed that it was the Asp^7 residue that was critical for the cell-surface transport of this receptor as its mutation resulted in an almost complete abolishment of plasma membrane D_5 receptor expression [34].

The elimination of N-linked glycosylation by mutation or tunicamycin resulted in a reduction of both total cellular or plasma membrane expression of D_2 receptors [14, 35]. Additionally, post-ER glycosylation has also been implicated in the discrete trafficking of the D_2 receptor isoforms, D_{2short} (D_{2S}) and D_{2Long} (D_{2L}). Using pulse-chase procedures it was demonstrated that, under certain conditions, D_{2S} was rapidly processed from a newly synthesized protein to a partially, and then fully glycosylated mature state. In contrast, a significant amount (approximately 20%) of the D_{2L} isoform was only partially glycosylated and remained intracellularly sequestered [36]. In concordance with these findings, it has recently been demonstrated that, in the absence of agonist, D_{2S} was predominantly localized to the plasma membrane, whereas the D_{2L} isoform was found both at the cell surface and intracellularly [37]. Similarly, it has been demonstrated that D_{2L} was retained further upstream and more strongly than D_{2S} in early compartments of the secretory pathway [35]. The D_{2S} and D_{2L} isoforms differ from one another by a 29 amino acid insertion found in the third intracellular loop of D_{2L}. Therefore, it is plausible that the differential trafficking rates and targeting of these receptor isoforms are associated with this sequence of amino acids. Specifically, it is possible that this sequence, in full or in part, may function as a retainment motif, whereby interactions of the motif with an as yet unidentified accessory protein may serve to retain the D_{2L} isoform intracellularly. Given that D_{2S} and D_{2L}, respectively, function pre- and postsynaptically [38], and work together to mediate dopamine transmission, understanding the mechanisms underlying the differential trafficking of these

two isoforms may have significant implications in understanding the physiological regulation of dopamine transmission in the brain.

8.2.2 Stabilization of Dopamine Receptors at the Cell Surface

It has been demonstrated that GPCRs are not static within the plasma membrane but can move in the plane of the membrane by the passive process of lateral diffusion [39–41]. One of the key factors that govern the dynamics of lateral diffusion of GPCRs is their association with other cellular proteins. The formation of these protein complexes can serve to restrict the movements of the GPCRs, effectively stabilizing the receptors in specific microdomains within the membrane, such as the synapse for example. Numerous protein–protein associations of this type have been reported between dopamine receptors and a variety of cellular proteins, examples of which are discussed below.

8.2.2.1 The NMDA-D_1 Receptor Trap

Movement by lateral diffusion has been reported for several GPCRs, including vasopressin V_2 [39], serotonin 1_A [40], and dopamine D_1 receptors [41]. In cultured neurons, approximately 65% of D_1 receptors are mobile with the remaining receptors anchored within the membrane [41]. This ratio of mobile to anchored receptors is not fixed, but fluctuating, and has recently been shown to be influenced by other receptors within the plasma membrane. One such receptor is the NMDA receptor whose activation has been reported to recruit D_1 receptors to the cell surface from intracellular compartments [42] and, furthermore, restrict the lateral movements of D_1 receptors within the membrane [41]. The modulation of D_1 receptor flow dynamics by NMDA activation at the cell surface stems in part from the ability of the two receptors to physically interact. Particular regions of the D_1 receptor carboxyl tail have been shown to bind to the NR-1 and NR-2 subunits of the NMDA receptor [43]. It is the interface with the NR-1 subunit, however, that has been identified as the critical region for both NMDA-mediated cell-surface trafficking and trapping of D_1 receptors [41]. With regard to the functional mechanisms underlying D_1 receptor trapping, the influx of calcium that results from NMDA receptor activation does not appear to be involved. Rather it appears to be the result of an allosteric transformation, induced through occupation of the NMDA binding site, which facilitates the interaction between the D_1 receptor and the NR-1 subunit [41]. It has been established that NMDA receptors are stabilized at the membrane via anchorage to the postsynaptic density. Thus, the formation of NMDA–D_1 receptor complexes functions in localizing and stabilizing the D_1 receptor at the synapse. Presumably, increased synaptic localization would make these receptors more susceptible to activation, culminating in enhanced signal transduction and neuronal responsiveness.

8.2.2.2 Role of Scaffolding Proteins in Dopamine Receptor Cell-Surface Stability

Perhaps one of the most well-known functions of scaffolding proteins is to maintain the structural integrity of the cell membrane. However, it is becoming more evident that scaffolding proteins may also serve additional important functions, one of which involves the stabilization of GPCRs at the plasma membrane. With regard to dopamine receptors, several scaffolding proteins have been identified that serve in this capacity. Two examples are protein 4.1 N and filamin A (actin-binding protein 280), both of which have been shown to influence the expression of D_2 and D_3 receptors through interactions at their third intracellular loops [44–47].

The association of a truncated mutant of protein 4.1 N with D_2 and D_3 receptors significantly reduced the expression of either receptor in cells, results that implicate a positive role for protein 4.1 N in the cell-surface stabilization of these receptors [44]. Similarly, the actin-binding protein filamin A positively contributed to D_2 receptor plasma membrane expression [46, 47], and moreover, has been shown to play a role in enhanced D_2 receptor-mediated signaling [45]. It is possible that the functional mechanism underlying this increase in signaling stems from filamin A-assisted formation of D_2 receptor clusters at specific locales on the cell surface. Such clusters may function to increase the efficiency of receptor–effector coupling by aggregating components of the signaling pathway. Filamin A also has been reported to contribute to efficient signaling and sequestration of the D_3 receptor [48], although to date, a role for this protein in D_3 receptor cluster formation has not been reported.

In addition to increasing receptor stability, scaffolding proteins can also destabilize receptors at the plasma membrane. The cytoskeletal subunit neurofilament-M (NF-M), for example, has been shown to negatively affect the cell-surface expression of the D_1 receptor [49]. Specifically, coexpression of NF-M with the D_1 receptor in cells resulted in a significant reduction in D_1 receptor cell-surface number and ligand-mediated cyclic AMP (cAMP) accumulation. Interestingly, receptors that remained at the cell surface exhibited a resistance to agonist-induced desensitization. Although the mechanism underlying this insensitivity was not identified, it was postulated that formation of the NF-M/D_1 complex might preclude associations of the D_1 receptor with kinases and/or arrestin, two events that are fundamental in the initiation of receptor trafficking into the cell.

8.3 Desensitization

8.3.1 D_1-Like Receptors

Desensitization of D_1-like receptors (D_1,D_5) has been extensively studied over the past several years and indicates that dopamine-induced attenuation of signaling by these receptors occurs within minutes of exposure [50–56]. As with the majority of

GPCRs, the predominant form of D_1 receptor desensitization has been identified as being mediated through GRKs. Truncated mutant constructs of the rat D_1 receptor have shown that multiple residues located downstream of Gly^{379} in the distal carboxyl terminus regulated dopamine-mediated phosphorylation and desensitization of the D_1 receptor, which was suggested to reflect the removal of potential GRK2 and/or GRK3 phosphorylation sites [50]. Carboxyl terminal sequences located upstream of Gly^{379} (between Cys^{351} and Gly^{379}) were shown to be important for phosphorylation but not for desensitization [50]. Site-directed mutagenesis studies of the human D_1 receptor, on the other hand, have provided evidence to suggest that GRK2 acts as a critical regulator of rapid agonist-induced receptor desensitization through phosphorylation of a single motif containing the residues Thr^{360} and Glu^{359} in the proximal segment of the carboxyl terminus [51]. Both of these studies have used differential methodology which may play a role in the discrepant results observed. Site-directed mutagenesis studies may be a more reliable method for identifying the importance of specific residues since there is little change in the intact structure of the receptor. Carboxyl terminal truncations, however, can alter the structure of the receptor which permits access to previously sterically hindered receptor domains, such as the third intracellular loop.

The third cytoplasmic loop has also been implicated in desensitization of the D_1 receptor. It was previously demonstrated that the mutation of specific residues in the third intracellular loop did not affect desensitization of the D_1 receptor [51]. However, a subsequent report has demonstrated that these same residues were involved in D_1 receptor phosphorylation and desensitization [57]. A possible reason for this discrepancy may be the use of differential cell lines, where one study used CHO cells and the other HEK 293 cells. It has been shown that the rate of agonist-induced desensitization of the D_1 receptor in CHO cells occurs more slowly than in other cell types [58]. Thus, it has been postulated that D_1 receptor phosphorylation may be GRK isoform dependent and these isoforms may be lacking in the CHO cell line [57].

Given the evidence demonstrating the importance of the carboxyl terminus and third intracellular loop, it has been proposed that D_1 receptor phosphorylation takes place in both the carboxyl terminus and the third intracellular loop in a sequential manner, where primary phosphorylation of the carboxyl terminus is permissive for secondary third intracellular loop phosphorylation, which then allows for the desensitization response [57].

In contrast to GRK2 phosphorylation, which requires receptor activation, GRK4 has been shown to regulate the constitutive phosphorylation and desensitization of the D_1 receptor [59] suggesting that specific GRK isoforms may serve discrete functions in the regulation of dopamine receptor activity.

Other kinases, such as protein kinase A (PKA), may also play a role in homologous or agonist-specific forms of GPCR desensitization (reviewed [11]). Although it has been demonstrated that the mutation of a potential D_1 receptor PKA phosphorylation site reduced the rate of agonist-induced desensitization [60], and moreover, that D_1 receptor desensitization was blunted in cells deficient in PKA [58], it has also been shown that the inhibition of PKA, either by substitution mutations

[51, 52] or pharmacologically [52], appeared to have no effect on D_1 receptor-mediated increases in cAMP.

The D_5 receptor exhibits very high sequence homology with the D_1 receptor; however, there are major differences in the intracellular loops and carboxyl termini. Unlike the D_1 receptor, the D_5 receptor exhibits higher levels of constitutive activity [61–63], a characteristic that has been shown to be regulated by the third cytoplasmic loop [64], as well as sequence-specific motifs within the carboxyl terminus [61, 62]. Similarly, the D_5 receptor also exhibits higher affinity for agonists, such as dopamine, than the D_1 receptor (reviewed [65]). A series of truncation/deletion mutants of the D_5 receptor identified the region encoded by amino acids 438–448 and particularly Gln^{439} as necessary and sufficient for full expression of higher agonist affinities and constitutive activity relative to the D_1 receptor. The last 40 amino acids of the D_5 receptor, on the other hand, were shown unnecessary for the observed distinguishing pharmacological and functional characteristics [61]. Given that the carboxyl terminus has been implicated in a variety of GPCR regulatory events (reviewed [66]) elucidation of further motifs or residues within the carboxyl terminus of the D_5 receptor is needed to help delineate the specific mechanisms underlying D_5 receptor trafficking.

8.3.2 D_2-Like Receptors

Early studies examining the functional desensitization of D_2-like receptors (D_2, D_3, D_4) have generated variable results but, in general, indicate that they desensitize much more slowly than D_1-like receptors and require prolonged agonist treatment [53, 67]. Similar to the D_1 receptor, the mechanisms underlying D_2-like receptor desensitization appear to involve GRKs, although their role in D_3 receptor desensitization as yet remains uncertain. Only by overexpression of GRK2, GRK5 [68], or GRK3 [69] was there increased phosphorylation of the human D_2 receptor and receptor internalization, indicating the sensitivity of the D_2 receptor as a substrate for GRK phosphorylation is lower than the D_1 receptor. These kinases, however, did not appear to influence phosphorylation and desensitization of the D_3 receptor [48, 69]. Indeed, only when D_3 chimeras were generated containing the second and third cytoplasmic loops of the D_2 receptor, was GRK-mediated phosphorylation evident, possibly revealing the importance of these receptor domains in GRK functioning [69]. It has also been reported, however, that GRK2 and GRK3 levels may regulate the stability of the D_3 receptor interaction with filamin A [70], a scaffolding protein that has been shown to be involved in the stability of D_3 receptor expression at the plasma membrane [48]. In addition, gene deletion of GRK6 was shown to lead to enhanced coupling of D_2-like receptors to their respective G proteins in vivo, an effect that was associated with increased susceptibility to the locomotor-activating effects of psychostimulants [71], and suggests that GRK6 plays a role in regulating the responsiveness of the D_2 and/or D_3 receptors.

The second messenger kinase, protein kinase C (PKC), has also been suggested to regulate the D_2 and D_3 receptors in a heterologous manner since PKC activation was shown to attenuate the ability of both these receptors to inhibit cAMP accumulation [48, 72]. PKC phosphorylation of the D_2 receptor was demonstrated to take place on two internal domains within the third intracellular loop, but only one residue, Ser^{355}, was shown to be involved in the PKC-induced desensitization response [72]. Site-directed mutagenesis of all the possible phosphorylation sites within the intracellular loops of the D_3 receptor identified Ser^{229} and Ser^{257} as the critical amino acids responsible for PKC-induced phosphorylation, desensitization, and internalization [48]. Additionally, PKC activation was shown to induce specific effects on each D_2 receptor isoform (D_{2L} and D_{2S}) with regard to receptor-stimulated calcium mobilization [73]. It has been reported that although PKC is able to effectively desensitize D_{2S}-induced increases in intracellular calcium, the D_{2L} isoform is insensitive to PKC-induced desensitization of calcium signaling due to the presence of a pseudosubstrate domain. A pseudosubstrate domain is a site that resembles a substrate domain except that the serine phosphorylation site is replaced by alanine or other residues and therefore may permit association with the kinase without resulting in functional phosphorylation [73]. This difference in substrate sensitivity of D_{2S} and D_{2L} appeared to be the result of intramolecular competition between different substrate domains on the D_{2L} receptor for PKC recognition and a pseudosubstrate domain, which is not found in the D_{2S} receptor. Given the importance of the D_2 receptor in numerous physiological processes, the presence of pseudosubstrate domains may potentially have significant implications for the regulation of the receptor by PKC.

8.3.3 The D_1–D_2 Heteromer

Although D_1 and D_2 receptors are biochemically and functionally distinct, some physiological functions require the coactivation of both receptors [74, 75]. At a mechanistic level this has been difficult to reconcile since coactivation of the D_1 and D_2 receptors can result in both opposing and synergistic physiological responses. The recent discovery, however, of a common functional output generated by the concurrent activation of D_1 and D_2 receptors within the same cells resulting in activation of a novel Gq/11-linked phospholipase C-dependent calcium signal [76] has provided a possible biochemical mechanism by which the D_1 and D_2 receptors work in concert to mediate these molecular and behavioral functions. Additionally, in cultured cells coexpressing both receptors, the existence of D_1–D_2 heteromers was established by fluorescence resonance energy transfer [77], cotrafficking studies [77], and visualization of D_1–D_2 heteromers in live cells [78]. Accordingly, a heteromeric D_1–D_2 signaling complex that could rapidly activate the Gq/11 protein and result in intracellular calcium release was demonstrated to exist in the adult rodent striatum [76, 77, 79].

Although little is known regarding the regulation of D_1–D_2 heteromer responsiveness, it has been shown that desensitization of the agonist-induced calcium signal occurs within minutes of agonist exposure and is initiated by agonist occupancy of either receptor subtype, even though the signal is generated only by occupancy of both receptors [80]. Additionally, the attenuation of receptor internalization did not result in a concomitant decrease in the extent of signal desensitization, suggesting desensitization of the signal occurred prior to recruitment of the complex into vesicles by endocytic machinery. Although GRK5 or GRK6 or any of the second messenger kinases did not play a role in the desensitization, GRK2 and GRK3 appeared to have a role in the extent of desensitization. Inhibition of GRK-mediated phosphorylation, however, did not inhibit this desensitization [80], suggesting that, in addition to phosphorylating receptors, GRKs may also mediate signal desensitization by phosphorylation-independent mechanisms. It has been suggested that GRK2 and GRK3 may sequester Gq/11 proteins, which interact with the RGS domain on these GRKs [81]. Thus, this may provide a mechanism by which GRK2 and GRK3 contribute to desensitization of the calcium signal mediated by the D_1–D_2 receptor heteromer [80]. It is of note, however, that heteromeric D_1 and D_2 receptors exhibit conformations that permitted cross-phosphorylation of the D_2 receptor by D_1 receptor activation [77], a finding that implicates a discrete mechanism by which the D_1 receptor within the D_1–D_2 complex may regulate heteromer functioning.

8.4 Internalization

8.4.1 D_1-Like Receptors

The acute administration of dopamine agonists has been demonstrated to induce robust internalization of the D_1 receptor in both cultured cells and neurons [82, 83], as well as in vivo [84]. While in the absence of agonist the D_1 receptor remained predominantly on the cell surface, the addition of dopamine induced rapid internalization of approximately 70% of the receptors, with a half-life of less than 5 min [56, 85]. Although endocytosis of the D_1 receptor has been consistently documented in both heterologous expression systems and neuronal cultures, the underlying mechanisms have shown to be more variable. While earlier studies have identified a role for PKA-mediated internalization in cells endogenously expressing the D_1 receptor [86], mutagenesis of the PKA sites of the human D_1 receptor [51], the rat D_1 receptor [60], and the non-human primate D_1 receptor [52] did not affect agonist-induced internalization.

Consistent with the role of GRKs in D_1 receptor desensitization, this group of kinases appears to play an essential role in D_1 receptor internalization, although the residues identified as being important for desensitization are not the same as for internalization. Receptor mutagenesis has revealed that specific residues in the distal portion of the carboxyl terminus (Thr^{446}, Thr^{439}, and Ser^{431}) are involved in GRK2-mediated internalization of the human D_1 receptor [51]. However, rat D_1 receptor

mutants with carboxyl terminal truncations implied that sequences located between Cys^{351} and Gly^{379} are pivotal to receptor internalization [50]. Although there appear to be discrepancies regarding the relative importances of specific residues in D_1 receptor internalization, the carboxyl terminus seems to be essential in this stage of the endocytic trafficking pathway. It has also been postulated, however, that GRK-mediated D_1 receptor phosphorylation on the third intracellular loop may be of relevance in promoting receptor interactions with arrestins [57], adaptor proteins that have been shown to be essential for the internalization of a number of GPCRs including the D_1 receptor (reviewed [87]). Specifically, it has been suggested that the phosphorylation of residues within the carboxyl terminus and third intracellular loop dissociates the two domains, allowing for arrestin to bind to the activated third loop [57]. Activation of the D_1 receptor leads to translocation of both arrestin2 and arrestin3 to the cell membrane, with arrestin3 being the more predominant translocated subtype. Following arrestin membrane localization, the D_1 receptor is internalized and arrestin subsequently dissociates from the receptor at or near the membrane [57, 88, 89]. Similarly, colocalization between endogenous D_1 receptors and arrestins in rat neostriatal neuronal cultures demonstrated that the D_1 receptor preferentially interacts with arrestin3 [90].

In addition to arrestins, studies assessing the internalization pathway of D_1 receptor membrane trafficking have demonstrated the involvement of numerous other proteins, including the scaffolding proteins PSD-95, clathrin, and caveolin-1, and the GTPase dynamin [85, 91, 92]. In cultured cells, the coexpression of PSD-95 with the D_1 receptor resulted in a robust internalization of the receptor in the absence of agonist. Additionally, the abolishment of PSD-95 in mice accentuated D_1 receptor-mediated behavioral responses, suggesting that PSD-95 may also serve an inhibitory role in the regulation of D_1 receptor signaling in vivo [92]. Evidence suggests that facilitation of D_1 receptor internalization by PSD-95 is mediated through interactions with the carboxyl terminus of the D_1 receptor and furthermore is dependent on the presence of dynamin [92]. As dynamin has been previously shown to be involved in dopamine-induced clathrin-mediated endocytosis of the D_1 receptor [56, 85], these findings implicate the clathrin-mediated endocytic pathway in the internalization of the D_1 receptor.

In addition to clathrin-mediated internalization, it has been shown in cultured cells that the D_1 receptor can be localized to low-density caveolin-enriched membrane domains and can associate with caveolin-1 in rat brain through a specific binding motif found in transmembrane domain 7 [91]. Agonist stimulation of the D_1 receptor caused its translocation into caveolin-1-enriched membrane fractions, which was determined to be the result of D_1 receptor endocytosis through caveolae. However, unlike the relatively rapid clathrin-dependent mechanism of internalization in which approximately 70% of activated receptors were internalized within 5 min [85], caveolin-dependent D_1 receptor endocytosis appeared to be kinetically slower, reaching approximately 55% internalization within 45 min of agonist stimulation [91]. These findings suggest that both clathrin- and caveolin-mediated processes may play functionally distinct roles in regulating D_1 receptor responsiveness in vivo. It would be of clinical relevance to determine whether

the relative contribution of each of these pathways differs in specific regions of the brain.

8.4.2 D_2-Like Receptors

The endocytosis of the D_2 receptor is a highly complex process that has been shown to be both isoform and cell specific, as well as to exhibit both dynamin-dependent and independent mechanisms [1, 57, 69, 85, 93].

Internalization of the D_2 receptor requires increased levels of GRKs in heterologous cells and appears to be a relatively slow process taking approximately 2 h to plateau [68, 93, 94]. Whereas little or no internalization was observed in the absence of exogenous GRKs or in the presence of the dominant negative GRK2 (DN-GRK2), coexpression of GRK2, GRK5 [68], or GRK3 [69] caused significant D_2 receptor internalization.

Similar to the D_1 receptor, internalization of the D_2 receptor involves GRK-dependent receptor phosphorylation, followed by the translocation of arrestin2 and arrestin3 to the cell membrane [69, 95] which function to promote receptor internalization [57]. The endogenous dopamine D_2 receptor in neurons, however, has been shown to preferentially interact with arrestin2 [95]. The D_2 receptor isoforms also showed differential regulatory mechanisms for internalization. For example, although both isoforms displayed a similar level of phosphorylation and arrestin translocation, the actual internalization of the two isoforms was differentially regulated by GRKs and arrestins, where the internalization of the D_{2S} receptor was preferentially enhanced by GRK2 or GRK3, but the D_{2L} receptor was preferentially enhanced by arrestin3 [96]. As discussed previously, given that the two receptor isoforms differ by a 29 amino acid insertion in the third intracellular loop of the D_{2L} receptor, it is plausible that this region may play a role in isoform-specific trafficking.

In contrast to the D_1 receptor, D_2 receptor internalization appears to be mediated by specific dynamin isoforms, suggesting specificity between dynamin isoforms and dopamine receptor subtypes. It has been reported that the internalization of the D_{2S} receptor is dynamin dependent, implicating the clathrin-coated endocytic pathway in the internalization of this receptor [57, 69, 93]. There are conflicting reports, however, as to the importance of dynamin-mediated mechanisms in the internalization of the D_{2L} receptor. While it has been suggested that the D_{2L} receptor internalizes in a dynamin-independent manner [57, 85], these studies assessed only the role of the dynamin1 isoform, whereas the dynamin2 isoform has been more recently implicated. In cultured cells and primary striatal neurons dynamin2 was shown to localize to sites of D_2 receptor internalization and associate with the D_2 receptor in the rat brain [1]. Furthermore, when high-resolution immunoelectron microscopy was used to study internalization patterns of the D_2 receptor in the primate prefrontal cortex, the D_2 receptor was demonstrated to undergo clathrin-mediated endocytosis via clathrin-coated pits and clathrin-coated vesicles [97].

In contrast to the D_2 receptor, the D_3 receptor demonstrated little internalization in response to dopamine stimulation and only in the presence of overexpressed

GRKs [48, 69, 96]. In addition, there appeared to be no noticeable translocation of arrestin in cells expressing the D_3 receptor [96].

PKC activation also led to 50% of the D_2 receptor being internalized when PKCβ was overexpressed [72]. Mutagenesis studies suggest that both of the PKC phosphorylation domains identified within the third intracellular loop were involved in regulating its internalization from the cell surface [72]. It has also been demonstrated that PKC activation induced significant internalization of the D_3 receptor that appeared to be dependent on both dynamin and filamin A [48], a result that supported previous evidence of a clathrin-mediated mechanism in D_3 receptor endocytosis [69].

Mechanisms underlying the regulation of the D_4 receptor are poorly understood. An interesting feature of the D_4 receptor, however, is the highly polymorphic region within the third cytoplasmic loop. The human D_4 receptor has a variable number of tandem repeats within the third intracellular loop as well as multiple putative Src homology 3 (SH3)-binding motifs that may regulate the trafficking properties of this receptor. Deletion of all the putative SH3-binding domains, but not the tandem repeat, in the third intracellular loop of the D_4 receptor resulted in constitutive internalization [98]. Similarly, deletion of one of the two SH3-binding sites abolished arrestin3 translocation [96], suggesting that the SH3 domains may be important in the regulation of D_4 receptor responsiveness.

8.4.3 The D_1–D_2 Heteromer

Given the relatively recent discovery of the D_1–D_2 receptor heteromer, there is much yet unknown regarding the trafficking properties of this complex. It has been determined that selective agonist occupancy by either a D_1 agonist or a D_2 agonist leads to D_1–D_2 heteromer cointernalization [77]. This is an interesting finding that indicates activation of only one receptor within the D_1–D_2 complex is sufficient for internalization, whereas coactivation of the D_1 and D_2 receptors is required for the PLC-mediated calcium signal. It was also shown that heteromerization resulted in altered steady-state cellular distribution of the D_1 and D_2 receptors within cells that were distinct from that of the D_1 and D_2 receptor homomers [77]. Together, these findings emphasize the unique trafficking responses of the heteromer compared to its constituent D_1 and D_2 receptors, a characteristic that may elucidate differences in physiological function.

8.5 Resensitization

8.5.1 D_1-Like Receptors

Investigations into the trafficking fate of the D_1 receptor after agonist-induced internalization have generally reported that the D_1 receptor recycles back to the plasma membrane [50, 51, 83–85, 99, 100], as opposed to being targeted to lysosomes for receptor degradation. With the use of immunohistochemistry or fluorescence

microscopy, the D_1 receptor expressed in cultured cells or neurons was demonstrated to recycle back to the plasma membrane after removal of agonist within approximately 20–30 min [83, 85, 99]. In accordance with these studies, dopamine-stimulated D_1 receptor phosphorylation has been shown to be rapidly reversed within 30 min, although resensitization of the D_1 receptor-mediated cAMP response occurred much more slowly, taking a minimum of 5 h to reach control levels. It was suggested that internalization was not mandatory for D_1 receptor dephosphorylation since pretreatment of the cells with hypertonic sucrose or concanavalin A did not alter D_1 receptor dephosphorylation after agonist removal [54]. The efficient recycling of the D_1 receptor, however, was recently reported to require a specific sequence within the proximal portion of the carboxyl terminus of the receptor [99]. This sequence spans amino acid residues 360–382 of the human D_1 receptor and is distinct from those previously identified as being required for efficient recycling of other GPCRs [101–105]. The importance of this sequence as a sorting signal was further established by demonstrating that the motif could induce the recycling of the δ-opioid receptor, a receptor that traffics preferentially to lysosomes after agonist-induced internalization [99].

Attempts have been made to elucidate the accessory proteins that may contribute to the regulation of D_1 receptor postendocytic sorting. GPCR-associated sorting protein (GASP) has been shown to interact with the D_1 receptor, and to a greater degree, the D_2 receptor [100, 106]. However, while GASP was demonstrated to promote receptor degradation in the D_2 receptor, a role in D_1 receptor sorting was not observed [100]. A recent study involving a number of mutant GPCRs, including the D_1 receptor, demonstrated that the presence of a GASP interaction in and of itself is not sufficient to induce receptor degradation but rather it is the robustness of the GASP–receptor interaction that regulates the targeting to lysosomes [106]. It was shown that although deletion of the recycling motif in the D_1 receptor prevented recycling, it also was not targeted for degradation, suggesting that preventing recycling does not necessarily promote D_1 receptor degradation unless affinity for sorting proteins such as GASP that mediate degradation is altered as well [106].

Although the endocytic sorting mechanisms of the D_5 receptor are poorly understood, it has been shown that the carboxyl terminus of the receptor binds to sorting nexin 1 (SNX1) [107], a trafficking protein that has been implicated in the lysosomal targeting of the thrombin receptor PAR1 [108]. Although the role of SNX1 in D_5 receptor trafficking has yet to be defined, these results suggest that the postendocytic sorting of the D_5 receptor may involve sorting to lysosomes that is mediated by SNX1.

8.5.2 D_2-Like Receptors

While it has been previously demonstrated that the D_2 receptor can recycle within approximately 30 min of dopamine exposure in cultured cells [85], more recent evidence suggests that the D_2 receptor is predominantly degraded after dopamine

exposure in cells and neurons [100]. As discussed previously, unlike the D_1 receptor, the sorting fate of the D_2 receptor appears to be mediated by GASP in non-neuronal cells. Moreover, it was shown that dopaminergic neurons endogenously expressing GASP did not exhibit a functional recovery of neuronal responses following D_2 receptor agonist administration, whereas disrupting the GASP–D_2 receptor interaction facilitated the recovery of functional D_2 receptor responses [100]. In addition to GASP, the PKC-interacting protein, ZIP, has been shown to associate with the D_2 receptor in both cultured cells and endogenous brain tissue. Overexpression of ZIP reduced D_2 receptor cell-surface expression via enhanced trafficking of the receptors to lysosomes, suggesting that the ZIP protein functions as a negative modulator of D_2 receptor expression [109]. Such differences in D_2 receptor sorting as compared to the D_1 receptor may have significant implications with regard to dopaminergic signaling since in vivo both the D_1 and D_2 receptors respond to dopamine and thus may lead to an altered signaling profile depending on the extent of previous dopamine exposure.

8.6 Dysregulation of Receptor Trafficking in Health and Disease

In many instances the acute activation of dopamine receptors leads to a reduction in receptor number as they internalize into the cell. For some receptors, such as the D_1 receptor, this effect is generally transitory with a subsequent recovery of receptors back to the cell surface. However, evidence indicates that the D_2 receptor may be preferentially targeted to degradation pathways (see Section 8.5.1), suggesting that persistent alterations in extracellular dopamine could potentially have long-term consequences on dopamine receptor trafficking and responsiveness. Indeed, chronic alterations in dopamine tone have been associated with brain region-specific alterations in dopamine receptor expression for a number of neurological disorders, including schizophrenia (reviewed [110]), Parkinson's disease (reviewed [111]), and drug abuse [112] and have been readily observed in animal models of enhanced dopamine transmission [113–115].

The pharmacological modification of dopamine transmission has long been employed as a therapeutic tool in the treatment of many dopamine-related disorders. It has been repeatedly shown, however, that the long-term use of dopamine receptor drugs can lead to the development of negative secondary symptoms that may be linked with changes in dopamine receptor trafficking. Long-term levodopa administration, for example, can be associated with the development of dyskinesias, a behavioral manifestation that has recently been postulated to be the result of impaired D_1 receptor desensitization processes [116]. Similarly, the chronic administration of various antipsychotics also has been reported to result in the manifestation of extrapyramidal motor symptoms (EPS) (reviewed [117, 118]) that may be associated with altered dopamine receptor densities in the brain [119–122].

As a result of the current undesirable consequences underlying some of the pharmaceutical agents used in the treatment of dopamine disorders, new avenues of

research continue to be explored in an attempt to identify novel drug therapies that have pharmacological benefits in the absence of behavioral side effects. Our improved understanding of dopamine receptor trafficking has greatly contributed to this goal. One such area of research involves the development of agonists that exhibit functional selectivity, that is, agonists that exhibit discrete receptor affinities, receptor trafficking profiles, and/or differ in their ability to induce specific downstream signaling events. Several agonists have been identified for the D_1 receptor, for instance, that differentially induce internalization and/or the targeting of internalized receptors to discrete trafficking pathways [123, 124]. Furthermore, while antipsychotics generally function as D_2 receptor antagonists (reviewed [125]), the antipsychotic aripiprazole has been shown to act as an agonist at presynaptic D_2 receptors [126] and additionally exhibit cell line-specific partial agonism, or functional antagonism, at postsynaptic D_2 receptors [126–128]. In a cell line where the drug displayed partial agonist activity, aripiprazole exhibited functional selectivity for both D_2 receptor-mediated trafficking and signaling. In contrast to dopamine, aripiprazole did not induce D_2 receptor internalization and, unlike typical agonists, this drug exhibited low potency for the mitogen-activated protein kinase effector pathway compared to other D_2 receptor-mediated signaling pathways [129]. As aripiprazole has been demonstrated to have an excellent side-effect profile (reviewed [130]), the differential mediation of D_2 receptor trafficking and signaling by the drug may be of relevance to its ability to provide therapeutic benefits without inducing the manifestation of EPS.

Another group of functionally selective agonists are those that affect the receptor export pathways by acting as pharmacological chaperones. These membrane permeable molecules would bind to partially folded or misfolded dopamine receptor monomers or oligomers, correct their folding, and rescue them from ER retention. It has been reported, for instance, that membrane permeable agonists could rescue an intracellularly sequestered D_1 receptor homomer by inducing a conformational change that permitted its cell-surface trafficking from the ER [7]. Similarly, the antipsychotic pipamperone has been shown to both increase the trafficking of D_4 receptors to the plasma membrane and also effectively rescue a D_4 receptor mutant from intracellular sequestration [131]. Thus, the exogenous manipulation of ER retention mechanisms through the use of pharmacological chaperones may provide another useful means to regulate dopamine receptor trafficking.

8.7 Concluding Remarks

Although the detailed mechanisms of trafficking of the five individual dopamine receptors and their isoforms have yet to be fully elucidated, what is currently known clearly highlights the complexity of dopamine receptor trafficking and its role in cellular responsiveness. From the biosynthesis of dopamine receptor complexes to desensitization and internalization, it is now apparent that dopamine receptor trafficking is regulated by an incredibly wide array of protein–protein interactions

that can be highly specific not only to each receptor subtype but also within each subcellular compartment. The importance of oligomerization in dopamine receptor trafficking has also become increasingly evident, and together with the exciting discovery of a novel D_1–D_2 receptor heteromer, these findings have provided new insights into the mechanisms of receptor transport. Undoubtedly, it is an exciting time for dopamine receptor research as intracellular accessory proteins, and oligomeric receptor models are incorporated into strategies for drug discovery. It is hopeful that the next decade will clarify further specific aspects of dopamine receptor trafficking, their contribution to cellular responsiveness, and their role in health and disease.

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References

1. Kabbani N, Jeromin A, Levenson R. Dynamin-2 associates with the dopamine receptor signalplex and regulates internalization of activated D2 receptors. Cell Signal 2004;16(4): 497–503.
2. Dong C, Filipeanu CM, Duvernay MT, Wu G. Regulation of G protein-coupled receptor export trafficking. Biochim Biophys Acta 2007;1768(4):853–70.
3. Hasbi A, Nguyen T, Fan T, et al. Trafficking of preassembled opioid mu-delta heterooligomer-Gz signaling complexes to the plasma membrane: coregulation by agonists. Biochemistry 2007;46(45):12997–3009.
4. Herrick-Davis K, Weaver BA, Grinde E, Mazurkiewicz JE. Serotonin 5-HT2C receptor homodimer biogenesis in the endoplasmic reticulum: real-time visualization with confocal fluorescence resonance energy transfer. J Biol Chem 2006;281(37):27109–16.
5. Issafras H, Angers S, Bulenger S, et al. Constitutive agonist-independent CCR5 oligomerization and antibody-mediated clustering occurring at physiological levels of receptors. J Biol Chem 2002;277(38):34666–73.
6. Hague C, Uberti MA, Chen Z, Hall RA, Minneman KP. Cell surface expression of alpha1D-adrenergic receptors is controlled by heterodimerization with alpha1B-adrenergic receptors. J Biol Chem 2004;279(15):15541–9.
7. Kong MM, Fan T, Varghese G, O'Dowd BF, George SR. Agonist-induced cell surface trafficking of an intracellularly sequestered D1 dopamine receptor homo-oligomer. Mol Pharmacol 2006;70(1):78–89.
8. Lopez-Gimenez JF, Canals M, Pediani JD, Milligan G. The alpha1b-adrenoceptor exists as a higher-order oligomer: effective oligomerization is required for receptor maturation, surface delivery, and function. Mol Pharmacol 2007;71(4):1015–29.
9. Salahpour A, Angers S, Mercier JF, Lagace M, Marullo S, Bouvier M. Homodimerization of the beta2-adrenergic receptor as a prerequisite for cell surface targeting. J Biol Chem 2004;279(32):33390–7.
10. White JH, Wise A, Main MJ, et al. Heterodimerization is required for the formation of a functional GABA(B) receptor. Nature 1998;396(6712):679–82.
11. Ferguson SS. Evolving concepts in G protein-coupled receptor endocytosis: the role in receptor desensitization and signaling. Pharmacol Rev 2001;53(1):1–24.
12. Moore CA, Milano SK, Benovic JL. Regulation of receptor trafficking by GRKs and arrestins. Annu Rev Physiol 2007;69:451–82.

13. Rashid AJ, O'Dowd BF, Verma V, George SR. Neuronal Gq/11-coupled dopamine receptors: an uncharted role for dopamine. Trends Pharmacol Sci 2007;28(11):551–5.
14. Free RB, Hazelwood LA, Cabrera DM, et al. D1 and D2 dopamine receptor expression is regulated by direct interaction with the chaperone protein calnexin. J Biol Chem 2007;282(29):21285–300.
15. Brothers SP, Janovick JA, Conn PM. Calnexin regulated gonadotropin-releasing hormone receptor plasma membrane expression. J Mol Endocrinol 2006;37(3):479–88.
16. Lanctot PM, Leclerc PC, Escher E, Guillemette G, Leduc R. Role of N-glycan-dependent quality control in the cell-surface expression of the AT1 receptor. Biochem Biophys Res Commun 2006;340(2):395–402.
17. Ou WJ, Bergeron JJ, Li Y, Kang CY, Thomas DY. Conformational changes induced in the endoplasmic reticulum luminal domain of calnexin by Mg-ATP and Ca2+. J Biol Chem 1995;270(30):18051–9.
18. Petaja-Repo UE, Hogue M, Laperriere A, Walker P, Bouvier M. Export from the endoplasmic reticulum represents the limiting step in the maturation and cell surface expression of the human delta opioid receptor. J Biol Chem 2000;275(18):13727–36.
19. Duvernay MT, Zhou F, Wu G. A conserved motif for the transport of G protein-coupled receptors from the endoplasmic reticulum to the cell surface. J Biol Chem 2004;279(29):30741–50.
20. Gaborik Z, Mihalik B, Jayadev S, Jagadeesh G, Catt KJ, Hunyady L. Requirement of membrane-proximal amino acids in the carboxyl-terminal tail for expression of the rat AT1a angiotensin receptor. FEBS Lett 1998;428(3):147–51.
21. Oksche A, Dehe M, Schulein R, Wiesner B, Rosenthal W. Folding and cell surface expression of the vasopressin V2 receptor: requirement of the intracellular C-terminus. FEBS Lett 1998;424(1–2):57–62.
22. Pankevych H, Korkhov V, Freissmuth M, Nanoff C. Truncation of the A1 adenosine receptor reveals distinct roles of the membrane-proximal carboxyl terminus in receptor folding and G protein coupling. J Biol Chem 2003;278(32):30283–93.
23. Tetsuka M, Saito Y, Imai K, Doi H, Maruyama K. The basic residues in the membrane-proximal C-terminal tail of the rat melanin-concentrating hormone receptor 1 are required for receptor function. Endocrinology 2004;145(8):3712–23.
24. Bermak JC, Li M, Bullock C, Zhou QY. Regulation of transport of the dopamine D1 receptor by a new membrane-associated ER protein. Nat Cell Biol 2001;3(5):492–8.
25. Robert J, Clauser E, Petit PX, Ventura MA. A novel C-terminal motif is necessary for the export of the vasopressin V1b/V3 receptor to the plasma membrane. J Biol Chem 2005;280(3):2300–8.
26. Schulein R, Hermosilla R, Oksche A, et al. A dileucine sequence and an upstream glutamate residue in the intracellular carboxyl terminus of the vasopressin V2 receptor are essential for cell surface transport in COS.M6 cells. Mol Pharmacol 1998;54(3):525–35.
27. Bermak JC, Li M, Bullock C, Weingarten P, Zhou QY. Interaction of gamma-COP with a transport motif in the D1 receptor C-terminus. Eur J Cell Biol 2002;81(2):77–85.
28. Bethune J, Wieland F, Moelleken J. COPI-mediated transport. J Membr Biol 2006;211(2):65–79.
29. Bednarek SY, Ravazzola M, Hosobuchi M, et al. COPI- and COPII-coated vesicles bud directly from the endoplasmic reticulum in yeast. Cell 1995;83(7):1183–96.
30. Fiedler K, Veit M, Stamnes MA, Rothman JE. Bimodal interaction of coatomer with the p24 family of putative cargo receptors. Science 1996;273(5280):1396–9.
31. Nishimura N, Balch WE. A di-acidic signal required for selective export from the endoplasmic reticulum. Science 1997;277(5325):556–8.
32. Yuan H, Michelsen K, Schwappach B. 14-3-3 dimers probe the assembly status of multimeric membrane proteins. Curr Biol 2003;13(8):638–46.
33. Dupre DJ, Robitaille M, Richer M, Ethier N, Mamarbachi AM, Hebert TE. Dopamine receptor-interacting protein 78 acts as a molecular chaperone for Ggamma subunits before assembly with Gbeta. J Biol Chem 2007;282(18):13703–15.

34. Karpa KD, Lidow MS, Pickering MT, Levenson R, Bergson C. N-linked glycosylation is required for plasma membrane localization of D5, but not D1, dopamine receptors in transfected mammalian cells. Mol Pharmacol 1999;56(5):1071–8.
35. Prou D, Gu WJ, Le Crom S, Vincent JD, Salamero J, Vernier P. Intracellular retention of the two isoforms of the D(2) dopamine receptor promotes endoplasmic reticulum disruption. J Cell Sci 2001;114(Pt 19):3517–27.
36. Fishburn CS, Elazar Z, Fuchs S. Differential glycosylation and intracellular trafficking for the long and short isoforms of the D2 dopamine receptor. J Biol Chem 1995;270(50): 29819–24.
37. Sedaghat K, Nantel MF, Ginsberg S, Lalonde V, Tiberi M. Molecular characterization of dopamine D2 receptor isoforms tagged with green fluorescent protein. Mol Biotechnol 2006;34(1):1–14.
38. Usiello A, Baik JH, Rouge-Pont F, et al. Distinct functions of the two isoforms of dopamine D2 receptors. Nature 2000;408(6809):199–203.
39. Jans DA, Peters R, Jans P, Fahrenholz F. Vasopressin V2-receptor mobile fraction and ligand-dependent adenylate cyclase activity are directly correlated in LLC-PK1 renal epithelial cells. J Cell Biol 1991;114(1):53–60.
40. Pucadyil TJ, Kalipatnapu S, Harikumar KG, Rangaraj N, Karnik SS, Chattopadhyay A. G-protein-dependent cell surface dynamics of the human serotonin1A receptor tagged to yellow fluorescent protein. Biochemistry 2004;43(50):15852–62.
41. Scott L, Zelenin S, Malmersjo S, et al. Allosteric changes of the NMDA receptor trap diffusible dopamine 1 receptors in spines. Proc Natl Acad Sci USA 2006;103(3): 762–7.
42. Scott L, Kruse MS, Forssberg H, Brismar H, Greengard P, Aperia A. Selective up-regulation of dopamine D1 receptors in dendritic spines by NMDA receptor activation. Proc Natl Acad Sci USA 2002;99(3):1661–4.
43. Lee FJ, Xue S, Pei L, et al. Dual regulation of NMDA receptor functions by direct protein-protein interactions with the dopamine D1 receptor. Cell 2002;111(2):219–30.
44. Binda AV, Kabbani N, Lin R, Levenson R. D2 and D3 dopamine receptor cell surface localization mediated by interaction with protein 4.1 N. Mol Pharmacol 2002;62(3): 507–13.
45. Li M, Bermak JC, Wang ZW, Zhou QY. Modulation of dopamine D(2) receptor signaling by actin-binding protein (ABP-280). Mol Pharmacol 2000;57(3):446–52.
46. Lin R, Canfield V, Levenson R. Dominant negative mutants of filamin A block cell surface expression of the D2 dopamine receptor. Pharmacology 2002;66(4):173–81.
47. Lin R, Karpa K, Kabbani N, Goldman-Rakic P, Levenson R. Dopamine D2 and D3 receptors are linked to the actin cytoskeleton via interaction with filamin A. Proc Natl Acad Sci USA 2001;98(9):5258–63.
48. Cho EY, Cho DI, Park JH, Kurose H, Caron MG, Kim KM. Roles of protein kinase C and actin-binding protein 280 in the regulation of intracellular trafficking of dopamine D3 receptor. Mol Endocrinol 2007;21(9):2242–54.
49. Kim OJ, Ariano MA, Lazzarini RA, Levine MS, Sibley DR. Neurofilament-M interacts with the D1 dopamine receptor to regulate cell surface expression and desensitization. J Neurosci 2002;22(14):5920–30.
50. Jackson A, Iwasiow RM, Chaar ZY, Nantel MF, Tiberi M. Homologous regulation of the heptahelical D1A receptor responsiveness: specific cytoplasmic tail regions mediate dopamine-induced phosphorylation, desensitization and endocytosis. J Neurochem 2002;82(3):683–97.
51. Lamey M, Thompson M, Varghese G, et al. Distinct residues in the carboxyl tail mediate agonist-induced desensitization and internalization of the human dopamine D1 receptor. J Biol Chem 2002;277(11):9415–21.
52. Mason JN, Kozell LB, Neve KA. Regulation of dopamine D(1) receptor trafficking by protein kinase A-dependent phosphorylation. Mol Pharmacol 2002;61(4):806–16.

53. Ng GY, Varghese G, Chung HT, et al. Resistance of the dopamine D2L receptor to desensitization accompanies the up-regulation of receptors on to the surface of Sf9 cells. Endocrinology 1997;138(10):4199–206.
54. Gardner B, Liu ZF, Jiang D, Sibley DR. The role of phosphorylation/dephosphorylation in agonist-induced desensitization of D1 dopamine receptor function: evidence for a novel pathway for receptor dephosphorylation. Mol Pharmacol 2001;59(2):310–21.
55. Ng GY, Mouillac B, George SR, et al. Desensitization, phosphorylation and palmitoylation of the human dopamine D1 receptor. Eur J Pharmacol 1994;267(1):7–19.
56. Ng GY, Trogadis J, Stevens J, Bouvier M, O'Dowd BF, George SR. Agonist-induced desensitization of dopamine D1 receptor-stimulated adenylyl cyclase activity is temporally and biochemically separated from D1 receptor internalization. Proc Natl Acad Sci USA 1995;92(22):10157–61.
57. Kim OJ, Gardner BR, Williams DB, et al. The role of phosphorylation in D1 dopamine receptor desensitization: evidence for a novel mechanism of arrestin association. J Biol Chem 2004;279(9):7999–8010.
58. Ventura AL, Sibley DR. Altered regulation of the D(1) dopamine receptor in mutant Chinese hamster ovary cells deficient in cyclic AMP-dependent protein kinase activity. J Pharmacol Exp Ther 2000;293(2):426–34.
59. Rankin ML, Marinec PS, Cabrera DM, Wang Z, Jose PA, Sibley DR. The D1 dopamine receptor is constitutively phosphorylated by G protein-coupled receptor kinase 4. Mol Pharmacol 2006;69(3):759–69.
60. Jiang D, Sibley DR. Regulation of D(1) dopamine receptors with mutations of protein kinase phosphorylation sites: attenuation of the rate of agonist-induced desensitization. Mol Pharmacol 1999;56(4):675–83.
61. Demchyshyn LL, McConkey F, Niznik HB. Dopamine D5 receptor agonist high affinity and constitutive activity profile conferred by carboxyl-terminal tail sequence. J Biol Chem 2000;275(31):23446–55.
62. Iwasiow RM, Nantel MF, Tiberi M. Delineation of the structural basis for the activation properties of the dopamine D1 receptor subtypes. J Biol Chem 1999;274(45):31882–90.
63. Sugamori KS, Scheideler MA, Vernier P, Niznik HB. Dopamine D1B receptor chimeras reveal modulation of partial agonist activity by carboxyl-terminal tail sequences. J Neurochem 1998;71(6):2593–9.
64. Charpentier S, Jarvie KR, Severynse DM, Caron MG, Tiberi M. Silencing of the constitutive activity of the dopamine D1B receptor. Reciprocal mutations between D1 receptor subtypes delineate residues underlying activation properties. J Biol Chem 1996;271(45):28071–6.
65. Missale C, Nash SR, Robinson SW, Jaber M, Caron MG. Dopamine receptors: from structure to function. Physiol Rev 1998;78(1):189–225.
66. Luttrell LM. Transmembrane signaling by G protein-coupled receptors. Methods Mol Biol 2006;332:3–49.
67. Zhang LJ, Lachowicz JE, Sibley DR. The D2S and D2L dopamine receptor isoforms are differentially regulated in Chinese hamster ovary cells. Mol Pharmacol 1994;45(5):878–89.
68. Ito K, Haga T, Lameh J, Sadee W. Sequestration of dopamine D2 receptors depends on coexpression of G-protein-coupled receptor kinases 2 or 5. Eur J Biochem 1999;260(1): 112–9.
69. Kim KM, Valenzano KJ, Robinson SR, Yao WD, Barak LS, Caron MG. Differential regulation of the dopamine D2 and D3 receptors by G protein-coupled receptor kinases and beta-arrestins. J Biol Chem 2001;276(40):37409–14.
70. Kim KM, Gainetdinov RR, Laporte SA, Caron MG, Barak LS. G protein-coupled receptor kinase regulates dopamine D3 receptor signaling by modulating the stability of a receptor-filamin-beta-arrestin complex: a case of autoreceptor regulation. J Biol Chem 2005;280(13):12774–80.
71. Gainetdinov RR, Bohn LM, Sotnikova TD, et al. Dopaminergic supersensitivity in G protein-coupled receptor kinase 6-deficient mice. Neuron 2003;38(2):291–303.

72. Namkung Y, Sibley DR. Protein kinase C mediates phosphorylation, desensitization, and trafficking of the D2 dopamine receptor. J Biol Chem 2004;279(47):49533–41.
73. Morris SJ, Van Ham II, Daigle M, Robillard L, Sajedi N, Albert PR. Differential desensitization of dopamine D2 receptor isoforms by protein kinase C: the importance of receptor phosphorylation and pseudosubstrate sites. Eur J Pharmacol 2007;577(1–3):44–53.
74. Capper-Loup C, Canales JJ, Kadaba N, Graybiel AM. Concurrent activation of dopamine D1 and D2 receptors is required to evoke neural and behavioral phenotypes of cocaine sensitization. J Neurosci 2002;22(14):6218–27.
75. Kita K, Shiratani T, Takenouchi K, Fukuzako H, Takigawa M. Effects of D1 and D2 dopamine receptor antagonists on cocaine-induced self-stimulation and locomotor activity in rats. Eur Neuropsychopharmacol 1999;9(1–2):1–7.
76. Lee SP, So CH, Rashid AJ, et al. Dopamine D1 and D2 receptor Co-activation generates a novel phospholipase C-mediated calcium signal. J Biol Chem 2004;279(34):35671–8.
77. So CH, Varghese G, Curley KJ, et al. D1 and D2 dopamine receptors form heterooligomers and cointernalize after selective activation of either receptor. Mol Pharmacol 2005;68(3):568–78.
78. O'Dowd BF, Ji X, Alijaniaram M, et al. Dopamine receptor oligomerization visualized in living cells. J Biol Chem 2005;280(44):37225–35.
79. Rashid AJ, So CH, Kong MM, et al. D1–D2 dopamine receptor heterooligomers with unique pharmacology are coupled to rapid activation of Gq/11 in the striatum. Proc Natl Acad Sci USA 2007;104(2):654–9.
80. So CH, Verma V, O'Dowd BF, George SR. Desensitization of the dopamine D1 and D2 receptor hetero-oligomer mediated calcium signal by agonist occupancy of either receptor. Mol Pharmacol 2007;72(2):450–62.
81. Iwata K, Luo J, Penn RB, Benovic JL. Bimodal regulation of the human H1 histamine receptor by G protein-coupled receptor kinase 2. J Biol Chem 2005;280(3):2197–204.
82. Martin-Negrier M, Charron G, Bloch B. Agonist stimulation provokes dendritic and axonal dopamine D(1) receptor redistribution in primary cultures of striatal neurons. Neuroscience 2000;99(2):257–66.
83. Martin-Negrier ML, Charron G, Bloch B. Receptor recycling mediates plasma membrane recovery of dopamine D1 receptors in dendrites and axons after agonist-induced endocytosis in primary cultures of striatal neurons. Synapse 2006;60(3):194–204.
84. Dumartin B, Caille I, Gonon F, Bloch B. Internalization of D1 dopamine receptor in striatal neurons in vivo as evidence of activation by dopamine agonists. J Neurosci 1998;18(5):1650–61.
85. Vickery RG, von Zastrow M. Distinct dynamin-dependent and -independent mechanisms target structurally homologous dopamine receptors to different endocytic membranes. J Cell Biol 1999;144(1):31–43.
86. Bates MD, Olsen CL, Becker BN, et al. Elevation of cAMP is required for down-regulation, but not agonist-induced desensitization, of endogenous dopamine D1 receptors in opossum kidney cells. Studies in cells that stably express a rat cAMP phosphodiesterase (rPDE3) cDNA. J Biol Chem 1993;268(20):14757–63.
87. Luttrell LM, Lefkowitz RJ. The role of beta-arrestins in the termination and transduction of G-protein-coupled receptor signals. J Cell Sci 2002;115(Pt 3):455–65.
88. Oakley RH, Laporte SA, Holt JA, Caron MG, Barak LS. Differential affinities of visual arrestin, beta arrestin1, and beta arrestin2 for G protein-coupled receptors delineate two major classes of receptors. J Biol Chem 2000;275(22):17201–10.
89. Zhang J, Barak LS, Anborgh PH, Laporte SA, Caron MG, Ferguson SS. Cellular trafficking of G protein-coupled receptor/beta-arrestin endocytic complexes. J Biol Chem 1999;274(16):10999–1006.
90. Macey TA, Liu Y, Gurevich VV, Neve KA. Dopamine D1 receptor interaction with arrestin3 in neostriatal neurons. J Neurochem 2005;93(1):128–34.

91. Kong MM, Hasbi A, Mattocks M, Fan T, O'Dowd BF, George SR. Regulation of D1 dopamine receptor trafficking and signaling by caveolin-1. Mol Pharmacol 2007;72(5):1157–70.
92. Zhang J, Vinuela A, Neely MH, et al. Inhibition of the dopamine D1 receptor signaling by PSD-95. J Biol Chem 2007;282(21):15778–89.
93. Iwata K, Ito K, Fukuzaki A, Inaki K, Haga T. Dynamin and rab5 regulate GRK2-dependent internalization of dopamine D2 receptors. Eur J Biochem 1999;263(2):596–602.
94. Itokawa M, Toru M, Ito K, et al. Sequestration of the short and long isoforms of dopamine D2 receptors expressed in Chinese hamster ovary cells. Mol Pharmacol 1996;49(3):560–6.
95. Macey TA, Gurevich VV, Neve KA. Preferential Interaction between the dopamine D2 receptor and Arrestin2 in neostriatal neurons. Mol Pharmacol 2004;66(6):1635–42.
96. Cho DI, Beom S, Van Tol HH, Caron MG, Kim KM. Characterization of the desensitization properties of five dopamine receptor subtypes and alternatively spliced variants of dopamine D2 and D4 receptors. Biochem Biophys Res Commun 2006;350(3):634–40.
97. Paspalas CD, Rakic P, Goldman-Rakic PS. Internalization of D2 dopamine receptors is clathrin-dependent and select to dendro-axonic appositions in primate prefrontal cortex. Eur J Neurosci 2006;24(5):1395–403.
98. Oldenhof J, Vickery R, Anafi M, et al. SH3 binding domains in the dopamine D4 receptor. Biochemistry 1998;37(45):15726–36.
99. Vargas GA, Von Zastrow M. Identification of a novel endocytic recycling signal in the D1 dopamine receptor. J Biol Chem 2004;279(36):37461–9.
100. Bartlett SE, Enquist J, Hopf FW, et al. Dopamine responsiveness is regulated by targeted sorting of D2 receptors. Proc Natl Acad Sci USA 2005;102(32):11521–6.
101. Cao TT, Deacon HW, Reczek D, Bretscher A, von Zastrow M. A kinase-regulated PDZ-domain interaction controls endocytic sorting of the beta2-adrenergic receptor. Nature 1999;401(6750):286–90.
102. Cong M, Perry SJ, Hu LA, Hanson PI, Claing A, Lefkowitz RJ. Binding of the beta2 adrenergic receptor to N-ethylmaleimide-sensitive factor regulates receptor recycling. J Biol Chem 2001;276(48):45145–52.
103. Gage RM, Kim KA, Cao TT, von Zastrow M. A transplantable sorting signal that is sufficient to mediate rapid recycling of G protein-coupled receptors. J Biol Chem 2001;276(48):44712–20.
104. Kishi M, Liu X, Hirakawa T, Reczek D, Bretscher A, Ascoli M. Identification of two distinct structural motifs that, when added to the C-terminal tail of the rat LH receptor, redirect the internalized hormone-receptor complex from a degradation to a recycling pathway. Mol Endocrinol 2001;15(9):1624–35.
105. Tanowitz M, von Zastrow M. A novel endocytic recycling signal that distinguishes the membrane trafficking of naturally occurring opioid receptors. J Biol Chem 2003;278(46): 45978–86.
106. Thompson D, Pusch M, Whistler JL. Changes in G protein-coupled receptor sorting protein affinity regulate postendocytic targeting of G protein-coupled receptors. J Biol Chem 2007;282(40):29178–85.
107. Heydom A, Sondergarrd BP, Hadrupn N, Holst B, Haft CR, Schwartz TW. Distinct in vitro interaction pattern of dopamine receptor subtypes with adaptor proteins involved in post-endocytotic receptor targeting. FEBS LETT 2004;556 (1-3):276–80.
108. Gullapalli A, Wolfe BL, Griffin CT, Magnuson T, Trejo J. An essential role for SNX1 in lysosomal sorting of protease-activated receptor-1: evidence for retromer-, Hrs-, and Tsg101-independent functions of sorting nexins. Mol Biol Cell 2006;17(3):1228–38.
109. Kim OJ, Ariano MA, Namkung Y, et al. D(2) dopamine receptor expression and trafficking is regulated through direct interactions with ZIP. J Neurochem 2008;106(1):83–95.
110. Seeman P, Kapur S. Schizophrenia: more dopamine, more D2 receptors. Proc Natl Acad Sci USA 2000;97(14):7673–5.
111. Hurley MJ, Jenner P. What has been learnt from study of dopamine receptors in Parkinson's disease? Pharmacol Ther 2006;111(3):715–28.

112. Martinez D, Broft A, Foltin RW, et al. Cocaine dependence and d2 receptor availability in the functional subdivisions of the striatum: relationship with cocaine-seeking behavior. Neuropsychopharmacology 2004;29(6):1190–202.
113. Dumartin B, Jaber M, Gonon F, Caron MG, Giros B, Bloch B. Dopamine tone regulates D1 receptor trafficking and delivery in striatal neurons in dopamine transporter-deficient mice. Proc Natl Acad Sci USA 2000;97(4):1879–84.
114. Stefanski R, Ziolkowska B, Kusmider M, et al. Active versus passive cocaine administration: differences in the neuroadaptive changes in the brain dopaminergic system. Brain Res 2007;1157:1–10.
115. Thanos PK, Michaelides M, Benveniste H, Wang GJ, Volkow ND. Effects of chronic oral methylphenidate on cocaine self-administration and striatal dopamine D2 receptors in rodents. Pharmacol Biochem Behav 2007;87(4):426–33.
116. Guigoni C, Doudnikoff E, Li Q, Bloch B, Bezard E. Altered D(1) dopamine receptor trafficking in parkinsonian and dyskinetic non-human primates. Neurobiol Dis 2007;26(2):452–63.
117. Gao K, Kemp DE, Ganocy SJ, Gajwani P, Xia G, Calabrese JR. Antipsychotic-induced extrapyramidal side effects in bipolar disorder and schizophrenia: a systematic review. J Clin Psychopharmacol 2008;28(2):203–9.
118. Haddad PM, Dursun SM. Neurological complications of psychiatric drugs: clinical features and management. Hum Psychopharmacol 2008;23(Suppl 1):15–26.
119. Dean B, Hussain T, Scarr E, Pavey G, Copolov DL. Extended treatment with typical and atypical antipsychotic drugs differential effects on the densities of dopamine D2-like and GABAA receptors in rat striatum. Life Sci 2001;69(11):1257–68.
120. Florijn WJ, Tarazi FI, Creese I. Dopamine receptor subtypes: differential regulation after 8 months treatment with antipsychotic drugs. J Pharmacol Exp Ther 1997;280(2):561–9.
121. Moran-Gates T, Gan L, Park YS, Zhang K, Baldessarini RJ, Tarazi FI. Repeated antipsychotic drug exposure in developing rats: dopamine receptor effects. Synapse 2006;59(2): 92–100.
122. Tarazi FI, Florijn WJ, Creese I. Differential regulation of dopamine receptors after chronic typical and atypical antipsychotic drug treatment. Neuroscience 1997;78(4):985–96.
123. Ryman-Rasmussen JP, Griffith A, Oloff S, et al. Functional selectivity of dopamine D1 receptor agonists in regulating the fate of internalized receptors. Neuropharmacology 2007;52(2):562–75.
124. Ryman-Rasmussen JP, Nichols DE, Mailman RB. Differential activation of adenylate cyclase and receptor internalization by novel dopamine D1 receptor agonists. Mol Pharmacol 2005;68(4):1039–48.
125. Laruelle M, Frankle WG, Narendran R, Kegeles LS, Abi-Dargham A. Mechanism of action of antipsychotic drugs: from dopamine D(2) receptor antagonism to glutamate NMDA facilitation. Clin Ther 2005;27(Suppl A):S16–24.
126. Kikuchi T, Tottori K, Uwahodo Y, et al. 7-(4-[4-(2,3-Dichlorophenyl)-1-piperazinyl]butyloxy)-3,4-dihydro-2(1H)-qui nolinone (OPC-14597), a new putative antipsychotic drug with both presynaptic dopamine autoreceptor agonistic activity and postsynaptic D2 receptor antagonistic activity. J Pharmacol Exp Ther 1995;274(1):329–36.
127. Burris KD, Molski TF, Xu C, et al. Aripiprazole, a novel antipsychotic, is a high-affinity partial agonist at human dopamine D2 receptors. J Pharmacol Exp Ther 2002;302(1):381–9.
128. Shapiro DA, Renock S, Arrington E, et al. Aripiprazole, a novel atypical antipsychotic drug with a unique and robust pharmacology. Neuropsychopharmacology 2003;28(8):1400–11.
129. Urban JD, Vargas GA, von Zastrow M, Mailman RB. Aripiprazole has functionally selective actions at dopamine D2 receptor-mediated signaling pathways. Neuropsychopharmacology 2007;32(1):67–77.
130. Swainston Harrison T, Perry CM. Aripiprazole: a review of its use in schizophrenia and schizoaffective disorder. Drugs 2004;64(15):1715–36.
131. Van Craenenbroeck K, Gellynck E, Lintermans B, et al. Influence of the antipsychotic drug pipamperone on the expression of the dopamine D4 receptor. Life Sci 2006;80(1):74–81.

Chapter 9
Dopamine Receptor-Interacting Proteins

Lisa A. Hazelwood, R. Benjamin Free, and David R. Sibley

Abstract Historically, dopamine receptors (DARs) and other G protein-coupled receptors (GPCRs) were believed to be independent signaling units in the plasma membrane, interacting only transiently with G proteins to initiate a downstream signaling cascade. However, in recent years it has become clear that DARs do not function in isolation, but in fact exist as members of macromolecular protein complexes. The DAR protein complex, or the signalplex, consists of a variety of protein interactors that may be transient or stable in nature and that are collectively referred to as dopamine receptor-interacting proteins (DRIPs). Ultimately, the goal of the signalplex is to organize the cellular machinery or signaling components that are critical to the DAR at any given time in an orderly fashion. This higher level of organization around the DAR enables the receptor to process all information, from extracellular ligands or intracellular signaling molecules, and propagate the necessary cellular response in a timely and efficient manner.

Keywords Interacting proteins · Signalplex · Yeast two-hybrid · Immunoprecipitation · Proteomics

9.1 Introduction to the Signalplex

Historically, dopamine receptors (DARs) and other G protein-coupled receptors (GPCRs) were believed to be independent signaling units in the plasma membrane, interacting only transiently with G proteins to initiate a downstream signaling cascade. However, in recent years it has become clear that DARs do not function in isolation, but in fact exist as members of macromolecular protein complexes [1, 2]. The DAR protein complex, or the signalplex, consists of a variety of protein interactors that may be transient or stable in nature and that are collectively

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referred to as dopamine receptor-interacting proteins (DRIPs). Once assembled into the signalplex, DARs and DRIPs can operate as a distinct unit to receive and process information critical to the cell from sources including ligands and cytosolic molecules (Fig. 9.1). To process this wide array of informational input, the constituents of the signalplex must change throughout the lifecycle of the receptor. The purpose of the signalplex then is to enable the DAR to adapt and function optimally and rapidly within a changing cellular milieu, based on factors as diverse as receptor maturity, cytosolic pH, calcium concentration, and ligand binding. In the absence of the signalplex, cellular signaling would be dependent on diffusion of a multitude of protein effectors, thus greatly reducing the speed and efficiency of the cellular response. Indeed, the signalplex provides a heightened level of organization within the cell for spatial and temporal control of signaling, and likely facilitates the diverse functions mediated by dopamine in different organs and even different cell types. Although we are only beginning to unravel the components of the DAR signalplex, it has become clear that the DRIPs involved may determine a multitude of receptor properties, including preference for signal transduction pathways and organization into cellular compartments or microdomains.

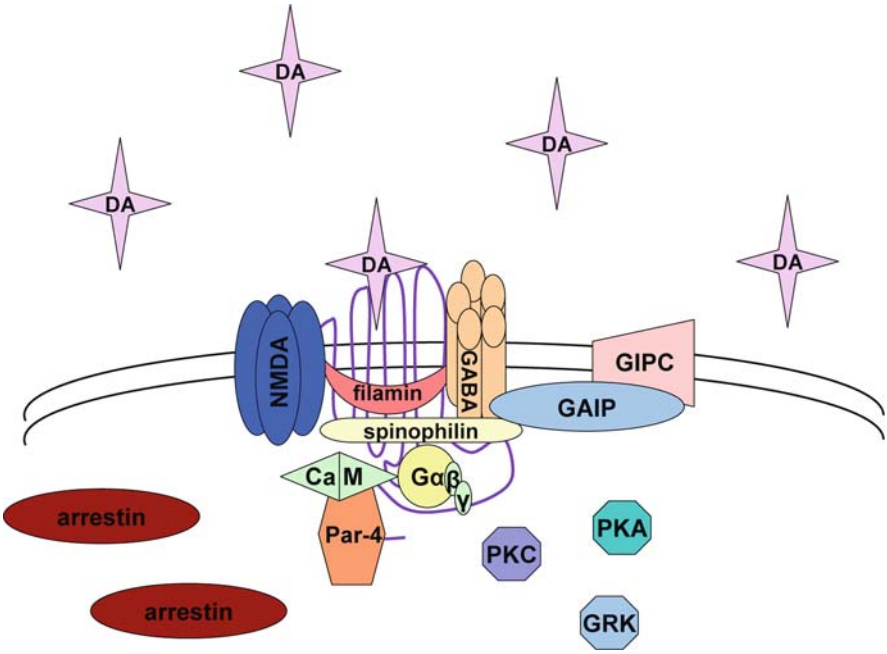

Fig. 9.1 The dopamine receptor has been demonstrated to interact with a multitude of proteins, including trafficking, scaffolding, and adaptor proteins, kinases, ion channels, and various signaling molecules, a few of which are illustrated in this "signalplex." The complement of interacting proteins likely changes throughout the life span of the cell, and can be impacted by dopamine stimulation and other extracellular events

9.1.1 Constituents of the Signalplex – DRIPs and DRAPs

The discrete components of the DAR signalplex likely change throughout the life span of the receptor, and depend upon the state of receptor maturation, ligand binding to the receptor, and various components of the cellular milieu. For a newly synthesized receptor, trafficking and targeting proteins such as calnexin [3] can begin their association with DARs at the translational machinery and remain with the receptor until insertion in the plasma membrane. This mechanism ensures correct spatial and temporal placement of the DAR in a direct manner. Receptor retention at the plasma membrane, as well as compartmentalization into microdomains, may be governed by scaffolding and accessory proteins such as spinophilin [4]. These scaffolding/accessory proteins may also act as tethers, linking the DAR to other proteins by indirect association (tethered proteins are termed DRAPs or dopamine receptor-associated proteins). Ligand binding to the DAR can initiate interaction with a variety of signaling proteins, including G proteins and kinases. Intracellular calcium levels can also activate subsets of signaling proteins, such as calcineurin [5], that interact with DARs. In both cases, the signaling proteins may directly interact with the receptor, facilitated by proximity within a microdomain, or they may be transiently tethered to the receptor by a scaffolding protein. Finally, after the DAR has been desensitized via interactions with protein kinases, it interacts with proteins such as arrestin that lead to internalization of the receptor. Once internalized, the DARs are sorted for recycling or degradation based on their interaction with specific trafficking proteins such as GASP [6] or GIPC [7].

As mentioned above, the association between DARs and members of the signalplex may be direct (DRIPs) or indirect (DRAPs). Most indirect interactors are likely downstream effectors, such as ion channels or adenylyl cyclase. While it is not necessary for DARs and DRAPs to be in direct contact with one another, signal propagation is far more efficient because the signalplex maintains the effector in close proximity to the receptor–G protein complex. In contrast, DRIPs must interact directly with the DAR, as is the case with kinases or G proteins. Scaffolding proteins are also DRIPs that may interact stably with the DAR in order to promote associations between DARs and a variety of other proteins, including DRAPs. In all cases, the signalplex provides the most efficient use of space and time for optimal cellular efficacy.

9.1.2 Points of Interaction for DRIPs

DRIPs may associate with various portions of the DAR to form the signalplex. The most common area for these protein–protein interactions to occur is on the intracellular face of the DAR, specifically within the third intracellular loop or the carboxyl terminus. In addition to facing the cytosol and, therefore, the vast majority of putative protein interactors, the third intracellular loop and carboxyl terminus are also the largest soluble spans of the DARs and are considered to be quite flexible. These

large regions of accessible protein provide ample room for binding of DRIPs and, indeed, many identified DRIPs have been proven to associate with the DAR in these two regions. In addition to the size and accessibility of these two receptor domains, they are also both targets of multiple protein kinases; many putative interactors utilize such phosphorylated residues to anchor protein associations. Finally, crystallography has recently uncovered a short eighth transmembrane domain within the carboxyl terminus of many GPCRs that appears to be important for ligand activation of the receptor and, subsequently, G protein coupling [8]. This region thus takes on significance as an activator "switch," and the possibility that proteins interacting with the DAR in this region may respond directly to ligand stimulation is quite intriguing. Aside from the intracellular face of the DAR, it is also possible for other membrane-spanning proteins to associate with the DAR through their transmembrane domains. In fact, this has recently been demonstrated as the likely mechanism for D_2 receptor oligomerization [9]. Importantly, the transmembrane domains involved in protein interactions are predicted to undergo conformational shifts upon ligand binding, allowing the receptor to communicate this activation directly to the interacting partner without intermediary signaling molecules. For interactions between DARs and other large, membrane-spanning proteins, the transmembrane surfaces provide ample surface and opportunities for interactions and direct communication without second messengers.

9.1.3 The Signalplex as the Most Efficient Unit for Transmission

Ultimately, the goal of the signalplex is to organize the cellular machinery or signaling components that are critical to the DAR at any given time in an orderly fashion. This higher level of organization around the DAR enables the receptor to process all information, from extracellular ligands or intracellular signaling molecules, and propagate the necessary cellular response in a timely and efficient manner. By altering the components of the signalplex throughout the life span of the DAR and in different physiological environments, the DAR is able to respond best to the needs of the specific organ and cell at that moment.

9.2 Discovery Mechanisms

The protein constituents of the DAR signalplex can be quite dynamic with respect to space and time. Elucidating the complete array of interacting proteins involved in the regulation and signaling mediated by DARs has become an important goal of this field. This identification presents a formidable task; first and foremost is the dynamic nature of the signalplex. As mentioned above, the signalplex is very likely constantly changing depending on the needs of the cellular environments, the state of the receptor, or in response to signaling. Therefore, the very nature of the signalplex itself greatly complicates discovery of proteins that interact with DARs.

Second, cellular and tissue-specific environments may very well dictate the components of a given DAR signalplex. Different tissues and cell types are likely to contain a mixture of different specific proteins. Proteins that are integral parts of the signalplex in one tissue may not even be present in another tissue. Luckily, a number of different techniques have been developed to address some of these concerns. While all of the current techniques have their pitfalls, they all also have powerful value in determining interacting proteins. Taken together these represent diverse ways of looking at a similar problem – discovery of the DAR signalplex.

9.2.1 Membrane-Based Two-Hybrid and Split-Ubiquitin Systems

The yeast two-hybrid, bacterial two-hybrid, and split-ubiquitin systems address the issue of cellular/tissue-specific interactions being a problem for identifying signalplex components by taking cellular environment completely out of the assay, and screening entire libraries of potential protein interactors. These systems use either yeast or bacteria as a model organism to determine potential protein interactions on a genomic scale. The general basic premise of the classical yeast two-hybrid revolves around transcriptional activation of screenable reporter genes. The classical yeast two-hybrid system has limited usefulness for membrane proteins such as DARs since the transcription factors are in the nucleus and membrane proteins are generally trafficked away from the nucleus. However, fragments of the receptor can be used as bait in this system and have been useful in identifying some interactors to non-membranous regions of the DAR. Therefore, classical two-hybrid is mostly useful for identifying soluble protein–protein interactions. However, modifications of these techniques such as the split-ubiquitin system [10] or membrane yeast two-hybrid [11] have allowed for use of this kind of technology for intact membrane proteins. In this system instead of using parts of transcription factors, ubiquitin can be split and separate parts attached to bait and prey proteins. The bait protein also contains a transcription factor for a reporter gene. If the bait and prey proteins interact (i.e., bind), then the ubiquitin pieces come together and are then cleaved by the yeast's ubiquitin-specific proteases and the transcription factor is liberated, where it travels to the nucleus and activates the reporter gene. If the two proteins do not interact, there is no transcription of the reporter gene since the transcription factor is locked at the membrane surface. This allows for selection of proteins that interact with the intact DAR.

The bacterial two-hybrid system is based on the transcriptional activation in *E. coli*, where an arbitrary pair of interacting proteins can mediate transcription of a synthetic promoter – one protein is tethered near the promoter via fusion to a DNA-binding domain, while the other protein is fused to a subunit of the *E coli* RNA polymerase. The resulting transcription can be measured as an increase in expression of a selectable reporter gene. The most significant benefit to this system is the ability to rapidly assess very large libraries greater than 10^8 in size – however, the technique is limited by the inability of host bacteria to post-translationally modify

or properly express eukaryotic proteins, however, it has proved possible to identify protein–protein interactions using this technique [12].

This assay has proven a powerful technique in identifying direct protein interactions and is able to examine interactions with entire proteomes at one time. However there are also multiple pitfalls of the system. The system is notorious for false positives, requiring that all found interactions be confirmed by additional methods. The system is also only able to identify proteins that directly interact with the receptor. It is possible, and maybe even likely, that many members for the signalplex form a complex that would not allow them to directly bind to the receptor, but bind to other members of the signalplex. Furthermore, this system cannot take into account modifications of a protein that may be needed for binding to the bait. Therefore it is difficult to envision that this technique alone would be able to find all the signalplex members. Regardless, several DAR-interacting proteins have been identified this way, and the technique can also be used to identify regions of protein interaction by utilizing truncated protein pieces.

9.2.2 Biochemical Approaches: GST-Fusion Protein Pull Downs

Another technique that has been developed and proven to be effective in elucidating protein–protein interactions are pull-down assays. Glutathione (GSH) is a protein that contains a free thiol group, normally involved in protection of cells against oxidative stress. Glutathione-*S*-transferase (GST) is a protein that participates in detoxification of many reactive substances by forming adducts through the thiol group. GST binds GSH with very high affinity. This natural chemistry is exploited in the GST pull-down assay where a recombinant protein of interest is made containing GST. This protein can then be coupled to GSH agarose for affinity chromatography to pull down interacting proteins, thus making an ideal way to separate and purify proteins that interact with your GST bait protein [13]. Because of the difficulty in identifying the interacting proteins through subsequent immunoblots, this assay has been used in a largely confirmatory way to show that two known proteins interact, the problem being that one has to know or at least suspect what the proteins are so that you can choose an appropriate antibody. However, if this is coupled with mass spectroscopy (MS) for identification of the interacting proteins (see more in Section 9.2.4) it becomes a more powerful technique for identifying novel interactions.

There are several primary advantages to this technique over the more traditional two-hybrid systems described above. These include: the isolation correlates to the strength of the interaction, the isolation is independent of a down-stream detection (such as transcriptional activation), the technique can internally be controlled for specificity by utilizing an unrelated negative control protein, one can isolate more than one interacting partner at a time, and entire genomes can be queried with the sequencing results found via MS.

While this highlights the power of this approach, there are indeed also significant pitfalls that make this approach miss some interacting proteins and make it entirely

unsuitable for others. The approach depends entirely on a recombinant/non-native protein. There is no guarantee that the protein will express properly in bacteria or fold properly to allow for native interacting partners to bind either in the host bacteria or after coupling to the agarose. Furthermore, weak or low-abundant interactions are likely to be missed or not interact with a strong enough affinity to be pulled down after washing in the chromatography steps. Interference can also arise from detergents or pre-formed protein complexes in the samples that are being used as prey. Finally, this is clearly a non-native artificial environment and many interactions likely rely on the cellular environment of the proteins. Therefore, not all proteins found will be direct members of the signalplex, but may be alternative binding partners of one of the signalplex members, that may form in solution before the bait protein is even tested.

9.2.3 Protein Microarrays

Protein microarrays have emerged as a complementary procedure for studying protein interactions in vitro. Microarray-based assays require purified proteins, which are usually obtained as tagged fusion proteins. By arraying sets of recombinant proteins in the microarray format one can screen dozens or even thousands of potential interactors. Prefabricated arrays can be purchased and used for detection. When you screen proteins like this you can either use an antibody against your bait protein of interest, or use a fusion protein bait with an available antibody to the fusion tag. The principle comes down to a miniaturized ligand-binding reaction, with your bait protein serving as the ligand [14].

While this approach in principle is very powerful it is limited by several factors, including the need for dedicated very expensive microarrayers and arraying robots. Current technology allows for arraying of only mostly soluble proteins, and purification both positive and negative controls for the assay is still being optimized. Regardless, the use of protein microarrays promises to be a powerful technique to screen many possible interaction partners in the future.

9.2.4 Mass Spectroscopy-Coupled Co-immunoprecipitation Proteomics

In MS-based proteomics, the protein itself is used as an affinity reagent to isolate its binding partners. The primary advantages over the above-mentioned methods are that the protein is used in its fully processed form, the interactions are in the protein's native environment, and multi-component complexes can be isolated in a single step. With the sequencing of the human genome and the emergence of advanced peptide-based mass spectroscopy (MS), recent studies have documented that these approaches may be more useful for identifying proteins involved in the biological regulation of neuronal responses.

Recently, MS proteomic-based methods have proven useful for identifying interacting proteins with neurotransmitter receptors, including NMDA [15], $P2X_7$ [16], and 5-HT_{2C} [17] receptors. Additionally, both D_1 and D_2 receptor-interacting proteins [3] have been successfully identified using this approach. There are three essential components for successfully identifying neuronal proteins using MS-based proteomics: immunoprecipitation of the protein bait, purification of the complex, and the identification of the interacting partners.

The ability to immunoprecipitate a protein bait of interest from the desired system must be established before further isolation of the complex can occur. Ideally, an antibody is available allowing immunoprecipitation of the native protein from tissues. If this is the case, then the bait can be an endogenous protein in native neuronal tissue. However, suitable antibodies are often not readily available. In these instances, the bait protein can be affinity tagged and expressed in an appropriate cell system, followed by immunoprecipitation with commercially available antibodies directed against the affinity tag.

Successful immunoprecipitation of the bait is followed by a series of washes of varying stringency and then separation of proteins by denaturing polyacrylamide gel electrophoresis. The coupling of MS technologies with successful co-immunoprecipitation allows rapid and specific identification of discrete members of the protein complex and provides an attractive alternative for the discovery of novel interacting partners that cannot be detected using two-hybrid or fusion pull-down analyses.

While MS-based proteomics can be a sensitive and effective tool for identification of interacting proteins, this procedure relies on a relatively strong affinity between bait and targets. Furthermore, many biological interactions are transient in nature and depend on cellular environment. Proper controls, manipulations, and optimization techniques can help limit some of these pitfalls [18]. When used to its full capability, co-immunoprecipitation coupled with MS analysis provides a useful assay for discovering previously unknown protein partners, thus expanding our existing knowledge of dynamic protein regulation and control.

9.3 Experimental Manipulations

Following the initial identification of a putative DRIP, much care must be taken to confirm the interaction and to determine the significance of the DRIP association with DARs. After verifying the occurrence and determining the functional significance of the interaction, the physiological location of the interaction should be assessed. It is possible that for many different DRIPs association with the DAR occurs in specific organs, and may even vary across cell types within the tissues. Determining the physiological location of the interaction will greatly assist in ascertaining the role of the DRIP in cellular or receptor regulation. Finally, the ultimate goal of DRIP discovery is to enhance the understanding of the workings and regulation of the DAR as a unit and its role in governing various cellular processes.

Thus, once we understand the function and location of the DAR–DRIP interaction, we can begin to ascribe a unique role in governing normal physiological processes to each DRIP. Moreover, we can determine the relevance of each DRIP–DAR interaction to known disease states and etiologies, perhaps even altering current forms of pharmacotherapy or creating novel drugs to better treat DAR-associated disorders.

9.3.1 Verification and Significance of the Interaction

After initial DRIP identification, preliminary experiments must be conducted to ensure that the interaction is valid, and not a false positive result. Many of the assays described in Section 9.2 can be employed in this secondary screening stage. In the DRIP literature, the most common methods for DRIP verification are co-immunoprecipitation or GST-fusion pull-down assays. In addition, FRET and BRET analyses can be used to verify DAR interactors and are also essential tools for determining direct interaction between DARs and DRIPs (discussed further in Section 9.3.2).

After verifying the interaction, the experimenter should next examine the significance of the DAR–DRIP association. This is commonly performed in cultured cell expression systems, and ideally utilizes cells that do not endogenously express either DAR or DRIP. If such cells are available, then multiple parameters of DAR and DRIP can be investigated individually and after co-expression, including protein localization and function. If such an expression system is unavailable, overexpression of one or both proteins is also a feasible mechanism to examine the relevance of the DAR–DRIP interaction. These are popular approaches, and were successfully utilized in the characterization of multiple DRIPs, including calnexin and S100B [3, 19].

In the event that all available cell systems contain both proteins, multiple investigation methodologies are still available. The DAR can be pharmacologically manipulated in conjunction with measurements of DRIP function and/or trafficking response. Another promising method for systems that express both proteins is the inhibition of endogenous DAR or DRIP using siRNA technology. This approach enables investigation of various cellular parameters and protein function both with and without the interacting partner. Many of the proteins highlighted in the following sections utilized these investigational tactics – studies of prostate apoptosis response 4 and the Na^+,K^+-ATPase employed siRNA technology [20, 21], and dopamine transporter experimentation employed pharmacological manipulations of both the transporter and DARs [22].

Finally, using a peptide inhibitor approach, it is possible to biochemically disrupt the interaction of DAR and DRIP without altering the expression levels of either protein. This is a valuable tool for cellular environments where full expression of both proteins is necessary for normal biological function, as it enables functional investigation of the consequence of targeted DAR–DRIP disruption. This approach was successfully employed in the study of DAR interactions with the dopamine transporter and G protein inwardly rectifying potassium channels [22, 23].

9.3.2 Location of the Interaction – Tissues and Protein Domains

Occasionally, elucidation of important organs for DAR–DRIP interactions is straightforward – when the DRIP is only present in a particular tissue, for example. However, because of the wide physiological distribution of DARs, and because many DRIPs have multiple homologues expressed across a variety of tissues, identifying significant organs for a given DAR–DRIP association can be challenging. If it is known that DAR and DRIP are both expressed in a given organ, then immunohistochemistry can be used to look at protein distribution at the cellular level. Once a cellular source for DAR–DRIP co-localization is identified, it is possible to generate primary cultures of these cells for functional analyses. Functional experimentation in primary cell cultures is likely to incorporate many of the methods detailed in the previous section, including pharmacological manipulation and biochemical inhibition of the proteins. Such experiments were performed by investigators studying DAR interactions with S100B and the G protein-coupled receptor-associated sorting protein [6, 19].

In addition to determining organ localization for the DAR–DRIP association, the DAR and DRIP protein domains that mediate the interaction are also of relevance. Many DRIPs were discovered using yeast or bacterial two-hybrid systems. In these instances, the DAR "bait" is the receptor-interacting domain and only the DRIP interaction point remains to be determined. For some DRIPs, especially scaffolding and anchoring proteins, the protein sequence contains a series of well-defined domains including protein-interaction domains. For such proteins, interaction mapping is straightforward and mutagenesis experiments can be employed. The task of mapping interaction domains is far more challenging if the DRIP, like DARs, is a transmembrane protein. In these circumstances all regions of both DAR and DRIP, including the hydrophobic transmembrane regions, are potential interacting sites. Mutagenesis and truncation mapping studies were performed when determining the DAR–DRIP interaction sites for many soluble DRIPs, including GAIP-interacting protein C terminus [7], as well as for soluble regions of the transmembrane-spanning dopamine transporter [22, 24].

Determining the domains that are critical for the protein–protein interaction does not necessarily indicate that the proteins directly interact – it is possible that the mapped regions on each protein are required for binding to an intermediary, scaffolding protein, as is the case with DRAPs. Indeed, most methods for examining DAR–DRIP interaction only support that the two proteins are in a complex and in close proximity to one another, but not that they can directly interact. The closest approximations of direct DAR–DRIP interactions are ascertained from co-localization studies and FRET or BRET analysis [25].

Co-localization studies require either transfection of fluorescent-tagged proteins or fluorescent-conjugated antibody staining of endogenous proteins to determine whether the DAR and DRIP are found in overlapping regions of the cell. This method for determining direct protein–protein interaction is most convincing if there is evidence for alternative distribution patterns of at least one of the proteins – for example, if the DRIP is commonly stored in vesicles, but when interacting with the

DAR it is localized to the plasma membrane. Such a finding was presented with the interaction of the transient receptor potential channel 1 with DARs [26].

FRET or BRET analysis provides the most detailed information on the proximity of DAR to DRIP. For these assays, the interacting proteins must be within 10 Å of one another in order to achieve proper luminescence transmission between the two tagged proteins. However, even in such close range, it is still possible that a scaffolding or adaptor protein may govern the association of DAR and DRIP. Nonetheless, FRET and BRET are currently the best estimation of protein proximity and, when possible, have been employed to assess whether DAR–DRIP interactions are direct. Notably, FRET and BRET analysis have been used to study DAR interactions with other GPCRs, a topic that will be covered in Chapter 10. BRET was also used to study interactions between the D_2 DAR and arrestin3 [27].

9.3.3 Model Systems and Disease Relevance

After complete biochemical characterization of the DAR–DRIP interaction, it is often desirable to generate a model system for further study. This is most notably the case when the DAR–DRIP complex has important cell regulatory functions that imply a role for the interaction in human disease. Transgenic and knockout mouse models have been utilized in the characterization of several DRIPs, including prostate apoptosis response 4 and arrestin3 [21, 28]. Experimentation with animal models has revealed formerly unappreciated aspects of dopaminergic signaling and regulation, and revealed new potential pathways for future therapeutic intervention in a variety of disease states including depression and addiction [6, 21, 22].

9.4 Protein Members of the Dopamine Receptor Signalplex

As the members of the DAR signalplex have been identified, it has become clear that DRIPs are not necessarily shared across the D1 and D2 families of DARs. In fact, even within the D1 and D2 families, protein interactors may associate with one DAR subtype and not another. This phenomenon may help to explain the wide array of physiological functions that are performed by DARs with the same G protein-coupling profile. It is likely, then, that unique signalplexes exist not only across cell types and throughout the lifecycle of the receptor, but also depend upon the complement of receptors present within the cell. Continued identification of proteins within the DAR signalplex will undoubtedly shed light on the unique signaling and regulative processes for each DAR.

9.4.1 Targeting and Trafficking Proteins

Proteins in this category can be subdivided into two groups – DRIPs that assist with trafficking and correct targeting of a newly synthesized DAR to the cell surface, and

those that are involved in the desensitization/internalization/recycling pathway of the DAR. DRIPs that target newly synthesized DARs to the cell surface are likely to first associate with the receptor in the ER; these DRIPS may also play a role in the proper folding and membrane insertion of the DAR. After receptor stimulation, a subset of DRIPs is necessary for binding to the receptor for removal from the plasma membrane. After endocytosis of the DAR, the fate of the receptor must be determined – resensitization and recycling to the cell surface versus lysosomal degradation of the receptor. These post-endocytic determinations of DAR fate are made by the complement of DRIPs that associate with the receptor.

9.4.1.1 Calnexin

The protein calnexin functions as both an endoplasmic reticulum (ER) retention protein and chaperone, thus enabling the proper folding and assembly of glycoproteins prior to their export to the Golgi [29–31]. Both D_1 and D_2 DARs have been found to interact with calnexin via co-immunoprecipitation-based mass spectroscopy studies [3]. The interaction with calnexin in the ER is critical for the optimum expression of both DARs, and interestingly appears to be tightly regulated; there is an optimal degree or duration of calnexin interaction that results in the maximum amount of receptor being expressed at the cell surface. The interaction of calnexin with the DARs appears to be complex and dependent upon at least two different mechanisms. This has been demonstrated by using receptor mutants and pharmacological approaches. Most calnexin interactions are associated with glycoproteins based on their N-linked oligosaccharide side chains; however, some newer data suggest that calnexin may also target and bind non-glycosylated membrane proteins [3]. These data support the idea that the DARs actually associate with calnexin via both mechanisms: interaction of protein components as well as their carbohydrate side chains. Demonstration that calnexin interacts with the DARs via two distinct mechanisms raises the possibility that these different interactions may serve distinct biological functions. Although calnexin functions as a chaperone protein to assist in protein folding and assembly, it also serves as an ER retention protein that prevents misfolded proteins from exiting the ER and going on to the Golgi. Furthermore, it is possible that it is involved in heteromeric and homomeric assembly of dimers in the ER, and some data suggest that it may play a role in the proper assembly of these complexes. It has been suggested based on data with trafficking-deficient DAR mutant receptors, as well as glycosylation-deficient DAR mutant receptors, that glycan-dependant receptor–calnexin interactions may mediate the chaperone functions of calnexin, whereas the glycan-independent protein–protein interactions may be more relevant to ER retention and quality control [3]. Regardless of mechanisms it is clear that optimal receptor–calnexin interactions critically regulate D_1 and D_2 receptor trafficking and expression at the cell surface, a mechanism that is likely to be of importance for many GPCRs. This highlights the essential nature of trafficking DRIPs in DAR physiology.

9.4.1.2 Dopamine Receptor-Interacting Protein-78

One of the first targeting and trafficking proteins identified to be a member of the D_1 DAR signalplex was DRIP78, so named because of its dopamine receptor interaction and its molecular mass of 78 kDa. It was identified in a yeast two-hybrid screen using the carboxyl terminus of the D_1 DAR as bait [32]. The specificity of this interaction location was subsequently verified with GST pull-down assays. Studies have shown that DRIP78 is an important member of the signalplex and is not likely to be totally specific to the D_1 DAR. DRIP78 closely co-localizes intracellularly with several ER membrane proteins and is clearly distinct from the Golgi. When DRIP78 is co-expressed with the D_1 DAR in heterologous cell lines, there is a marked change in D_1 DAR localization from the cell surface to intracellular stores containing DRIP78. Interestingly, when a mutant form of DRIP78 that does not bind to the D_1 DAR was overexpressed with the receptor, the D_1 DARs remained on the cell surface. These findings indicate the ability of DRIP78 to have a negative regulatory function when overexpressed with the D_1 DAR. Experiments that disrupt the DRIP78–D_1 DAR interaction also showed effects on transport to the cellular surface–hence, disruptions in the endogenous levels of this interaction appear to compromise the normal transport of the receptor to the cellular surface [32]. DRIP78 most likely acts as an ER retention protein by binding to, and thereby masking, a portion of the D_1 DAR carboxyl-terminal tail that is essential for export from the ER. If the completely formed receptors are not transported from the ER in a timely fashion then they are rapidly degraded, and it appears that the DRIP78 interaction is an essential part of this quality control mechanism. D_1 DAR transport to the cell surface is highly sensitive to the levels of DRIP78, and either interference or augmentation of those levels slows the ER export of the receptors. This suggests a dual function in both ER retention and quality control, as well as assistance in transport, reminiscent of the D_1 DAR interaction with calnexin described above.

9.4.1.3 ALG-2-Interacting Protein 1

Recently ALG-2-interacting protein 1 (AIP1) was identified as a D_1-interacting protein through a yeast two-hybrid screen using the carboxyl-terminal domain of the receptor. AIP1 is a known modulator of caspase-dependent and caspase-independent neuronal cell death. The protein is also known to be part of the endosomal transport system. AIP1 was also found to interact with the D_3 DAR in a similar yeast two-hybrid experiment. This interaction was verified for both receptors via GST pull-down assays and co-immunoprecipitation from mouse brain lysates [33]. Interestingly, AIP1 may represent an important functional link between D_1 and D_3 DAR-mediated signaling. It has been suggested that the D_1 and D_3 DARs and AIP1 proteins may all form a single complex. These findings have led to studies investigating the interaction of AIP1 in affecting the trafficking, stability, and recycling of these two DARs. Overexpression of all three proteins in heterologous cell lines leads to the accumulation of the proteins in the cytoplasm, indicating a possible effect on trafficking. Furthermore, it has been suggested that AIP1 could also be involved in

recycling of the receptors because AIP1 is known to interact with ESCRT (endosomal sorting complex required for transport) proteins [34]. This protein is known for sorting proteins for degradation or recycling to the plasma membrane. While the exact influence of AIP1 is not yet fully characterized, it is a DRIP that may play a prominent role in DAR biology.

9.4.1.4 Neurofilament-M

Neurofilament-M (NF-M), a neuronal cytoskeletal protein, was identified in a yeast two-hybrid screen to be a DRIP with the D_1 DAR. It was not found to interact with the third cytoplasmic loops (the region of interaction with the D_1 DAR) of the D_2, D_3, or D_4 receptors, but it did show weak interaction with the D_5 DAR. Co-expression of NF-M with the D_1 receptor resulted in a decrease in both expression and function of the receptor in heterologous cell lines. The other subtypes were not affected by NF-M expression in the same system. NF-M also appears to play a role in D_1 DAR trafficking and desensitization. When NF-M is expressed with the D_1 DAR in cells there is a decrease in cell surface expression and apparent accumulation of the receptor in the cytosol. Interestingly, the D_1 DARs expressed at the cell surface in the presence of NF-M were resistant to agonist-induced desensitization. These findings indicate that this interaction may play a critical role in both receptor expression and desensitization [35]. The D_1 DAR–NF-M interaction has also been investigated in vivo where approximately 50% of medium-sized striatal neurons express both proteins. Co-localization was also observed in pyramidal cells and interneurons within the frontal cortex. In addition, when NF-M-deficient mice were examined via immunohistochemistry, it was determined that they showed a decrease in D_1 receptor expression as compared to control mice. These data support a role for NF-M in the brain modifying D_1 DAR expression and regulation [35]. NF-M is encoded by the NEF3 gene. Point mutations in the NEF3 gene have been identified in patients with Parkinson's disease, however, no association of these variants and the disease has been shown [36]. Of note, when a clinical antipsychotic response study was conducted in schizophrenic patients, it was found that patients carrying specific genetic variants of NEF3 may be more likely to respond early to treatment than those not carrying the variants [37]. These findings demonstrate some potential clinical aspects of the importance of this DRIP.

9.4.1.5 Dynamin-2

It is well established that many GPCRs are desensitized and internalized via the arrestin–clathrin pathway. One of the key players in this pathway is the protein dynamin. In addition to its role in the clathrin endocytic pathway, dynamin can also assist with internalization of GPCRs via caveolae and in late-endosomal trafficking [38, 39]. Because of its important role in GPCR trafficking, dynamin was investigated as a potential interactor with the D_2 DAR. The D_2 DAR and dynamin-2 were found to co-localize in both synaptic and non-synaptic regions of rat striatum and could be co-immunoprecipitated from transfected cells [40]. Interestingly,

only the dominant negative dynamin-2 construct prevented D_2 DAR internalization; the dominant negative dynamin-1 construct did not alter D_2 internalization. This is in contrast to findings with the D_1 DAR, where both dynamin-1 and dynamin-2 were found to associate with and contribute to receptor internalization [40]. These findings highlight the critical role of dynamin-2 in D_2 DAR internalization. The difference in dynamin requirements for the D_2 versus the D_1 DAR may be mediated by the cellular location of protein expression, or may be caused by differences in protein interaction domains between the receptors. It is also interesting to note that dynamin isoforms can dimerize, and this dimerization process may be critical to DAR–dynamin pairing. It is possible that interaction of DARs with dynamin is not only necessary for receptor internalization and trafficking, but also serves to scaffold the receptor to other members of the DAR signalplex.

9.4.1.6 GAIP-Interacting Protein, C Terminus

GAIP-interacting protein C terminus (GIPC) was first identified as an interacting protein with RGS19 (regulator of G protein signaling 19) or GAIP [41]; GAIP specifically acts as a GTPase for $G\alpha_{i/o}$ G proteins and has been shown to interact with D_2 DARs (Section 9.4.3.9). GIPC also interacts with other membrane-spanning receptors [42, 43] and transporters [44] and has been hypothesized to play a role in receptor expression, trafficking, and G protein signaling. Using the carboxyl terminus of the D_3 DAR as bait in yeast two-hybrid assays, GIPC was identified as a DRIP [7]. Interestingly, GIPC was found to interact with the D_2 and D_3 receptors, but not with the D_4 DAR, both in vitro and in rat striatum. In a transfected cell system, the D_2 and D_3 DARs caused translocation of GIPC to the plasma membrane and GIPC was co-internalized with the DARs following agonist treatment; the D_4 DAR neither recruited nor co-internalized with GIPC. In addition to its role in D_2 and D_3 DAR internalization, GIPC was also found to diminish the D_3 receptor response; this is likely caused by GIPC recruiting GAIP to the signaling form of the receptor. Finally, GIPC increased the number of D_2 and D_3, but not D_4, binding sites in transfected cells. This is likely the result of an increase in receptor recycling, as opposed to DAR degradation, following co-internalization with GIPC. The variance observed in GIPC-D2 family interactions implies that differences in D2 DAR internalization kinetics may be produced by a different complement of trafficking DRIPs associated with each receptor. Taken together, these data suggest that GIPC plays a role in trafficking of the D_2 and D_3 DARs via internalization and recycling of the receptors to the plasma membrane. These results also implicate GIPC as a scaffolding protein because of its known ability to recruit GAIP (discussed below in signaling proteins, Section 9.4.3) and other membrane-bound receptors.

9.4.1.7 *N*-Ethylmaleimide-Sensitive Factor

N-Ethylmaleimide-sensitive factor (NSF) is an ATPase shown to be involved in membrane vesicle trafficking [45]. This protein has also been found to interact with the β_2 adrenergic receptor where it is likely involved in both internalization and

recycling of the receptor [46]. NSF has also been identified via GST pull-down assays to be a DAR DRIP. It binds tightly to both the D_1 and D_5 receptor's carboxyl termini, but was reported to bind weakly to D2-like receptors [47]. Regardless, it is likely that NSF also affects trafficking and recycling of these DARs. Despite the reportedly weak interaction in GST pull-down assays between NSF and the D_2 DARs, co-immunoprecipitation from solubilized rat hippocampal tissue documents that D_2 DARs and NSF do, in fact, form a complex [48]. Interestingly, when the interaction sites were mapped using GST-pull-down assays, the NSF interaction site identified on the D_2 DAR is the same site that mediates the receptor's interaction with the AMPA receptor (see Section 9.4.4.5). It has been further proposed that, in fact, NSF mediates the interaction of the D_2 DAR and the AMPA receptor. This complex appears to play an important role in controlling glutamate-mediated excitotoxicity. Activation of the D_2 receptor results in a decrease in toxicity and this effect appears to be mediated by the shuttling of NSF between the D_2 DAR and the AMPA receptor [48]. This highlights how a DRIP can potentially mediate other interactions and demonstrates how two receptors can communicate through common interacting proteins.

9.4.1.8 Sorting Nexin-1

Sorting nexin-1 (SNX1) was originally found to interact with the epidermal growth factor receptor and was subsequently shown to be responsible for the efficient targeting of the thrombin receptor, PAR1, from sorting endosomes to lysosomes [49]. SNX1 has also been found to bind strongly to the carboxyl terminus of the D_5 DAR and weakly to the D_1 and D_3 DAR [47]. The overall importance of the DAR interactions with sorting nexin proteins continues to be investigated.

9.4.1.9 G Protein-Coupled Receptor-Associated Sorting Protein

The DRIP, G protein-coupled receptor-associated sorting protein (GASP), was first discovered as an interactor with the δ-opioid receptor [50]. Because the D_2 receptor, like the δ-opioid receptor, can be down-regulated in response to agonist treatment, the ability of GASP to interact with the D_2 DAR was also investigated [6]. Interestingly, the D_1 DAR has not been consistently shown to down-regulate in response to agonist. As such, GASP was found to interact with the carboxyl terminus of the D_2 but not the D_1 DAR [6, 51]. This D_2–GASP interaction occurred in both transfected cell lines and endogenously in the rat brain. Both a dominant negative form of GASP and an antibody against GASP prevented degradation of D_2 DARs, but had no impact on desensitization or internalization of the receptor. This supports the hypothesis that GASP is solely involved in sorting the D_2 DARs to a degradative pathway, but not in desensitizing or internalizing the receptor. In fact, examination of neurons from the rat ventral tegmental area revealed that treatment with the GASP antibody prevented receptor degradation, and allowed desensitization and internalization, followed by resensitization and recovery of D_2 DAR response to agonist [6]. This successful manipulation of the GASP–D_2 DAR

interaction provides insight into potential therapeutic utility for selective blockade of GASP–D_2 interactions in addiction and other disorders where recovery of the D_2 DAR response to agonist is critical.

9.4.2 Anchoring, Scaffolding, and Adaptor Proteins

DRIPs in this category may associate with the cytoskeletal architecture. Often, this cytoskeletal association occurs via binding to actin, and functions to assist with anchoring of the DAR to the plasma membrane and DAR retention at the cell surface. Such DRIPs may also act to retain the DAR within discrete membrane microdomains. DRIPs in this category may also contain multiple protein-binding domains. DRIPs with multiple interaction sites can associate with the DAR and recruit other DRIPs and DRAPS to the signalplex, thus acting as an adaptor or scaffold protein between the DAR and its interactors. Finally, it is possible for proteins in this category to serve both purposes – they may tether the DAR to the cell surface and simultaneously scaffold the DAR to other DRIPs and signaling machinery.

9.4.2.1 Filamin-A

Filamin-A was initially termed actin-binding protein 280 and was discovered as a DRIP with the D_2 DAR [52]. The interaction between filamin-A and the D_2 DAR was found to occur on the third intracellular loop of the D_2 DAR by yeast two-hybrid analysis. It was subsequently determined that filamin-A can interact with both the D_2 and the D_3 DARs, but not with the D_1 or D_4 DARs [52–54]. Transfection of D_2 DARs into cells without endogenous filamin-A revealed both an intracellular and a diffuse membrane-bound distribution of the DAR [52, 54]. Upon transfection of filamin-A into these cells, DAR expression became localized to discrete regions of the plasma membrane, and the intracellular stores of DAR were eliminated [52, 54]. These data indicate that filamin-A is important for anchoring the DAR to the plasma membrane, and that it may play a critical role in tethering the DAR to specific membrane microdomains. It is interesting that while many GPCRs require membrane-anchoring by an actin-binding protein, a single actin-binding protein is not capable of interacting with all DARs, even within the D_2 family of receptors. It may be that the ability to interact with specific anchoring proteins is based on similar distributions and co-localization. Of note, D_2 DAR and filamin-A were found to co-localize in neuronal soma and astrocytes from cultured rat striatum [54]. In brain regions where all members of the D_2 DAR family are expressed, there may be a single actin-associated protein which is able to interact with all receptors.

9.4.2.2 Protein 4.1 N

Protein 4.1 N was identified as a DRIP for D_2 and D_3 DARs using the yeast two-hybrid assay with the third intracellular loop of D_2 or D_3 DARs as bait [55]. The

interaction between DARs and protein 4.1 N was subsequently confirmed by co-immunoprecipitation of the two proteins from transfected cells. Protein 4.1 N is known to interact with actin via an actin-binding domain that is separate from the region of the protein necessary for DAR interaction. As such, it was determined that protein 4.1 N and the D_2 and D_3 DARs co-localize at the plasma membrane of transfected cells. Importantly, a dominant negative version of 4.1 N caused a largely intracellular distribution of both D_2 and D_3 DARs, indicating that DAR interaction with 4.1 N is critical for receptor stability at the plasma membrane. It is notable that both protein 4.1 N and filament-A bind to the same region of the third intracellular loop of D_2 and D_3 DARs [52–55]. As such, the ability of 4.1 N and filament-A to compete for DAR binding was assessed. It was determined that the presence of 4.1 N did not interfere with the binding of filament-A to the DARs [55], indicating that the binding sites are, in fact, different. It is not presently known if the different DAR isoforms preferentially bind to either filament-A or 4.1 N for membrane-retention, or if there are alterations in DAR interactions with these DRIPs across various cell types.

9.4.2.3 Spinophilin

Using the third intracellular loop of the D_2 DAR as bait, spinophilin was identified as a DRIP in a yeast two-hybrid screen [4]. The interaction between D_2 and spinophilin was confirmed by co-immunoprecipitation and co-localization studies in transfected cells. Spinophilin is an actin-binding protein with multiple interaction domains for protein binding. It is has been shown to interact with a variety of other proteins, including protein phosphatase 1 (PP1). In fact, experiments revealed that spinophilin can simultaneously bind both D_2 DAR and PP1, indicating that PP1 may also be a part of the D_2 DAR signalplex. Spinophilin serves as a membrane anchor for the D_2 DAR, and most likely performs the important function of scaffolding a variety of other protein partners to the D_2 signalplex in a time- and cell-dependent manner.

9.4.2.4 Radixin

Radixin belongs to the ERM (ezrin, radixin, moerin) family of proteins and is capable of simultaneously binding actin and interacting proteins [56]. Radixin was discovered as a D_3 DAR DRIP by yeast two-hybrid assay and was found to interact with the D_3 DAR carboxyl terminus [57]. The cellular role of radixin with the D_3 DAR is not presently known, but it likely acts as both a membrane anchoring protein and a scaffold to other members of the D_3 DAR signalplex.

9.4.2.5 Multi-PDZ-Domain-Containing Protein 1

Multi-PDZ-domain-containing protein 1, or MUPP1, is a scaffolding protein consisting of 13 protein-binding PDZ-domains. It has been shown to interact with a variety of other proteins, including the serotonin 5-HT_{2C} receptor [58, 59]. Recently,

it was identified by yeast two-hybrid assay as an interactor with the carboxyl terminus of the D_3 DAR [57]. The precise function or localization of this interaction has not yet been determined, but MUPP1 most likely serves as a scaffold between the D_3 DAR and other proteins in the DAR signalplex.

9.4.2.6 Heart-Type Fatty Acid Binding Protein

Heart-type fatty acid binding protein (H-FABP) is expressed in a variety of tissues, including heart, kidney, and brain [60]; it is known to bind and traffic fatty acids and other hydrophobic ligands [61]. H-FABP was identified as a DRIP using the third intracellular loop of the D_{2L} receptor as bait in yeast two-hybrid screens [62]. Interestingly, H-FABP did not co-localize with or interact with the D_{2S} receptor in co-immunoprecipitation assays. Of note, Takeuchi and Fukunaga demonstrated that the D_{2L} isoform was predominantly intracellularly distributed, while the D_{2S} isoform was localized to the plasma membrane in a transfected cell system. Although this study indicates that H-FABP is important for differential D_2 DAR isoform distribution, it is also plausible that, given H-FABPs known role in fatty acid binding, it is significant in D_2-mediated arachidonic acid signaling. While the role of H-FABP in the D_2 DAR signalplex has not been defined, DRIPs that interact with the variable region of the D_2 DARs third intracellular loop may determine subtle differences in localization or functionality between these two receptors.

9.4.2.7 Caveolin-1

Caveolae are subtypes of lipid rafts that exist as distinct invaginations in the plasma membrane. This dynamic structure is thought to play a role in cell-surface signaling, possibly by forming a signaling platform for the integration of various signaling molecules. The caveolin proteins are found only in caveolae, and form a scaffolding complex that binds to a large number of receptors, signaling molecules, and adaptor proteins [63]. It was initially found that the D_1 DAR contains a caveolin-1 binding motif in the seventh transmembrane domain, thereby implying a role in D_1 DAR function and piquing interest in the study of this protein as a potential DRIP [64]. Subsequent studies determined that caveolin-1 and the D_1 DAR are localized in the same caveolae-related lipid raft domains. Co-immunoprecipitation assays from both heterologous cell lines and rat brain, as well as BRET studies in transfected cell lines, verified the direct nature of the interaction. It was subsequently determined that agonist-induced internalization through caveolae was dependent upon the direct interaction of the D_1 DAR and caveolin-1. When the caveolin-1 binding domains were mutated in the D_1 DAR, the receptor failed to internalize. Furthermore, this interaction with caveolin-1 also appears to be important for signal transduction as a mutated D_1 DAR lacking the caveolin-1 binding domain also showed diminished ability to stimulate cAMP production [64]. Taken together, these findings indicate that the caveolin-1–D_1 DAR interaction may have important implications for regulation and signaling by dopamine in the brain.

9.4.2.8 Arrestin

Arrestin proteins directly interact with DARs to mediate signal termination and internalization of the receptors, as well as to propagate alternate signaling pathways. Using GST-fusion protein pull-down assays, it was determined that arrestin2 and arrestin3, isolated from striatal homogenates, could bind to the third cytoplasmic loop of the D_2 DAR [65]. Similar experiments also showed that purified arrestin2 and arrestin3 could bind to the second and third cytoplasmic loops as well as the carboxyl terminus of the D_2 DAR [65]. Furthermore, the D_2 DAR was shown to co-immunoprecipitate with both arrestin2 and arrestin3 solubilized from neostriatal membranes [65]. Direct interactions between the D_1 DAR and arrestins have also been characterized. Both arrestin2 and arrestin3 interact (demonstrated using both GST-fusion protein and co-immunoprecipitation assays) with the carboxyl terminus of the D_1 receptor in striatal membranes, with arrestin3 binding more strongly [66].

Recently, arrestin has also been demonstrated to act as a scaffold between the D2 family of DARs and regulation of the Akt signaling pathway [28]. Akt is a serine/threonine kinase that negatively regulates glycogen synthase kinase 3 (GSK3) and has been shown to be regulated by dopamine [67, 68]. Arrestin has been found to scaffold other GPCRs to G protein-independent signaling pathways that are slower than traditional G protein-mediated signaling pathways [69, 70]. Arrestin3 knockout mice were thus employed to investigate the role of arrestin-scaffolded signaling in dopaminergic pathways [28], Experiments in mice lacking arrestin3 revealed that amphetamine-induced phosphorylation of Akt was greatly diminished in knockout mice as compared to wild-type littermates [28]. However, knockout versus wild-type mice exhibited no alterations in the phosphorylation of DARPP-32, a downstream target of the cAMP pathway. Using GST-pull-down assays, it was determined that Akt, arrestin3, GSK3, and protein phosphatase 2A can all interact [28]. The formation of this complex was also found to occur in vivo and was regulated by dopamine levels. Taken together, these data implicate arrestin3 as the scaffold necessary for linking the D_2 DAR with the Akt/GSK3 signaling pathway.

9.4.3 Signaling Proteins

This category of DRIPs contains the most classical DAR interactors – members of the downstream signaling cascade. Such DRIPs include all manner of signal initiators and propagators, from the traditional G proteins (discussed in Chapter 6) to the more recently discovered signaling proteins – calcium-sensing molecules and arrestin. In addition, this class of proteins also includes signal terminators, such as RGS proteins and kinases (see Chapter 8). To achieve maximal efficiency in signaling, it is important for the DARs to be near the machinery within their respective signaling cascades. As such, it has been demonstrated that several of the proteins in this category are scaffolded in close proximity to the receptor, and then recruited to the DAR when necessary by the DRIPs discussed in Section 9.4.2.

9.4.3.1 Calcium-Dependent Activator Protein for Secretion 1

Calcium-dependent activator protein for secretion 1, or CAPS1, is a calcium-triggered protein that assists in fusion and release of large dense-core vesicles (LDCV) to the plasma membrane [71]. LDCVs can package neurotransmitters, including dopamine, and are present along with CAPS1 and 2 in several cell lines. CAPS1 was discovered as a DRIP using the second intracellular loop of the D_2 dopamine receptor in yeast two-hybrid screens and was verified using co-immunoprecipitation assays [72]. Further investigation revealed that a serine within this second intracellular loop was necessary for CAPS1 binding, indicating a possible phosphorylation event that mediates D_2–CAPS1 interaction. Interestingly, no other DARs were found to interact with CAPS1, but D_2 DARs could also bind the closely related protein, CAPS2. CAPS1, CAPS2, and the D_2 DAR are all endogenously expressed, and co-localize, in rat pheochromocytoma PC12 cells. Transfecting PC12 cells with a truncated version of the D_2 receptor disrupted the D_2–CAPS interaction, thus allowing investigation of the importance of this DRIP in a D_2-specific manner. Significantly, disrupting the D_2–CAPS association caused a specific decrease in K^+-evoked dopamine release from PC12 cells, without altering release of any other neurotransmitters [72]. This implies that CAPS binding to the D_2 DAR or other GPCRs confers specific information regarding synaptic neurotransmitter requirements and may promote or delay LDCV fusion and exocytosis in a neurotransmitter-dependent fashion.

9.4.3.2 Neuronal Calcium Sensor-1

The neuronal calcium sensor (NCS) proteins are a family of EF-hand calcium-binding proteins that are highly evolutionarily conserved and bind calcium with high affinity [73]; within this grouping, NCS-1 belongs to the recoverin subfamily [73]. NCS-1 has been shown to regulate channel activity and, interestingly, recoverin can modulate GRK phosphorylation of rhodopsin [74]. Using the carboxyl terminus of the D_2 DAR as bait in a yeast two-hybrid assay, NCS-1 was identified as a DRIP [75]. Further investigation revealed that the D_3 and D_5 DARs could also interact with NCS-1, but not the closely related D_1 or D_4 DARs [75]. Although the significance of these selective interactions is not yet understood, it could be related to physiological distribution of the DARs and NCS-1. Investigations of the striatum revealed co-localization of NCS-1 and D_2 DAR. In a transfected cell system, NCS-1 overexpression was found to decrease the level of D_2 phosphorylation and internalization, even in the presence of GRK overexpression. This decrease in D_2 DAR phosphorylation and internalization was accompanied by a parallel increase in D_2 signaling. These data imply that NCS-1 can modulate the GRK phosphorylation-dependent desensitization of D_2 DARs. Indeed, all three proteins – D_2 DAR, GRK2, and NCS-1 – could be co-immunoprecipitated from transfected cells [75]. Moreover, the formation of this protein complex was regulated by forskolin-induced increases in cAMP and by increased Ca^{2+} concentration, highlighting the potential significance of this complex in neuronal signaling and receptor regulation.

9.4.3.3 S100B

S100B is a member of the diverse S100 family of proteins that bind calcium via two EF-hand domains [76]. S100 proteins have been implicated in an array of cellular processes, including regulation of protein phosphorylation and Ca^{2+} homeostasis; S100B has been shown to interact with and modulate the function of more than 20 other cellular proteins [76]. Recently, S100B was identified as a DRIP using the third intracellular loop of the D_2 DAR in a bacterial two-hybrid screen [19]. S100B was capable of interacting with the D_2, but not with the D_3 DAR, implicating a unique region of the D_2 third intracellular loop as the putative binding domain for S100B. The D_2 DAR and S100B could be co-immunoprecipitated from transfected cells or from striatum, and the two proteins were found to co-localize in neostriatal neurons. When S100B was overexpressed in transfected cells, D_2 DAR-stimulated ERK activation and D_2 DAR-mediated cAMP inhibition were both enhanced. These results imply that S100B can act as a D_2 DAR DRIP to augment the dopamine-stimulated D_2 DAR signaling response. Whether S100B produces this effect alone or as a dimer, scaffolding the DAR to another protein, is not yet known.

9.4.3.4 Calcineurin

Immunoprecipitation experiments have shown that protein phophatase 2B, also known as calcineurin, is a DRIP of the dopamine D_1 DAR [5]. It has become apparent (see Chapter 8) that phosphorylation and dephosphorylation play an essential role in the regulation of many aspects of DAR signaling, especially desensitization and resensitization as well as trafficking. Calcineurin has been linked to the dephosphorylation of a large number of proteins in the D_1 DAR signaling pathway, although its role in dephosphorylation of the D_1 DAR remains unclear [77]. These data indicate that, in addition to mediating signaling for the D_1 DAR, calcineurin is also physically linked to the receptor – thus making it a DRIP.

9.4.3.5 Calmodulin

Calmodulin [17] is a small protein that, upon calcium binding, undergoes large conformational changes that enable protein binding [78]. A CaM-binding motif in the third intracellular loop of the D_2 DAR led to the discovery that CaM is a DRIP [79]. CaM was able to bind either solubilized D_2 DAR [79] or to co-immunoprecipitate from transfected cells with the D_2 DAR [80], and agonist treatment increased the co-localization of D_2 and CaM in transfected cells or primary neuronal cultures [80]. Moreover, it was determined that the D_2 DAR could interact with CaM and the $G\alpha_{i1}$ G protein simultaneously, indicating that there is no competition for binding between these two proteins [79]. Furthermore, addition of Ca^{2+}/CaM, but not Ca^{2+} alone, to Ca^{2+}/CaM-depleted cells caused a decrease in G protein turnover [79]. These data indicate that CaM binding to the D_2 DAR can act to inhibit the receptor signal. Subsequent studies have shown that mutating the D_2 DAR residues required for CaM binding decreases D_2–CaM interactions without altering D_2-G

protein binding [80]. However, this CaM-binding mutation does cause a decrease in D_2-mediated cAMP and ERK signaling [80]. The conflicting results between these two groups may be explained by the role of other calcium-sensing DRIPs in receptor signaling and should be further investigated to determine the precise function of CaM–D_2 interactions.

9.4.3.6 Prostate Apoptosis Response 4

Prostate apoptosis response 4 (Par-4) was identified as a DRIP by yeast two-hybrid analysis using the D_2 DAR third intracellular loop as bait [21]. The D_3 DAR and all other GPCRs tested did not interact with Par-4. Par-4 is expressed in neurons and other tissues, though its role in these regions is unclear, and it contains a leucine zipper domain that is critical for interacting with the D_2 DAR [21]. The D_2–Par-4 interaction was verified by co-immunoprecipitation and co-localization in striatum. Silencing Par-4 expression by siRNA caused a decrease in D_2-mediated signaling in transfected cells. To examine the effect of disrupting this protein interaction in living animals, the D_2-binding leucine zipper domain of Par-4 was mutated and transgenic mice expressing this mutated Par-4 gene were generated. Cultured cells from these mice displayed elevated levels of cAMP after dopamine treatment, suggesting a loss of D_2 DAR signaling. These mice were also more prone to depressive behaviors as assessed by both Porsolt's forced swim test and the tail suspension test, with no alteration in anxiety-like behaviors [21]. These data present a possible role for Par-4 in the brain, and for the importance of Par-4–D_2 DAR interactions in neurological disorders. Of note, it was also determined that Par-4 and calmodulin compete for binding in the same region of the D_2 DAR third intracellular loop [21]. This implies that disrupting the Par-4–D_2 DAR interaction may have an appreciable impact on the association of D_2 DARs with other DRIPs, including calmodulin, and that the consequences of Par-4 inhibition may be due to other proteins interacting with D_2 DAR.

9.4.3.7 Post-synaptic Density 95

Ultrastructural studies have demonstrated that many D_1 DARs are located in the post-synaptic density of neurons. Post-synaptic density 95 (PSD-95) is a scaffolding protein that is highly enriched in post-synaptic densities and is a member of the guanylate kinase family. This complex protein contains several important domains that have been shown to be involved in its interaction with a number of different partners, most notably the NMDA receptor. Via these interactions, PSD-95 organizes proteins into functional signaling complexes in the PSD [81]. It has subsequently been appreciated that PSD-95 may also play an important role in dopaminergic signaling. PSD-95 directly interacts with the D_1 DAR thorough the amino terminus of PSD-95 and the carboxyl terminus of the D_1 DAR [82]. It was first determined that the two proteins interacted via co-immunoprecipitation assays which utilized both recombinant cell lines and mice (both wild-type and PSD-95 knockout mice). This interaction was then confirmed by localization studies using confocal microscopy

and the interaction sites were subsequently determined using GST pull-down assays and specific domain probes [83]. PSD-95 is able to regulate surface expression, and therefore subsequent signaling, by inducing agonist-independent internalization of D_1 DARs that are otherwise located on the plasma membrane. This provides an interesting mechanism by which localization and signaling is regulated by a DRIP in a cellular compartment – in this case the PSD. It has further been determined that mice lacking PSD-95 show enhanced effects of D_1 agonist treatment, indicating a functional significance to this protein–protein interaction that is possibly related to drugs of abuse [83].

9.4.3.8 Protein Kinases

In addition to serving as second messenger molecules, cAMP-dependent protein kinase (PKA) and protein kinase C (PKC) have both been shown to phosphorylate the DARs [77, 84, 85]. The DARs are also regulated by GPCR kinase (GRK) phosphorylation, particularly by GRKs 2, 3, 4, and 6 [86–89]. Phosphorylation of DARs is thought to uncouple the receptors from G proteins and to facilitate the recruitment of trafficking/scaffolding proteins, such as arrestins, that are necessary for receptor internalization. In addition to their role in DAR trafficking and signal termination, kinases can also regulate the binding sites for DRIPs and DAR insertion at the plasma membrane. The details of DAR phosphorylation and, therefore, interaction with protein kinases, has been detailed elsewhere in this volume and extensively reviewed in the literature [90, 91].

9.4.3.9 Protein Kinase C-ζ-Interacting Protein 1

Protein kinase C-ζ-interacting protein 1 (ZIP1) was identified as a DRIP in yeast two-hybrid screens using the third intracellular loop of the D_2 DAR as the bait protein [92]. Subsequent yeast two-hybrid assays revealed that ZIP1 does not interact with any other D2 or D1-family DARs. The D_2 DAR–ZIP1 interaction was verified by co-immunoprecipitation from both transfected cells and mouse brain. Of note, ZIP1 transfection caused a decrease in D_2 DAR expression and signaling in mammalian cells. This was apparently caused by an increase in lysosomal trafficking of D_2 DARs following ZIP1 overexpression. Interestingly, there was no alteration in PKCζ phosphorylation of D_2 DAR following ZIP1 overexpression, and the D_2 DAR was found to interact with the PKCζ-interaction domain of ZIP1 [92]. These data imply that, while there is a D_2 DAR-ZIP1 interaction, a D_2 DAR-ZIP1-PKCζ complex is not likely to form.

9.4.3.10 Regulator of G Protein Signaling 19

GAIP, or regulator of G protein signaling 19 (RGS19), specifically acts as a GTPase for $G\alpha_{i/o}$ G proteins, and has been shown to interact with GIPC [41]. Because GIPC was previously identified as a DRIP for both D_2 and D_3 receptors ([7];

Section 9.4.1.6), the possibility that GAIP was also a member of the DAR signalplex was investigated [93]. GAIP, GIPC, and the D_2 DAR were all found to co-immunoprecipitate from transfected cells. Furthermore, GAIP was found to co-localize with activated D_2 DARs in a GIPC-dependent manner. Importantly, recruitment of GAIP to activated D_2 DARs was shown to attenuate the D_2 DAR signal. When the GAIP GTPase domain was mutated, GAIP could still translocate to active D_2 DARs, but could not diminish the D_2 DAR signal. However, disrupting the interaction between GAIP and GIPC prevented translocation of GAIP to D_2 DARs. These data indicate that GAIP is recruited to the D_2 signalplex by GIPC which is both a trafficking and a scaffolding protein for the D_2 DAR. Once recruited to the signalplex, GAIP attenuates the D_2 signaling response via its GTPase activity.

9.4.4 Ion Channels and Pumps

It has long been accepted that the DARs can modulate the function of a variety of voltage- and ligand-gated ion channels. In recent years, several of these ion channels have been shown to associate directly with the DAR, allowing for rapid adjustments in cellular ionic balance by the DAR-activated signalplex. In addition to the interaction between DARs and ion channels, ATP-driven ionic pumps have also been shown to associate with the DARs. The ionic pumps are responsible for maintaining resting membrane potentials and normal ionic balance within cells. DARs are important regulators of neuronal excitability, and the association of DARs with ion channels and pumps is likely critical for appropriate ionic response to stimulus and recovery from events such as DAR-stimulated membrane depolarization. Their interaction with DARs allows ion channels and pumps to receive efficient signal cross talk from DARs, and also raises the interesting possibility of a more direct method of modulating ion channel function via DAR conformational changes.

9.4.4.1 Chloride Intracellular Channel 6

CLIC6, a member of the chloride intracellular channel family, was found to interact with the carboxyl terminus of the D_3 receptor through a yeast two-hybrid screen [57]. Little is known about the function of CLIC6, but the CLIC family has been implicated in the subcellular transport of chloride ions. In addition to interacting with the D_3 receptor, CLIC6 was also able to bind the carboxyl terminus of D_2 and D_4 DARs in yeast two-hybrid screens, and to co-localize with the D_3 DAR in transfected cells. Moreover, CLIC6 was also found to interact with the scaffolding proteins MUPP1 and radixin, raising the possibility of these three DRIPs scaffolding together within the DAR signalplex. Unfortunately, co-expression of the D_3 DAR with CLIC6 revealed no effect on chloride transport or D_3 function, therefore the role of CLIC6 in the DAR signalplex remains unclear. It is possible that the significance of CLIC6 is cell-type specific, and analysis of other cell lines or primary tissues will reveal a function for this DRIP.

9.4.4.2 Transient Receptor Potential Channel 1

Transient receptor potential channel 1 (TRPC1) was found to interact with the D_2 dopamine receptor by yeast two-hybrid analysis [26]. TRPC proteins have been shown to mediate Na^+ and Ca^{2+} entry, phospholipase C signaling, and lipid raft integrity [94]. TRPC proteins can also homo- or heterodimerize, with TRPC1 commonly forming heterodimers with TRPC4 and 5 [95]. As such, the ability of D_2 DARs to interact with TRPC4 and 5 was also evaluated, and it was determined that these two TRPC proteins are also DRIPs. The D_2–TRPC1 protein complex could be co-immunoprecipitated from rat brain and the two proteins were also found to co-localize in post-synaptic compartments of cortical neurons. Examination of human embryonic kidney cells that endogenously express TRPC1 revealed that D_2 DAR transfection caused an increase in plasma membrane expression of the TRPC1 protein, with no change in the total expression level of TRPC1. While the precise role of TRPC1 in the D_2 signalplex is unclear, it appears that D_2 expression is important for membrane expression of the TRP channel.

9.4.4.3 G Protein-Activated Inwardly Rectifying Potassium Channels

Dopamine receptors are known to regulate the activity of G protein-activated inwardly rectifying potassium channels (GIRKs or Kir3 [96]), likely via Gβγ activity. Based on the presence of G proteins and other effector molecules in various GPCR signalplexes, the presence of GIRKs in the DAR signalplex was investigated [23]. In these experiments, GIRK was found to co-immunoprecipitate with both D_2 and D_4 DARs from transfected cells and rat striatum. Agonist treatment and pertussis toxin treatment both had no effect on the DAR–GIRK interaction, indicating that the association was stable and not dependent upon the signaling cascade. Interestingly, the assembly of the DAR–GIRK complex was dependent upon the presence of Gβγ, but once assembled, the complex did not require Gβγ for maintained stability. It is likely that close association with GIRKs and other effectors enables rapid communication following agonist stimulation of the DARs.

9.4.4.4 Na^+,K^+-ATPase

The Na^+,K^+-ATPase (NKA, sodium pump) is a ubiquitous protein important for regulation of cellular ion balance. In the brain, NKA is critical in restoring neuronal polarity after membrane depolarization events. Dopamine signaling cascades have previously been shown to regulate the vesicular insertion or endocytosis of NKA at the plasma membrane [97, 98]. Recently, however, using mass spectroscopy coupled with co-immunoprecipitation from DAR-transfected cells, endogenous NKA was found to interact with both the D_1 and D_2 DARs [20]; the NKA-D_2 DAR interaction was also verified by co-immunoprecipitation of native proteins from striatum. Enhancing the association of NKA with DARs caused a decrease in both D_1 and D_2 DAR function. This could be reversed by pharmacological blockade of NKA, or by diminishing the NKA–DAR interaction via siRNA inhibition of NKA. The DARs

were also found to modulate NKA activity, even in the absence of ligand. This novel association of NKA with DARs in the signalplex allows for conformational changes in the proteins for exchange of cellular information, providing a more direct and rapid means of communication than traditional signaling pathways and allowing for reciprocal modulation of function between the two proteins in response to cellular stimuli.

9.4.4.5 AMPA Receptors

AMPA receptors are ligand-gated ion channels that are regulated via downstream second messenger signaling pathways stimulated through GPCRs [99, 100]. However, recent studies have revealed additional mechanisms of cross talk between AMPA receptors and GPCRs – specifically an interaction of the AMPA receptor with the D_2 DAR. This interaction presents a unique story of how DAR signalplex members can regulate one another via multiple mechanisms resulting in a complex regulatory paradigm between signaling systems. While data from in vitro binding assays suggest that the D_2 DAR and the AMPA receptor may not directly interact, they certainly form a protein complex that is mediated by the GluR2 subunit and the third intracellular loop of the D_2 DAR [48]. Co-immunoprecipitation and GST-fusion pull-down assays both indicate that the D_2 DAR and the AMPA receptor form a complex. Interestingly, NSF (see Section 9.4.1.7) interacts with both the D_2 DAR and the GluR2 subunits implicating it as the possible accessory protein in this D_2 DAR–AMPA interaction. There is considerable evidence that D_2 DAR receptors can modulate AMPA receptor-mediated neurotoxicity [101]. The mechanism for this physiological effect may partially involve the protein–protein coupling of these two systems as outlined above. It has been proposed that agonist stimulation of D_2 DARs promotes the formation of protein–protein interactions between the D_2 DAR and NSF, while at the same time uncoupling NSF from the carboxyl-terminal tail of the GluR2 subunit of the AMPA receptor, thereby resulting in a decrease in AMPA receptor membrane expression and subsequent inhibition of AMPA-mediated neurotoxicity. These findings demonstrate the importance of the D_2 DAR-NSF-AMPA interaction and show how a member of the D_2 DAR signalplex can regulate other receptors through a common interacting protein [48].

9.4.4.6 NMDA Receptors

The NMDA receptor complex has become a primary target for CNS drug development. Dopamine and glutamate are known to extensively interact in several brain areas and to regulate a number of physiological functions including attention, working memory, and positive reinforcement. Evidence continues to mount connecting these two signaling pathways in several pathologies including Parkinson's disease and schizophrenia. While different mechanisms are probably important in the

interaction of these pathways, including second messenger-mediated phosphorylation of the NMDA receptor by D_1 DAR activation and coordinated regulation of receptor trafficking, the D_1 DAR–NMDA receptor interaction makes the NMDA receptor an important member of the DAR signalplex. The direct interaction of the D_1 DAR and NMDA receptor has been reported in the striatum and hippocampus and has been shown to influence both signaling and trafficking of both receptors [24, 102, 103]. These direct interactions were primarily shown using co-immunoprecipitation experiments from various areas of the brain, including striatal PSDs. This interaction has been further characterized by using heterologous cell lines and fusion pull-down assays which demonstrated that both the NR1 and the NR2a subunits of the NMDA receptor interact directly with the D_1 DAR through the carboxyl-terminal domains. Two separate domains in the D_1 DAR's carboxyl terminus mediate the direct interaction with the two different NMDA receptor subunits. The direct nature of this interaction (in an attempt to rule out the possibility of linker proteins) was investigated using BRET assays. These assays confirmed the direct interactions between the D_1 DAR and the two NMDA receptor subunits while also determining that the D_1 DAR does not interact with the NR2b subunit of the NMDA receptor. Furthermore, stimulation of these receptors by dopamine, glutamate/glycine, or both, failed to change the interaction in any way, indicating the constitutive nature of this DRIP formation [102]. Functionally, the interaction of D_1 DAR with the NR1 subunit has been shown to cause an increase in trafficking to the membrane, inhibition of dopamine-mediated DAR internalization, and the suppression of NMDA-mediated cell death. The D_1 DAR interaction with the NR2A subunit has been demonstrated to cause a decrease in NMDA currents [24, 102, 103].

9.4.4.7 GABA Receptors

GABA receptors mediate fast inhibitory interneuronal synaptic transmission, and it is now realized that DARs can influence this system by dramatically regulating signal strength through a G protein-independent mechanism. This mechanism involves direct coupling of the DAR to the GABA receptor – making it another member of the DAR signalplex. This interaction was originally found by using GST fusion proteins of the D_5 DAR in rat hippocampal membranes to pull down the GABA receptor. This was subsequently verified via co-immunoprecipitation assays using specific antibodies [104]. This direct interaction suggests cross talk between these two systems. When GABA and D_5 DAR receptors were expressed in the same cells, D_5 DAR-mediated cAMP accumulation was decreased by GABA receptor stimulation. Furthermore, whole-cell current measurements of GABA function were decreased when dopamine was applied to the cells, indicating that dopamine stimulation can inhibit GABA function [104]. These data provide yet another finding of two distinct receptor classes that, in addition to being intimately linked via signaling pathways through second messengers, are also directly interacting in a manner to influence each other's physiology.

9.4.5 Neurotransmitter Transporters and Other GPCRs

Neurotransmitter transporters are located pre-synaptically and are responsible for clearing excess neurotransmitter from the synaptic cleft. Recent investigations have revealed an association between DARs and these transporters. It has also recently been shown that DARs are capable of direct interaction with other GPCRs, including other DARs, to form receptor oligomers; the topic of DAR oligomerization is covered extensively in Chapter 10.

9.4.5.1 Dopamine Transporter

The dopamine transporter (DAT) is located pre-synaptically and facilitates the re-uptake of synaptic dopamine for degradation or vesicular re-packaging. It is well documented that the DAT can be regulated by phosphorylation, including phosphorylation events downstream of DAR activation [105]. Recently, using co-immunoprecipitation assays, it was shown that the D_2 DAR can directly interact with the DAT [22]. Co-expression of the D_{2S} DAR with DAT enhanced re-uptake of dopamine. The increase in DAT-mediated dopamine clearance could be blocked by expressing a peptide that binds to the D_{2S} DAR and prevents its interaction with the DAT; transfection of this peptide in the absence of D_{2S} DAR had no impact on DAT function. Interestingly, the observed increase in DAT function was independent of agonist binding to the D_2 DAR, indicating that D_2 DAR signaling alone was not required for the enhanced DAR function. DAT localization was also impacted by D_2 DAR expression – the presence of D_2 DAR caused DAT to translocate from a diffuse intracellular distribution to the cell surface. This DAT translocation could be reversed by expression of the D_{2S}-DAT blocking peptide. Physiologically, the interaction of D_{2S} DAR and DAT would be predicted to enhance clearance of dopamine from the synapse, and disrupting this interaction would result in hyperlocomotion. To examine this possibility, the D_{2S}-DAT blocking peptide was administered to mice. As predicted, the peptide caused a decrease in synaptosomal dopamine uptake and an increase in activity and rearing behaviors. This direct interaction between D_{2S} DAR and DAT provides a mechanism for rapid regulation between the two proteins, and disruption of this interaction clearly impedes normal synaptic function.

9.5 Conclusions

The growing literature identifying and characterizing DAR-interacting proteins has revealed a great deal of information about the dynamic nature of the DAR signalplex. The components of the DAR–DRIP complex can vary with time and across cells and can influence many aspects of DAR and DRIP regulation, including signaling, pharmacology, and desensitization and resensitization/recycling pathways. Not only does this information about DAR–DRIP interactions reveal previously unappreciated aspects of both receptor and cellular regulation, it also provides a unique

perspective on DAR diversity and a new set of tools to the experimenter for receptor manipulation. In addition, the specificity of DRIP interactions to distinct DAR isoforms and cell types reveals a new set of therapeutic targets and a new arena for drug design.

The selective interaction between DRIPs and closely related DARs can provide investigators with new information about functional differences both across and within DAR families. Regardless of tissue distribution, it is clear that even within D1 and D2 receptor families not all DARs can interact with each DRIP, thus highlighting a previously unappreciated outcome of sequence variation. Given the ability of DARs to bind specific DRIPs and the limited distribution of many interactors, it is likely that physiological receptor location alone does not determine each DAR's unique functional properties. Instead, the array of DRIPs within a cell that can interact uniquely with a DAR isoform shapes the functional role of each receptor.

As illustrated by the DRIPs discussed in this chapter, specific reinforcement or disruption of DAR–DRIP interactions has already provided useful information to investigators regarding the mechanism underlying various aspects of receptor regulation and signaling. It may now be possible to exploit the individual properties of these DAR–DRIP interactions in the treatment of dopaminergic disorders. Indeed, manipulating DAR interactions with GASP, DAT, and Par-4 has already demonstrated potential therapeutic utility for such manipulations [6, 21, 22]. Many neurological and psychiatric disorders are characterized by abnormalities in dopaminergic transmission and/or responsiveness to dopaminergic drug therapy, including schizophrenia, Parkinson's disease, and depression. However, most of the currently available drug treatments produce adverse side effects that limit their therapeutic utility. It is possible that the underlying dopaminergic abnormality involves alterations in a specific DAR–DRIP interaction; indeed, there is evidence for alterations in the levels of the D_2 DRIP, NCS-1, in schizophrenic patients [106]. Manipulating this and other DAR–DRIP interactions may provide a new course of treatment with more limited side effects – by focusing on a specific, dysfunctional DAR–DRIP interaction, unaffected brain regions under normal dopaminergic control can be avoided, and undesirable consequences of therapy thus eliminated.

While identifying DRIPs and characterizing the various permutations of the DAR signalplex is a challenging endeavor, much progress has already been made. Certainly, the advent of proteomic-based protein identification will expedite the identification process even further and generate many new DRIP targets for characterization. Elucidating the role of DRIPs in an organ-, region-, and cell-specific manner will undoubtedly further our understanding of variation in dopaminergic signaling and provide novel molecular tools, as well as targets for drug design and therapeutic intervention.

References

1. Kabbani N, Hannan MA, Levenson R. Unraveling the dopamine receptor signalplex by DRIPs and DRAPs. Curr Proteomics 2005;2(3):209–23.

2. Kabbani N, Levenson R. A proteomic approach to receptor signaling: molecular mechanisms and therapeutic implications derived from discovery of the dopamine D2 receptor signalplex. Eur J Pharmacol 2007;572(2–3):83–93.
3. Free RB, Hazelwood LA, Cabrera DM, et al. D1 and D2 dopamine receptor expression is regulated by direct interaction with the chaperone protein calnexin. J Biol Chem 2007;282(29):21285–300.
4. Smith FD, Oxford GS, Milgram SL. Association of the D2 dopamine receptor third cytoplasmic loop with spinophilin, a protein phosphatase-1-interacting protein. J Biol Chem 1999;274(28):19894–900.
5. Adlersberg M, Hsiung SC, Glickstein SB, Liu KP, Tamir H, Schmauss C. Regulation of dopamine D-receptor activation in vivo by protein phosphatase 2B (calcineurin). J Neurochem 2004;90(4):865–73.
6. Bartlett SE, Enquist J, Hopf FW, et al. Dopamine responsiveness is regulated by targeted sorting of D2 receptors. Proc Natl Acad Sci USA 2005;102(32):11521–6.
7. Jeanneteau F, Diaz J, Sokoloff P, Griffon N. Interactions of GIPC with dopamine D2, D3 but not D4 receptors define a novel mode of regulation of G protein-coupled receptors. Mol Biol Cell 2004;15(2):696–705.
8. Rasmussen SG, Choi HJ, Rosenbaum DM, et al. Crystal structure of the human beta2 adrenergic G-protein-coupled receptor. Nature 2007;450(7168):383–7.
9. Guo W, Urizar E, Kralikova M, et al. Dopamine D2 receptors form higher order oligomers at physiological expression levels. Embo J 2008;27(17):2293–304.
10. Grefen C, Lalonde S, Obrdlik P. Split-ubiquitin system for identifying protein-protein interactions in membrane and full-length proteins. In: Gerfen C, Holmes A, Rogawski M, Sibley D, Skolnick P, Wray S, eds. Current Protocols in Neuroscience. Hoboken, NJ: John Wiley & Sons; 2007.
11. Lentze N, Auerbach D. Membrane-based yeast two-hybrid system to detect protein interactors. In: Coligan JE, Dunn BM, Speicher DW, Wingfield PT, eds. Current Protocols in Protein Science. Hoboken, NJ: John Wiley & Sons; 2008.
12. Joung JK. Identifying and modifying protein-DNA and protein-protein interactions using a bacterial two-hybrid selection system. J Cell Biochem Suppl 2001; 37(Suppl):53–7.
13. Brymora A. Protein-protein interactions identified by pull-down experiments and mass spectrometry. In: Bonifacino JS, Dasso M, Harford JB, Lippincott-Schwartz J, Yamada KM, eds. Current Protocols in Cell Biology. Hoboken, NJ: John Wiley & Sons; 2004.
14. Kramer S, Joos TO, Templin MF. Protein microarrays. In: Coligan JE, Dunn BM, Speicher DW, Wingfield PT, eds. Current Protocols in Protein Science. Hoboken, NJ: John Wiley & Sons; 2005.
15. Husi H, Ward MA, Choudhary JS, Blackstock WP, Grant SG. Proteomic analysis of NMDA receptor-adhesion protein signaling complexes. Nat Neurosci 2000;3(7):661–9.
16. Kim M, Jiang LH, Wilson HL, North RA, Surprenant A. Proteomic and functional evidence for a P2X7 receptor signalling complex. Embo J 2001;20(22):6347–58.
17. Becamel C, Alonso G, Galeotti N, et al. Synaptic multiprotein complexes associated with 5-HT(2C) receptors: a proteomic approach. Embo J 2002;21(10):2332–42.
18. Free RB, Hazelwood LA, Sibley DR. Identifying novel protein-protein interactions using co-immunoprecipitation and mass spectroscopy. In: Gerfen C, Holmes A, Rogawski M, Sibley D, Skolnick P, Wray S, eds. Current Protocols in Neuroscience. Hoboken, NJ: John Wiley & Sons; 2008.
19. Liu Y, Buck DC, Neve KA. Novel interaction of the dopamine D2 receptor and the Ca2+ binding protein S100B: role in D2 receptor function. Mol Pharmacol 2008;74(2):371–8.
20. Hazelwood LA, Free RB, Cabrera DM, Skinbjerg M, Sibley DR. Reciprocal modulation of function between the D1 and D2 dopamine receptors and the Na+,K+-ATPase. J Biol Chem 2008;283(52):36441–53.
21. Park SK, Nguyen MD, Fischer A, et al. Par-4 links dopamine signaling and depression. Cell 2005;122(2):275–87.

22. Lee FJ, Pei L, Moszczynska A, Vukusic B, Fletcher PJ, Liu F. Dopamine transporter cell surface localization facilitated by a direct interaction with the dopamine D2 receptor. Embo J 2007;26(8):2127–36.
23. Lavine N, Ethier N, Oak JN, et al. G protein-coupled receptors form stable complexes with inwardly rectifying potassium channels and adenylyl cyclase. J Biol Chem 2002;277(48):46010–9.
24. Lee FJ, Xue S, Pei L, et al. Dual regulation of NMDA receptor functions by direct protein-protein interactions with the dopamine D1 receptor. Cell 2002;111(2):219–30.
25. Pfleger KD, Eidne KA. Monitoring the formation of dynamic G-protein-coupled receptor-protein complexes in living cells. Biochem J 2005;385(Pt 3):625–37.
26. Hannan MA, Kabbani N, Paspalas CD, Levenson R. Interaction with dopamine D2 receptor enhances expression of transient receptor potential channel 1 at the cell surface. Biochim Biophys Acta 2008;1778(4):974–82.
27. Klewe IV, Nielsen SM, Tarpo L, et al. Recruitment of beta-arrestin2 to the dopamine D2 receptor: insights into anti-psychotic and anti-parkinsonian drug receptor signaling. Neuropharmacology 2008;54(8):1215–22.
28. Beaulieu JM, Sotnikova TD, Marion S, Lefkowitz RJ, Gainetdinov RR, Caron MG. An Akt/beta-arrestin 2/PP2A signaling complex mediates dopaminergic neurotransmission and behavior. Cell 2005;122(2):261–73.
29. Hammond C, Braakman I, Helenius A. Role of N-linked oligosaccharide recognition, glucose trimming, and calnexin in glycoprotein folding and quality control. Proc Natl Acad Sci USA 1994;91(3):913–7.
30. Molinari M, Eriksson KK, Calanca V, et al. Contrasting functions of calreticulin and calnexin in glycoprotein folding and ER quality control. Mol Cell 2004;13(1):125–35.
31. Swanton E, High S, Woodman P. Role of calnexin in the glycan-independent quality control of proteolipid protein. Embo J 2003;22(12):2948–58.
32. Bermak JC, Li M, Bullock C, Zhou QY. Regulation of transport of the dopamine D1 receptor by a new membrane-associated ER protein. Nat Cell Biol 2001;3(5):492–8.
33. Zhan L, Liu B, Jose-Lafuente M, et al. ALG-2 interacting protein AIP1: a novel link between D1 and D3 signalling. Eur J Neurosci 2008;27(7):1626–33.
34. Katoh K, Shibata H, Suzuki H, et al. The ALG-2-interacting protein Alix associates with CHMP4b, a human homologue of yeast Snf7 that is involved in multivesicular body sorting. J Biol Chem 2003;278(40):39104–13.
35. Kim OJ, Ariano MA, Lazzarini RA, Levine MS, Sibley DR. Neurofilament-M interacts with the D1 dopamine receptor to regulate cell surface expression and desensitization. J Neurosci 2002;22(14):5920–30.
36. Kruger R, Fischer C, Schulte T, et al. Mutation analysis of the neurofilament M gene in Parkinson's disease. Neurosci Lett 2003;351(2):125–9.
37. Strous RD, Greenbaum L, Kanyas K, et al. Association of the dopamine receptor interacting protein gene, NEF3, with early response to antipsychotic medication. Int J Neuropsychopharmacol 2007;10(3):321–33.
38. Lajoie P, Nabi IR. Regulation of raft-dependent endocytosis. J Cell Mol Med 2007;11(4):644–53.
39. Robinet P, Fradagrada A, Monier MN, et al. Dynamin is involved in endolysosomal cholesterol delivery to the endoplasmic reticulum: role in cholesterol homeostasis. Traffic 2006;7(7):811–23.
40. Kabbani N, Jeromin A, Levenson R. Dynamin-2 associates with the dopamine receptor signalplex and regulates internalization of activated D2 receptors. Cell Signal 2004;16(4):497–503.
41. De Vries L, Lou X, Zhao G, Zheng B, Farquhar MG. GIPC, a PDZ domain containing protein, interacts specifically with the C terminus of RGS-GAIP. Proc Natl Acad Sci USA 1998;95(21):12340–5.

42. Blobe GC, Liu X, Fang SJ, How T, Lodish HF. A novel mechanism for regulating transforming growth factor beta (TGF-beta) signaling. Functional modulation of type III TGF-beta receptor expression through interaction with the PDZ domain protein, GIPC. J Biol Chem 2001;276(43):39608–17.
43. Hu LA, Chen W, Martin NP, Whalen EJ, Premont RT, Lefkowitz RJ. GIPC interacts with the beta1-adrenergic receptor and regulates beta1-adrenergic receptor-mediated ERK activation. J Biol Chem 2003;278(28):26295–301.
44. Bunn RC, Jensen MA, Reed BC. Protein interactions with the glucose transporter binding protein GLUT1CBP that provide a link between GLUT1 and the cytoskeleton. Mol Biol Cell 1999;10(4):819–32.
45. Zhao C, Slevin JT, Whiteheart SW. Cellular functions of NSF: not just SNAPs and SNAREs. FEBS Lett 2007;581(11):2140–9.
46. Cong M, Perry SJ, Hu LA, Hanson PI, Claing A, Lefkowitz RJ. Binding of the beta2 adrenergic receptor to N-ethylmaleimide-sensitive factor regulates receptor recycling. J Biol Chem 2001;276(48):45145–52.
47. Heydorn A, Sondergaard BP, Hadrup N, Holst B, Haft CR, Schwartz TW. Distinct in vitro interaction pattern of dopamine receptor subtypes with adaptor proteins involved in post-endocytotic receptor targeting. FEBS Lett 2004;556(1–3):276–80.
48. Zou S, Li L, Pei L, et al. Protein-protein coupling/uncoupling enables dopamine D2 receptor regulation of AMPA receptor-mediated excitotoxicity. J Neurosci 2005;25(17): 4385–95.
49. Wang Y, Zhou Y, Szabo K, Haft CR, Trejo J. Down-regulation of protease-activated receptor-1 is regulated by sorting nexin 1. Mol Biol Cell 2002;13(6):1965–76.
50. Whistler JL, Enquist J, Marley A, et al. Modulation of postendocytic sorting of G protein-coupled receptors. Science 2002;297(5581):615–20.
51. Thompson D, Pusch M, Whistler JL. Changes in G protein-coupled receptor sorting protein affinity regulate postendocytic targeting of G protein-coupled receptors. J Biol Chem 2007;282(40):29178–85.
52. Li M, Bermak JC, Wang ZW, Zhou QY. Modulation of dopamine D(2) receptor signaling by actin-binding protein (ABP-280). Mol Pharmacol 2000;57(3):446–52.
53. Li M, Li C, Weingarten P, Bunzow JR, Grandy DK, Zhou QY. Association of dopamine D(3) receptors with actin-binding protein 280 (ABP-280). Biochem Pharmacol 2002;63(5): 859–63.
54. Lin R, Karpa K, Kabbani N, Goldman-Rakic P, Levenson R. Dopamine D2 and D3 receptors are linked to the actin cytoskeleton via interaction with filamin A. Proc Natl Acad Sci USA 2001;98(9):5258–63.
55. Binda AV, Kabbani N, Lin R, Levenson R. D2 and D3 dopamine receptor cell surface localization mediated by interaction with protein 4.1 N. Mol Pharmacol 2002;62(3):507–13.
56. Niggli V, Rossy J. Ezrin/radixin/moesin: versatile controllers of signaling molecules and of the cortical cytoskeleton. Int J Biochem Cell Biol 2008;40(3):344–9.
57. Griffon N, Jeanneteau F, Prieur F, Diaz J, Sokoloff P. CLIC6, a member of the intracellular chloride channel family, interacts with dopamine D(2)-like receptors. Brain Res Mol Brain Res 2003;117(1):47–57.
58. Parker LL, Backstrom JR, Sanders-Bush E, Shieh BH. Agonist-induced phosphorylation of the serotonin 5-HT2C receptor regulates its interaction with multiple PDZ protein 1. J Biol Chem 2003;278(24):21576–83.
59. Ullmer C, Schmuck K, Figge A, Lubbert H. Cloning and characterization of MUPP1, a novel PDZ domain protein. FEBS Lett 1998;424(1–2):63–8.
60. Heuckeroth RO, Birkenmeier EH, Levin MS, Gordon JI. Analysis of the tissue-specific expression, developmental regulation, and linkage relationships of a rodent gene encoding heart fatty acid binding protein. J Biol Chem 1987;262(20):9709–17.
61. Schroeder F, Jolly CA, Cho TH, Frolov A. Fatty acid binding protein isoforms: structure and function. Chem Phys Lipids 1998;92(1):1–25.

62. Takeuchi Y, Fukunaga K. Differential subcellular localization of two dopamine D2 receptor isoforms in transfected NG108-15 cells. J Neurochem 2003;85(4):1064–74.
63. Williams TM, Lisanti MP. The Caveolin genes: from cell biology to medicine. Ann Med 2004;36(8):584–95.
64. Kong MM, Hasbi A, Mattocks M, Fan T, O'Dowd BF, George SR. Regulation of D1 dopamine receptor trafficking and signaling by caveolin-1. Mol Pharmacol 2007;72(5): 1157–70.
65. Macey TA, Gurevich VV, Neve KA. Preferential Interaction between the dopamine D2 receptor and Arrestin2 in neostriatal neurons. Mol Pharmacol 2004;66(6):1635–42.
66. Macey TA, Liu Y, Gurevich VV, Neve KA. Dopamine D1 receptor interaction with arrestin3 in neostriatal neurons. J Neurochem 2005;93(1):128–34.
67. Beaulieu JM, Sotnikova TD, Yao WD, et al. Lithium antagonizes dopamine-dependent behaviors mediated by an AKT/glycogen synthase kinase 3 signaling cascade. Proc Natl Acad Sci USA 2004;101(14):5099–104.
68. Emamian ES, Hall D, Birnbaum MJ, Karayiorgou M, Gogos JA. Convergent evidence for impaired AKT1-GSK3beta signaling in schizophrenia. Nat Genet 2004;36(2):131–7.
69. Ahn S, Shenoy SK, Wei H, Lefkowitz RJ. Differential kinetic and spatial patterns of beta-arrestin and G protein-mediated ERK activation by the angiotensin II receptor. J Biol Chem 2004;279(34):35518–25.
70. Luttrell LM, Roudabush FL, Choy EW, et al. Activation and targeting of extracellular signal-regulated kinases by beta-arrestin scaffolds. Proc Natl Acad Sci USA 2001;98(5): 2449–54.
71. Ann K, Kowalchyk JA, Loyet KM, Martin TF. Novel Ca2+-binding protein (CAPS) related to UNC-31 required for Ca2+-activated exocytosis. J Biol Chem 1997;272(32): 19637–40.
72. Binda AV, Kabbani N, Levenson R. Regulation of dense core vesicle release from PC12 cells by interaction between the D2 dopamine receptor and calcium-dependent activator protein for secretion (CAPS). Biochem Pharmacol 2005;69(10):1451–61.
73. Burgoyne RD, Weiss JL. The neuronal calcium sensor family of Ca2+-binding proteins. Biochem J 2001;353(Pt 1):1–12.
74. Calvert PD, Klenchin VA, Bownds MD. Rhodopsin kinase inhibition by recoverin. Function of recoverin myristoylation. J Biol Chem 1995;270(41):24127–9.
75. Kabbani N, Negyessy L, Lin R, Goldman-Rakic P, Levenson R. Interaction with neuronal calcium sensor NCS-1 mediates desensitization of the D2 dopamine receptor. J Neurosci 2002;22(19):8476–86.
76. Donato R. Functional roles of S100 proteins, calcium-binding proteins of the EF-hand type. Biochim Biophys Acta 1999;1450(3):191–231.
77. Rex EB, Rankin ML, Ariano MA, Sibley DR. Ethanol Regulation of D(1) Dopamine Receptor Signaling is Mediated by Protein Kinase C in an Isozyme-Specific Manner. Neuropsychopharmacology 2008;32:2900–2911.
78. Hoeflich KP, Ikura M. Calmodulin in action: diversity in target recognition and activation mechanisms. Cell 2002;108(6):739–42.
79. Bofill-Cardona E, Kudlacek O, Yang Q, Ahorn H, Freissmuth M, Nanoff C. Binding of calmodulin to the D2-dopamine receptor reduces receptor signaling by arresting the G protein activation switch. J Biol Chem 2000;275(42):32672–80.
80. Liu Y, Buck DC, Macey TA, Lan H, Neve KA. Evidence that calmodulin binding to the dopamine D2 receptor enhances receptor signaling. J Recept Signal Transduct Res 2007;27(1):47–65.
81. Kim E, Sheng M. PDZ domain proteins of synapses. Nat Rev Neurosci 2004;5(10):771–81.
82. Yao WD, Gainetdinov RR, Arbuckle MI, et al. Identification of PSD-95 as a regulator of dopamine-mediated synaptic and behavioral plasticity. Neuron 2004;41(4):625–38.
83. Zhang J, Vinuela A, Neely MH, et al. Inhibition of the dopamine D1 receptor signaling by PSD-95. J Biol Chem 2007;282(21):15778–89.

84. Jiang D, Sibley DR. Regulation of D(1) dopamine receptors with mutations of protein kinase phosphorylation sites: attenuation of the rate of agonist-induced desensitization. Mol Pharmacol 1999;56(4):675–83.
85. Namkung Y, Sibley DR. Protein kinase C mediates phosphorylation, desensitization, and trafficking of the D2 dopamine receptor. J Biol Chem 2004;279(47):49533–41.
86. Fraga S, Jose PA, Soares-da-Silva P. Involvement of G protein-coupled receptor kinase 4 and 6 in rapid desensitization of dopamine D1 receptor in rat IEC-6 intestinal epithelial cells. Am J Physiol Regul Integr Comp Physiol 2004;287(4):R772–9.
87. Ito K, Haga T, Lameh J, Sadee W. Sequestration of dopamine D2 receptors depends on coexpression of G-protein-coupled receptor kinases 2 or 5. Eur J Biochem 1999;260(1): 112–9.
88. Rankin ML, Marinec PS, Cabrera DM, Wang Z, Jose PA, Sibley DR. The D1 dopamine receptor is constitutively phosphorylated by G protein-coupled receptor kinase 4. Mol Pharmacol 2006;69(3):759–69.
89. Tiberi M, Nash SR, Bertrand L, Lefkowitz RJ, Caron MG. Differential regulation of dopamine D1A receptor responsiveness by various G protein-coupled receptor kinases. J Biol Chem 1996;271(7):3771–8.
90. Krupnick JG, Benovic JL. The role of receptor kinases and arrestins in G protein-coupled receptor regulation. Annu Rev Pharmacol Toxicol 1998;38:289–319.
91. Tobin AB. G-protein-coupled receptor phosphorylation: where, when and by whom. Br J Pharmacol 2008;153(Suppl 1):S167–76.
92. Kim OJ, Ariano MA, Namkung Y, et al. D2 dopamine receptor expression and trafficking is regulated through direct interactions with ZIP. J Neurochem 2008;106(1):83–95.
93. Jeanneteau F, Guillin O, Diaz J, Griffon N, Sokoloff P. GIPC recruits GAIP (RGS19) to attenuate dopamine D2 receptor signaling. Mol Biol Cell 2004;15(11):4926–37.
94. Beech DJ. TRPC1: store-operated channel and more. Pflugers Arch 2005;451(1):53–60.
95. Ramsey IS, Delling M, Clapham DE. An introduction to TRP channels. Annu Rev Physiol 2006;68:619–47.
96. Werner P, Hussy N, Buell G, Jones KA, North RA. D2, D3, and D4 dopamine receptors couple to G protein-regulated potassium channels in Xenopus oocytes. Mol Pharmacol 1996;49(4):656–61.
97. Barnard ML, Olivera WG, Rutschman DM, Bertorello AM, Katz AI, Sznajder JI. Dopamine stimulates sodium transport and liquid clearance in rat lung epithelium. Am J Respir Crit Care Med 1997;156(3 Pt 1):709–14.
98. Brismar H, Asghar M, Carey RM, Greengard P, Aperia A. Dopamine-induced recruitment of dopamine D1 receptors to the plasma membrane. Proc Natl Acad Sci USA 1998;95(10):5573–8.
99. Anderson SM, Famous KR, Sadri-Vakili G, et al. CaMKII: a biochemical bridge linking accumbens dopamine and glutamate systems in cocaine seeking. Nat Neurosci 2008;11(3):344–53.
100. Mangiavacchi S, Wolf ME. D1 dopamine receptor stimulation increases the rate of AMPA receptor insertion onto the surface of cultured nucleus accumbens neurons through a pathway dependent on protein kinase A. J Neurochem 2004;88(5):1261–71.
101. Cepeda C, Levine MS. Dopamine and N-methyl-D-aspartate receptor interactions in the neostriatum. Dev Neurosci 1998;20(1):1–18.
102. Fiorentini C, Gardoni F, Spano P, Di Luca M, Missale C. Regulation of dopamine D1 receptor trafficking and desensitization by oligomerization with glutamate N-methyl-D-aspartate receptors. J Biol Chem 2003;278(22):20196–202.
103. Pei L, Lee FJ, Moszczynska A, Vukusic B, Liu F. Regulation of dopamine D1 receptor function by physical interaction with the NMDA receptors. J Neurosci 2004;24(5):1149–58.
104. Liu F, Wan Q, Pristupa ZB, Yu XM, Wang YT, Niznik HB. Direct protein-protein coupling enables cross-talk between dopamine D5 and gamma-aminobutyric acid A receptors. Nature 2000;403(6767):274–80.

105. Mortensen OV, Amara SG. Dynamic regulation of the dopamine transporter. Eur J Pharmacol 2003;479(1–3):159–70.
106. Bai J, He F, Novikova SI, et al. Abnormalities in the dopamine system in schizophrenia may lie in altered levels of dopamine receptor-interacting proteins. Biol Psychiatry 2004;56(6):427–40.

Chapter 10
Dopamine Receptor Oligomerization

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Abstract Each dopamine (DA) receptor subtype physically interacts with its own kind (homomers) or other receptors (heteromers) in the plasma membrane of neurons in the basal ganglia to form dimeric or high-order receptor oligomers, termed dimeric or high-order receptor mosaics (RMs). Two types of heteromeric DA RMs are primarily discussed, namely type 1 receptor mosaic (RM1) formed by different DA receptor (DA-R) subtypes that display classical cooperativity and type 2 receptor mosaic (RM2) formed by DA-R subtypes physically interacting with other receptors that display non-classical cooperativity. The D_2 receptor can form a RM1 with either D_1 or D_3 receptor subtypes as well as different types of RM2 with A_{2A}, mGluR5, CB_1, neuropeptide receptors (SSR5, NTS1, CCK-2), and *N*-methyl-D-aspartate (NMDA) receptors. Trimeric A_{2A}-D_2-mGluR5 and A_{2A}-D_2-CB_1 RM2 may exist in striatal neuronal networks and are also discussed. D_1 receptors can form RM1 with D_3 receptors and different types of RM2 with A_1, μ-opioid, and NMDA receptors. D_3 receptors can form a RM2 with A_{2A} receptors and D_5 receptors can form a RM2 with γ-aminobutyric acid (GABA)-A receptors. Through existing as part of a horizontal molecular network, RMs fine-tune multiple effector systems already at the level of the membrane, involving Ca^{2+}, Na^+, and K^+ and including G protein-regulated inwardly rectifying potassium channels (GIRK), adenylyl cyclase (AC), phospholipase C (PLC), and dopamine transporter activity. The synaptic strength is particularly modulated by DA receptors within DA receptor RM2 that involve ligand-gated ion channels such as GABA-A and NMDA receptors. The existence of a RM2 formed by D_2 receptors and receptor tyrosine kinase (RTK) receptors is also likely to exist and bears high relevance for the integration of trophic and informational signals within striatal networks. A novel neuropsychopharmacology may develop on the basis of DA receptor-containing RMs in the brain from the unique pharmacological properties afforded by their receptor–receptor interactions.

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10.1 Introduction

This chapter describes the functional existence of dopamine receptor (DA-R) homomers and heteromers in the brain, especially within the basal ganglia, while highlighting their clinical relevance (for general reviews, see [1–8]). Special focus will be placed on how DA receptor subtypes directly interact with other G protein-coupled receptors (GPCRs) and with two types of ligand-gated ion channels, specifically the *N*-methyl-D-aspartate (NMDA) and GABA-A receptors (see [2, 9, 10]). However, it should also be emphasized that D_2 and D_4 receptors immunoprecipitate with G protein-regulated inward rectifying potassium (GIRK) channels, form stable complexes with these channels that are opened via the G protein $\beta\gamma$ dimers from activated DA-R, and lead to marked reductions in neuronal excitability [11]. Furthermore, evidence also exists for a direct association of GABA-B receptors with GIRK (Kir3) channels and the regulator of G protein signaling RGS4 [12], forming a signaling complex also involving G proteins. DA-R oligomers likely participate in such signaling complexes.

DA receptor homomeric and heteromeric complexes participate in horizontal and vertical molecular networks including effectors such as GIRK channels and various types of DA-R-interacting proteins. At the prejunctional level, D_2 receptors have been demonstrated to form stable complexes with the dopamine transporter (DAT) [13], enabling the DA re-uptake process to be properly fine-tuned to the ongoing activity of the D_2 autoreceptor-formed heteromers including for example, the neurotensin receptor 1 (NTS1)/D_2 autoreceptor heteromer (see [14]) and the non-α7 nicotinic/D_2 receptor heteromer in the dorsal striatum [15–17]. The existence of unique DA-R-oligomeric receptor complexes at both the pre- and postjunctional level, due to the differential location of the D_{2L} and D_{2S} isoforms, may also give rise to a different neuropsychopharmacology at the pre- and postjunctional D_2 receptors. These differences may lead to novel DA agonists or antagonists for the treatment of neurological and mental disease (see [18]) with reduced side effects by fine-tuning DA transmission toward increases or reductions in specific DA-R heteromeric complexes [19, 20].

10.2 Receptor–Receptor Interactions

In previous papers [21–27], it has been demonstrated that direct interactions among GPCRs at the level of the plasma membrane can occur based on analysis of neuropeptide/monoamine receptor interactions. Initial observations at the level of

agonist recognition in membrane preparations from the CNS suggested the existence of receptor heteromers. In the past years, accumulating data have demonstrated that GPCRs can homodimerize and heterodimerize as well as form oligomers of unknown stoichiometry [1, 3, 4, 6–8, 20, 28–31]. However, the present techniques used to measure receptor–receptor interactions cannot distinguish between domain-swapped dimers and contact dimers [32, 33].

It is our opinion that both types of interactions (domain swapping and domain contact) may occur that allow the formation of high-order heterooligomers (high-order receptor mosaics) depending on the receptor type and the chemicophysical characteristics of the environments in which the receptors are embedded that affect GPCR conformation. It is likely that several points of contact exist between two receptors in view of the available data on protein–protein interactions [34]. Most interaction interfaces are made up of relatively large protein surfaces (larger than 600 $Å^2$) with complementary conformations and electrostatic salt bridging for enhanced stability. Furthermore, a small set of hot-spot residues at the interfaces contribute significantly to the free energy binding of the protein–protein interaction and are mainly clustered at the center of these interfaces.

It should also be pointed out that electrostatic and hydrogen bond-based interactions are weaker in a water medium than within the lipid environment plane of the membrane. Hence, the interactions in the plane of the membrane involving the transmembrane domains (TMD) may form the backbone of the receptor–receptor interactions. It is likely that additional interactions between receptors in the extra- and intracellular environments are more plastic and can be easily modified [35].

It is of basic importance to discuss not only the process through which receptors physically interact but also the factors affecting such interactions, thereby modulating the transfer of the information within the receptor mosaic (RM, dimeric, or high-order receptor oligomers). In fact, hydrogen bonds (whose strength depends on the charge of acceptor and donor atoms), electrostatic interactions (whose strength depends on distance and markedly on the dielectric constant of the medium), and van der Waals forces (whose strength clearly depends on the distance and falls rapidly beyond 4 Å of separation) have an energy that differs little from the average thermal energy of molecules. Since the assembly of protein modules is owed to the sequential formation of these types of atom–atom interactions, if two protein modules approach one another in a non-optimal way or if two protein modules that are not intended to interact initiate the interaction process, the thermal threshold can stop the process by disrupting the few weak bonds formed.

In addition, it should also be considered that receptors can assume different large-scale conformations according to several environmental conditions, including ionic strength, pH, and temperature, all of which influence receptor–receptor interactions. The existence of stable conformations, which differ on the larger scale, can be explained in terms of the free energy landscape of the receptor. The free energy landscape describes the free energy of all possible receptor protein structures for a given primary sequence in a space of $3N$ dimensions, where N is the number of atoms. Each individual conformation is represented by a valley with a given depth in this free energy hypersurface [36], and proteins can shift from one minimum to the

nearest one if the relevant parameters are changed. This motion would correspond to intervalley motion, compared with intravalley fluctuations [37].

The various stabilized conformations in such a free energy landscape are viewed as wells into which receptors may enter or leave according to energetic and entropic considerations. Since receptor–receptor interactions depend on receptor conformations, we propose that different receptor–receptor interactions develop according to the wells in which the interacting receptors are located and hence according to their free energy. It should again be pointed out that the extramembrane parts of a GPCR are highly flexible structures immersed in a polar medium (water). Therefore, they can be remodeled in their environment and be particularly suited for "fishing," e.g., for ligands and epitopes of other receptors and proteins in the extracellular and intracellular media, respectively. This will also lead to the transfer of information in the receptor mosaic via allosteric interactions causing development of negative or positive cooperativity in, e.g., the transmitter binding pockets of the RM.

10.3 The Concept of Receptor Mosaics

In the year 1982 we suggested that clusters of receptors could exist in the plasma membrane. These clusters of receptors, termed RMs [8, 38, 39], are built up of several receptors and interact via receptor–receptor interactions (i.e., via allosteric interactions). The denomination RM provides a better relationship to the biochemical fingerprint of the receptor cluster, i.e., the receptor–receptor interactions and its topology, than the term receptor oligomer (Fig. 10.1). In each RM, each receptor represents a single tessera within the mosaic; however, the receptor mosaic functions as an integrated unit with emergent properties unique to the intracellular biochemical machinery associated with each RM. We also introduced the RM hypothesis of the engram in 1982 that claims the existence of clusters of receptor proteins (G protein- and ion channel-coupled receptors) at the level of the plasma membrane that operates as computational units. These are hypothesized to play an important role in "information handling" by the cell and can contribute to the modulation of the synaptic weight of cells in neuronal networks thereby altering learning and memory processes [8, 38, 39].

A cluster of receptors operates as a high-order RM (high-order oligomer) only if each of the participating receptors modulates the biochemical/functional features of at least one other receptor within the cluster. The transitions of each receptor among its possible conformational states within the RM are constrained by the conformations of the other participating receptors through direct receptor–receptor interactions. Thereby, each receptor will respond to its ligand in a way that depends on its interactions with the other receptors in the RM. The term dimeric RM may be used instead of dimeric oligomers to consistently use the term RM [20].

A situation analogous to that of hemoglobin [40] may exist for RM consisting of the same receptor or isoreceptors, i.e., of receptors that share the same transmitter. We have defined these as RM1 (type 1 RM) representing RMs that are formed by

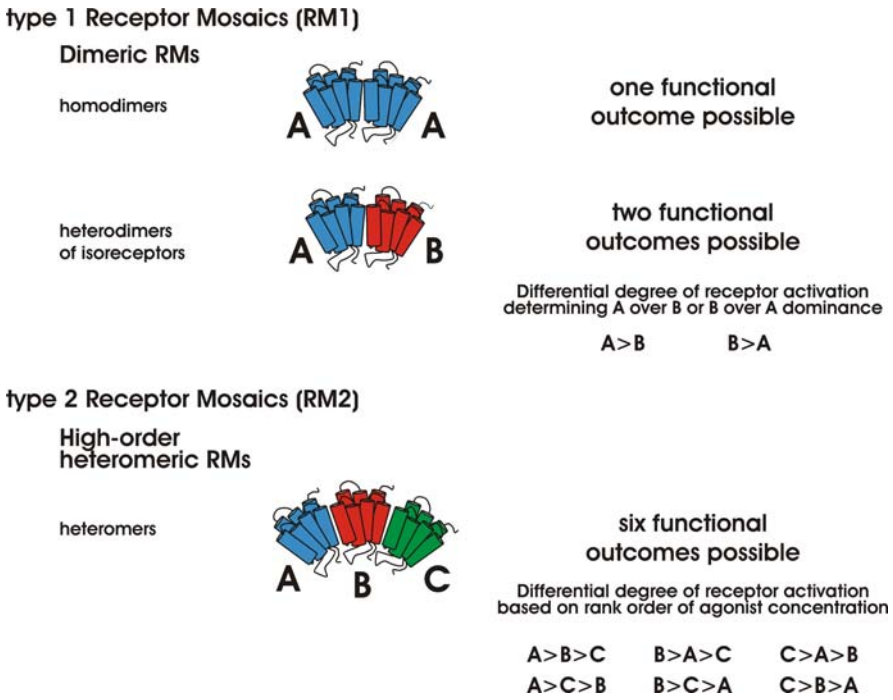

Fig. 10.1 Theoretical functional outcomes from homo- and heterodimeric (isoreceptors) type 1 receptor mosaics (RM1) and from high-order heteromeric type 2 receptor mosaics (RM2). The rank order of agonist concentration for each of the participating receptors determines the different functional outcomes. Thus, the rank order of receptor activation, dependent on agonist concentration, establishes the integration of receptor signaling that ultimately leads to the different emerging properties within RMs. In RM1 all the receptors consist of receptor subtypes having similar binding sites and share the same ligand. Thus, the *black* and *grey* receptors are subtypes of receptors sharing the same ligand such as DA. In RM2 the *black, grey,* and *light grey* receptors of the trimer have different ligands such as adenosine, dopamine, and glutamate activating for example A2A, D2, and mGluR5 receptors

one type of receptor (homomers) or by isoreceptors (heteromers formed by different subtypes of receptors for the same neurotransmitter). RM2 are those RMs formed by different types of receptors, i.e., receptors that bind different ligands [8, 41] (see Fig. 10.1). Dimers and tetramers for DA-R exist as discussed below. In fact, a tetrameric form of the DA-D_3 receptor has been reported to be the predominant form in the brain [42] but can also interact with the D_3 receptor splice variant D_{3nf} [43]. Furthermore, the early demonstration of negative cooperative interactions among the β-adrenergic receptors in frog erythrocyte membranes gave additional support to the existence of RM1 and gave functional implications [44]. Also, the findings of Armstrong and Strange demonstrated negative cooperativity in DA D_2-R ([45]; and

see [46]). Thus, RM1 formed by the same type (or subtype) of receptors shows classical cooperativity. Furthermore, the demonstrated receptor–receptor interactions in RM2 at the level of agonist recognition that involve receptors with different ligands gave evidence for the existence of non-classical cooperativity for these RMs (see [20, 41]). According to Koshland and Hamadani [40] classical cooperativity demands initial conditions with essentially identical binding sites.

10.4 On the Existence of Different Types of DA Receptor Mosaics

10.4.1 DA Type 1 Receptor Mosaics

Beginning in the early 1990s, evidence for the existence of sodium dodecyl sulfate (SDS)-resistant homodimers of D_1 and D_2 receptors was obtained in cell lines, and subsequently in brain tissue [47, 48]. High-order oligomers (high-order RM) of D_2 receptors may also exist [49]. Dimers may in fact be building blocks for high-order RMs [50, 51], and linear and ring models of RMs have been introduced based on atomic-force microscopy (see [8, 41]). Multiple sites of interactions participate in the D_2 homodimer formation [52] where the TM domain 4, but not the disulfide bonds, plays a major role in the interface [53]. It seems possible that contact dimers are formed from monomers in the endoplasmatic reticulum, allowing their expression in the cell surface membrane (see [1, 49]) and the development of negative cooperativity [45]. Evidence for the existence of D_3 dimers and D_3 tetramers in the brain has also been obtained (see above; [42]).

10.4.1.1 The D_2/D_3 Heteromer

Coimmunoprecipitation experiments in D_2 and D_3 cotransfected HEK-293 cells have given evidence for D_2/D_3 heteromeric complexes [54]. Furthermore, split D_2/D_3 heteromeric complexes may be formed through domain swapping mechanisms ([32]; see also [55]) which may contribute to their altered pharmacology allowing the D_2-like agonists including ropinirole and pramipexole to be more potent at the D_2/D_3 heteromer binding pockets [56]. The D_2/D_3 RM may also allow the D_3 receptor to become more strongly coupled to adenylyl cyclase (AC) with an increased inhibition of its activity [54]. It is also possible that D_2/D_3 RMs may exist in discrete nerve cell systems of the brain, since D_2 and D_3 receptors may be colocalized only in certain peptide nerve cells of the nucleus accumbens [57].

10.4.1.2 The D_1/D_2 Heteromer

Phospholipase C (PLC)-coupled D_1/D_2 receptor RM1s have been discovered in D_1/D_2 cotransfected cells [58, 59] and D_1 and D_2 receptors coimmunoprecipitate in rat striatal membranes [60]. Also, the two receptors may be colocated in discrete

ventral striatal nerve cell populations [61]. It is of substantial interest that the two receptors, when coactivated in the same cell, produce a novel PLC-mediated calcium signal not seen when the receptors are activated alone. The pharmacological analysis of this RM1 indicated a specific coupling to the $G_{q/11}$ pathway that produces a unique pharmacology of the D_1/D_2 RM1. The D_1-like agonist SKF 83959 exhibits a relative specificity for the $G_{q/11}$-coupled D_1-like receptor participating in D_1/D_2 heteromers with an additional ability to act as a partial agonist at the D_2 receptor. The unique pharmacology of this RM1 exhibits high PLC activation and low AC activation (see [62]). It seems possible that in the coactivated D_1/D_2 RM1, due to the receptor–receptor interaction, the conformational state of the two receptors is such that it will only allow G_q to couple to the D_1/D_2 heteromer. This is mainly detected in older animals and causes the activation of calcium/calmodulin-dependent protein kinase IIα in the ventral striatum, thereby contributing to plasticity changes in the local circuits of the striatum. This RM may be of special relevance for schizophrenia in view of the observations of a reduced link of D_1 and D_2 receptors in this disease [63].

10.4.1.3 The D_1/D_3 Heteromer

It has been shown that D_3 receptors are overexpressed in the D_1-enriched direct GABA pathway upon DA denervation and intermittent L-DOPA therapy. This D_3 receptor expression may therefore contribute to the D_1 sensitization and development of L-DOPA-induced dyskinesias [64, 65]. Thus, a facilitating receptor–receptor interaction between D_1 and D_3 receptors should be considered as well as a role for D_3 antagonists as an anti-dyskinetic therapy. It is therefore of substantial interest that it has been possible to demonstrate D_1/D_3 heteromers in living cells and to demonstrate striatal intramembrane D_3/D_1 receptor interactions [66, 67]. Heteromer formation was demonstrated by FRET and BRET techniques in cotransfected mammalian cells. Biochemical binding experiments performed in striatal membrane preparations exhibited facilitatory D_3/D_1 receptor interactions with D_3 agonist-induced increases in the affinity of the D_1 agonist-binding sites. In line with these results, D_3 receptor activation enhanced the motor stimulation effects of D_1 agonists in reserpinized mice. Thus, the D_1/D_3 heteromer may operate by enhancement of D_1 receptor recognition and signaling with relevance for D_1-mediated motor functions and when exaggerated by prolonged L-DOPA treatment may contribute to development of dyskinesias in Parkinson's disease [66].

In view of the above, it seems likely that the major functional forms of D_1, D_2, and D_3 receptors in brain are dimers and high-order oligomers such as tetramers. Thus, in the brain the DA-R subtypes can in part exist as RM1s built up of homo- and heterooligomers formed by D_1, D_2, and D_3 receptors (dimeric and high-order RM1). As discussed earlier in this section, these RMs can show classical cooperativity, since all the receptor binding sites are similar (see [8, 20, 41]). The same may also be true for D_4 and D_5 receptors. However, in all these DA type 1 receptor mosaics the stoichiometry and topography are unknown.

10.4.2 DA Type 2 Receptor Mosaics

10.4.2.1 The Somatostatin SSTR5/D_2 Receptor Heteromer

This RM2 was not constitutively formed, but formed after treatment with agonist in cotransfected CHO-K1 cells, demonstrated by photobleaching fluorescence resonance energy transfer (FRET) microscopy [68]. In this heteromer, D_2 agonist could enhance the binding affinity of somatostatin agonist at the SSTR5 and its G protein coupling while simultaneous agonist treatments increased the inhibitory cAMP signaling. These facilitatory intramembrane receptor–receptor interactions within this RM2 may help explain the positive somatostatin–DA interactions found in the brain after treatment with D_2 agonists or somatostatin. However, the population of nerve cells in the brain where SSTR5 and D_2 are colocated needs to be further established and defined. It is of substantial interest that a study using the mutant Δ318-SSTR5 receptor (a partially active C-tail deletion mutant of human SSTR5) in CHO-K1 cells indicated that a cross-activation of the D_2 receptor can occur in the absence of DA by a direct receptor–receptor interaction in the interface of the SSTR5/D_2 RM2 after somatostatin activation of the SSTR5. Similar to the DA receptor subtypes, the somatostatin receptor subtypes can assemble as functional homo- and heterodimers [69].

10.4.2.2 Putative Neuropeptide Receptor/D_2 Heteromers

Putative CCK2/D_2heteromers: It was demonstrated very early in the 1980s that both cholecystokinin-8 (CCK-8) and CCK-4 (selective ligands for CCK2 receptors) could increase the affinity of the D_2 antagonist-binding sites while reducing the affinity of the D_2 agonist-binding sites in striatal membrane preparations [24, 25, 70]. Such an antagonistic receptor–receptor interaction may contribute to the neuroleptic-like actions of CCK-8 and indicated the existence of CCK2/D_2 heteromers. A substantially stronger modulation of D_2 receptor affinity at its agonist-binding sites by CCK-8 was found in rat striatal sections most likely due to the intact cell structure including the plasma membrane [71]. The actions of CCK-8 on D_2 receptors were counteracted by a CCK2 receptor antagonist demonstrating its specificity [72]. These results underlined the previous evidence for the existence of CCK2/D_2 receptor interactions in the striatum and strongly supported the view of the existence of striatal CCK2/D_2 heteromers.

Further evidence for this view was obtained in receptor binding studies performed in a mouse fibroblast cell line (L-hD_{2L}/CCK2), expressing both human D_2 receptors (long form, D_{2L}) and human CCK2 receptors [73]. Thus, CCK-8 caused a significant decrease in the affinity of the D_2 agonist [^{3}H]NPA binding sites in the L-hD_{21}/CCK2 cell membranes, blocked by a CCK2 receptor antagonist. However, in contrast to rat neostriatal membranes where CCK-8 increases the affinity of D_2 antagonist binding [25], CCK-8 decreased the affinity of the D_2 antagonist [^{3}H]raclopride binding sites in the L-hD_{2L}/CCK2 cell membranes. This may

be related to the absence of the D_1 receptor in the D_2/CCK2 cell membranes. As a matter of fact, CCK-8 was found to increase the affinity of DA for the D_2 receptors labeled by the D_2 antagonist [^{3}H]raclopride in the rat neostriatal membranes when the D_1 receptors were also activated, but to decrease it when the D_1 receptors were blocked, indicating a control by D_1 receptors of the CCK receptor regulation of D_2 receptors. In view of the existence of D_1/D_2 heteromers ([58] and see above), it seems possible that a high-order RM may exist in the striatal membranes containing D_1, D_2, and CCK2 receptors. Within this novel postulated RM2, D_1 receptors will strongly modulate the CCK2/D_2 receptor interaction. Thus in vivo, the activity of D_1 receptors will determine whether CCK-8 via CCK2 receptors will act as an endogenous inhibitor or enhancer of D_2 receptor function.

Putative NTS1/D_2 heteromers: Experimental evidence indicates that neurotensin (NT) and in particular, but not exclusively, the NT receptor subtype 1 (NTS1) play a key role in the regulation of the functional activity of the basal ganglia and that NTS1 receptors are widely expressed on dopaminergic neurons in the substantia nigra. In the dorsal striatum, their expression appears to be limited to the dopaminergic terminals of the nigral neurons and glutamatergic terminals from cortical inputs (see [14, 74, 75]).

A large number of studies have well documented the existence of a functional neurotensin/dopamine interaction in the central nervous system. The regulation of dopaminergic transmission, especially the nigro-striatal and mesocorticolimbic DA pathways, by NT [76] is mainly due to an antagonistic action of the activated neurotensin receptor (NTR) on D_2 receptor recognition and signaling by decreasing the affinity of D_2 receptor agonist binding [14, 74, 75, 77–84]. The NT-induced changes in D_2 receptor agonist affinity have been demonstrated to be strong in striatal sections [78, 85] but exist also in striatal membrane preparations [86]. The effects are blocked by a NTS1 receptor antagonist (SR 48692). These results demonstrate a direct allosteric NTS1/D_2 receptor–receptor interaction that results in a reduction in the agonist affinity of the D_2 receptor and indicates the existence of a striatal NTS1/D_2 heteromer.

The microdialysis findings in the dorsal striato-pallidal GABA pathway have provided a functional in vivo correlate to the antagonistic NTS1/D_2 receptor–receptor interaction observed in ligand binding experiments. These microdialysis findings demonstrated the antagonistic effects of threshold concentrations of NT on striatal D_2 autoreceptor signaling and D_2 postjunctional receptors [14, 75, 79–81, 86, 87]. The NT-induced reduction of D_2-mediated signaling at both the striatal pre- and postjunctional levels leads to increased activity in the striato-pallidal GABA neurons, and DA transmission is switched toward a D_1-mediated transmission that leads to increased activity of the striatonigral GABA pathway. The former action will contribute to the motor inhibition and catalepsy exhibited after NT treatment and underlies the use of NT receptor antagonists as a novel treatment strategy for Parkinson's disease (see [14, 75]). The demonstration of the antagonistic NTS1/D_2 autoreceptor interaction in the DA terminals of the caudate-putamen gave the first indication of the existence of a D_2 autoreceptor heteromer in this case with NTS1 receptors.

10.4.2.3 The D_2-non-α7 nAChR Heteromer

Molecular interactions have been demonstrated between the β2 subunits of non-α7 nAChRs and the D_2 autoreceptor as determined in coimmunoprecipitation experiments on membrane preparations from cotransfected mammalian cells and striatum, suggesting the existence of a RM built up of non-α7 nAChR channels and D_2 autoreceptors [15, 17]. Thus, prejunctional dorsal striatal DA transmission is also modulated by these receptor heteromers, and previous studies had demonstrated that single nicotine injections could modulate the affinity of striatal D_2 receptors for antagonists [16]. The D_2/non-α7 nAChR heteromer makes it possible for the D_2 autoreceptor to decrease the stimulatory effects of non-α7 nAChR activation on DA release. The induction of DA release takes place via direct actions on the protein networks involved in vesicular fusion through increased calcium influx via the nicotinic channels and/or opening of N- and P/Q-type voltage-dependent calcium channels (see [15]).

10.4.2.4 The A_{2A}/D_2 Heteromer

A_{2A}/D_2 receptor heteromers forming type 2 RM (RM2) [88–91] may exist in the dorsal and ventral striato-pallidal GABA pathway in which activation of A_{2A} receptors reduces D_2 receptor recognition, coupling, and signaling [2, 7, 9, 26]. In the striato-pallidal GABA neurons, three types of RM may exist in equilibrium on the neuronal surface membrane, the type 1 RM formed by either A_{2A} or D_2 homomers and the type 2 RM formed by A_{2A}/D_2 heteromers. It seems possible that high-order A_{2A}/D_2 RM2 of unknown stoichiometry and topology may also exist, containing, e.g., D_2 homodimers and A_{2A} homodimers. In such a case, antagonistic A_{2A}/D_2 receptor interactions can still take place by assuming that the A_{2A} receptors can enhance the negative cooperativity in such participating D_2 receptor homodimers. Such events may also take place in the A_{2A}/D_3 and A_1/D_1 heteromers (see below). A major component of the interface in the A_{2A}/D_2 heteromer is the electrostatic interaction between the positively charged arginine-rich epitope in the N-terminal domain of the third intracellular loop (IC3) of the D_2 receptor and the negatively charged epitopes in the C-terminal tail of the A_{2A} receptor, especially the epitope (aa 370–378) containing a phosphorylated serine [89, 92]. Thus phosphorylation events may modulate the strength of the receptor–receptor interactions within these RMs. These results were also supported by studies using D_1/D_2 chimeras [93].

A_{2A} receptor activation results in an A_{2A}-induced inhibition of inhibitory D_2 receptor signaling. This produces a net increase in the activity of the striato-pallidal GABA neurons that leads to the reduced activity of the glutamate-mediated motor drive to the cortical motor regions with reduction of both motor and reward functions. In Parkinson's disease where D_2 receptor signaling is markedly reduced, A_{2A} receptor antagonists may therefore be used as a therapeutic agent to enhance D_2 receptor signaling in A_{2A}/D_2 receptor heteromers of the dorsal striato-pallidal GABA pathway [19, 94, 95]. In schizophrenia and drug addiction, with a likely pathological increase in D_2 receptor signaling in the A_{2A}/D_2 receptor heteromers

in the ventral striato-pallidal GABA pathway, A_{2A} receptor agonists may instead be useful as therapeutic agents [9, 20, 96–98]. In this way, reducing D_2 receptor signaling in the A_{2A}/D_2 receptor heteromer in the ventral striato-pallidal GABA pathway may inter alia counteract the deficit found in the glutamate drive to the prefrontal cortex in schizophrenia. The understanding of the structure and pharmacology of the A_{2A}/D_2 receptor heteromer may offer novel treatments of inter alia Parkinson's disease, schizophrenia, and drug addiction [2, 7, 8, 19, 20, 22, 26, 65, 95, 96, 99–103]

10.4.2.5 The Putative mGluR5/A_{2A}/D_2 Heteromer (High-Order RM2)

There exists evidence for the colocalization of mGluR5, A_{2A}, and D_2 receptors mainly located on dendritic spines of the striato-pallidal GABA neurons in perisynaptic zones around glutamate and DA boutons (see [15]). As discussed above, A_{2A}/D_2 heteromers have been demonstrated in striatal tissue. Furthermore, coimmunoprecipitation studies show A_{2A}/mGluR5 heteromeric receptor complexes in membrane preparations from cotransfected HEK-293 cells and in striatal membrane preparations [104]. It therefore seems possible that these three receptors can form a trimeric RM (Fig. 10.2) with the three receptors in direct contact with each other in the dendritic spines of the striato-pallidal GABA neurons and on corticostriatal glutamate terminals. Furthermore, indications of intramembrane receptor–receptor interactions between mGluR5/D_2 receptors have previously been obtained. Glutamate Group I mGlu receptor agonists and mGluR5 agonists have been found to decrease the affinity of the D_2 receptor for its agonists [105–107]. Furthermore, combined activation of A_{2A} and mGluR5 exerts enhanced effects on the antagonistic modulation of the D_2 agonist-binding site. In various behavioral and other biochemical models, significant antagonistic A_{2A}/D_2 and mGluR5/D_2 have been observed, as well as synergistic A_{2A}/mGluR5 receptor interactions [2, 7, 8, 15].

However, it remains to be identified to which extent this membrane cross talk may involve indirect interactions via scaffolding, adapter proteins, and cytoskeletal proteins such as protein 4.1 N [108], Filamin A [109, 110], and spinophilin [111]. It also seems possible that the A_{2A} and D_2 receptors, besides mGluR5 [112], could be linked to the NMDA receptor scaffold. There may be a docking to some extent of the mGluR5, D_2, and A_{2A} receptors to the NMDA receptor-associated PSD-95 complex, although they are predominantly located extrasynaptically. To support this notion, PSD-95 has in fact been identified as a regulator of DA-mediated synaptic and behavioral plasticity [113]. Furthermore, ischemic damage can be reduced by perturbing NMDA receptor/PSD-95 protein interactions [114], demonstrating its impact on excitotoxicity.

In this way, a horizontal molecular network [7] may be identified in dendritic microdomains at the membrane surface where heteromers of GPCRs, scaffolding proteins, adapter proteins (e.g., homers), and glutamate-gated ion channels (such as NMDA receptors) participate. This may allow the development of the necessary

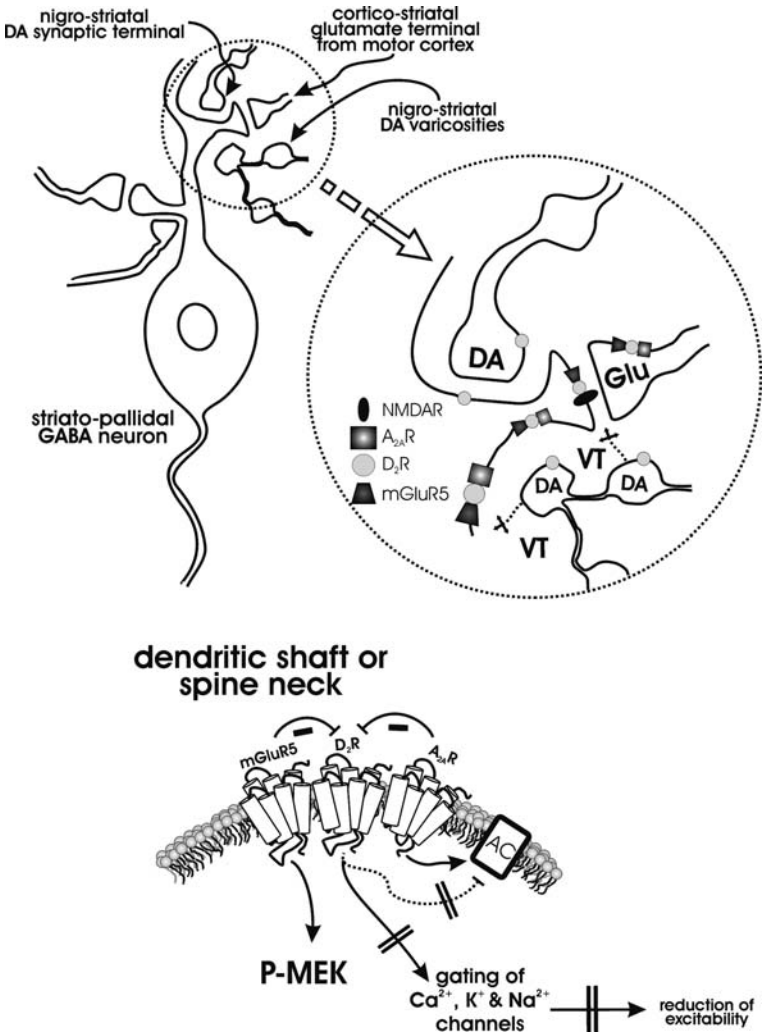

Fig. 10.2 Schematic representation of postulated A_{2A}/D_2/mGluR5 RMs mainly located in the striato-pallidal GABA neurons but also in corticostriatal glutamate terminals. They mainly exist extrasynaptically on the neck of the spines and on the dendritic shafts but also on the glutamate terminals. Junctional (shown) and extrajunctional (not shown) NMDA receptors may be regulated by D_2 receptors. Increased action potentials reaching the corticostriatal glutamate terminals will lead to increased glutamate spillover and increased formation of extracellular adenosine due to increased release of ATP from the glutamate terminals. This will lead to increased concurrent activation of mGluR5 and A_{2A} receptors in the extrasynaptic A_{2A}/D_2/mGluR5 RM, which will synergize to inhibit D_2 signaling in these RMs resulting in increases in glutamate release and reduced ability of postjunctional D_2 receptors to inhibit firing in the striato-pallidal GABA neurons via gating of Ca^{2+}, K^+, and Na^+ channels. (volume transmission; VT)

molecular circuits in the membrane and cytoplasm for the glutamate, adenosine, and DA cross talk where the receptor–receptor interactions importantly participate via direct (dimeric and high-order type 2 RMs) or indirect (adapter proteins) interactions. It now becomes possible to begin to understand the integrative molecular basis of the regulation of the excitability, firing rates, metabolism, and trophism in the striato-pallidal GABA neurons and the glutamate release process.

10.4.2.6 The A_{2A}/D_3 Heteromer

A recent study by Torvinen et al. [115] demonstrates a specific and high FRET efficiency in cells transiently cotransfected with A_{2A}-YFP and D_3-GFP^2 receptors, suggesting that A_{2A} and D_3 receptors form an A_{2A}/D_3 heteromer. Evidence was also obtained in membranes prepared from stably transfected CHO cell lines that A_{2A} activation reduces D_3 receptor agonist binding and D_3 receptor signaling. This provided the evidence for an antagonistic A_{2A}/D_3 receptor interaction in A_{2A}/D_3 heteromers similar to those observed in the A_{2A}/D_2 receptor heteromer. A_{2A}/D_3 receptor heteromers may therefore exist in the D_3-rich nucleus accumbens if coexpressed in the same neuron. In view of the existence of D_3 receptor dimers and tetramers in brain [42] and the existence of D_2/D_3 receptor heterodimers [54], the existence of high-order RM2 should be considered in this region where A_{2A}, D_3, and D_2 receptors may participate (see [8, 19, 20]). The D_3 receptor is considered a target for anti-schizophrenic drugs [116], and therefore A_{2A}/D_3 RMs offer possibilities for novel treatment strategies of this disease.

10.4.2.7 The CB_1/D_2 Heteromer and the Putative $A_{2A}/D_2/CB$ High-Order RM2

In the year 2003 it was proposed that the anti-parkinsonian actions of cannabinoid CB_1 receptor antagonists involve the counteraction of antagonistic CB_1/dopamine D_2 receptor interactions within CB_1/D_2 heteromeric complexes [2]. Subsequent work in 2005 indicated the existence of CB_1/D_2 heteromers in HEK-293 cell lines based on coimmunoprecipitation experiments and demonstrated that the putative CB_1/D_2 heteromers were enhanced through their concurrent agonist stimulation [117]. The existence of CB_1/D_2 heteromers was further established in living HEK-293 cells using FRET-based analysis and was found to be independent of receptor occupancy [118]. The CB_1 receptor agonist CP 55,940 reduced the affinity of D_2 receptor agonist binding sites in both the dorsal and the ventral striatum including the nucleus accumbens shell giving indications for the existence of antagonistic CB_1–D_2 receptor interactions taking place in striatal CB_1/D_2 heteromeric complexes. The evidence suggests a co-location of CB_1 and D_2 striatal receptors predominantly in the soma and dendrites of the ventral striato-pallidal GABA neurons and also in corticostriatal glutamate terminals where A_{2A} receptors are also present (see [119, 120]).

Antagonistic CB_1/D_2 interactions were also detected at the behavioral level through an analysis of quinpirole-induced locomotor hyperactivity in rats. The CB_1 receptor agonist CP 55,940 at a dose that did not alter basal locomotion was able to block quinpirole-induced increases in locomotor activity. In addition, not only the CB_1 receptor antagonist rimonabant but also the specific A_{2A} receptor antagonist MSX-3 blocked the inhibitory effect of CB_1 receptor agonist on D_2-like receptor agonist-induced hyperlocomotion. These results indicated the specific involvement of A_{2A} receptors in the behavioral inhibition exerted by the CB_1 receptor agonist on D_2 receptor agonist-induced hyperlocomotion [118].

In agreement with the above, A_{2A} receptor antagonists counteract the striatal CB_1 receptor-mediated motor depression, and striatal A_{2A} and CB_1 receptors form functional heteromeric complexes [121]. It is of substantial interest that CB_1 agonists like CP 55,940 and Δ^9-tetrahydrocannabinol can promote PKA-dependent phosphorylation of DARPP-32 in A_{2A}-expressing medium spiny neurons probably representing the striato-pallidal GABA neurons [122, 123], and this phosphorylation is dependent on the presence and likely tonic activation of both A_{2A} and D_2 receptors [122]. CB_1-induced DARPP-32 phosphorylation may be a consequence of the antagonistic CB_1/D_2 interaction demonstrated by Marcellino et al. [118]. A possible major mechanism for this event may be the ability of the antagonistic CB_1/D_2 receptor interaction to release the A_{2A}-activated AC from D_2-mediated inhibition [9, 20] in putative $CB_1/D_2/A_{2A}$ RMs. In fact, in the brain there exists a high molecular weight form of CB_1 devoid of G proteins, which may represent such a trimeric RM [124].

10.4.2.8 The A_1/D_1 Heteromer

Evidence suggests the existence of A_1/D_1 heteromers with antagonistic A_1/D_1 receptor interactions [8, 26, 125–128] in the basal ganglia and prefrontal cortex, and especially in the direct striatonigral–striatoentopeduncular GABA pathways. The neurochemical and behavioral findings demonstrating antagonistic A_1/D_1 receptor interactions can be explained by the existence of such A_1/D_1 heteromers and/or by antagonistic interactions at the level of second messengers [26, 126, 129, 130]. The topology and stoichiometry of the A_1/D_1 RMs are unknown. However, even if the number of D_1 receptors may dominate in these RMs with the formation of D_1 receptor dimers and tetramers, a single A_1 receptor in this RM may still a have powerful inhibitory influence. The A_1 receptor could still enhance the negative cooperativity in the activated D_1 receptor dimers and tetramers within the RM via direct interactions with one of the D_1 receptor subunits. These results suggest a role of A_1 receptor agonists and antagonists in the treatment of diseases with dysfunction of D_1 receptor signaling via their actions on the A_1/D_1 heteromers. Such diseases may include attention deficit hyperactivity disorder, drug addiction, and dyskinesias where A_1 receptor agonists may have a therapeutic value and Parkinson's disease where A_1 receptor antagonists may show anti-parkinsonian effects [19, 22, 26, 131].

10.4.2.9 The μ-Opioid Receptor/D_1 Heteromer

The μ-opioid receptor is colocated with the D_1 receptor in nerve cells of the dorsal striatum [132] to which a novel approach, using a method that harnessed the mechanism for transport of proteins to the nucleus, was used to demonstrate the heteromerization between these two receptors in living cells (see Chapter 8). The nuclear translocation pathway was adapted for the visualization of the μ-opioid receptor/D_1 heteromer, which caused an increased surface expression of the μ-opioid receptor [132]. The interface in this heteromer involved the carboxyl tail of the D_1 receptor, since its substitution with the carboxyl tail of the dopamine D_5 receptor failed to increase surface expression of the μ-opioid receptor. It is presently unknown how the μ-opioid receptor/D_1 heteromer is linked to the A_1/D_1 heteromer within the striatonigral–striatoentopeduncular GABA pathways.

10.4.2.10 The D_1/NMDA Receptor Mosaic

Dr. Fang Liu and collaborators [133, 134] have obtained evidence for a dual regulation of NMDA receptor function by direct receptor–receptor interactions with the DA D_1 receptor. Two regions of the C-terminal tail of the D_1 receptor interact with the NMDA receptor. One region has been found to directly interact with the NMDA receptor subunit NR1-1A. Upon D_1 receptor activation this direct interaction is disrupted making it possible for the C-terminal tail of NR1 to recruit calmodulin and PI-3 kinase, leading to PI-3 kinase activation causing a reduction of NMDA excitotoxicity. The other region of the D_1 receptor region directly interacts with the NR2A. This direct interaction seems to be involved in reducing NMDA receptor surface expression and leads to a reduction in NMDA receptor signaling witnessed by an inhibition of NMDA receptor-gated currents. In contrast, D_1-mediated phosphorylation mechanisms appear to enhance NMDA surface expression and signaling (see [92]). Further evidence for the direct D_1/NMDA receptor interaction has recently been obtained in cotransfected COS-7 cells using the bioluminence resonance energy transfer (BRET) technique [135]. A reciprocal D_1/NMDA receptor–receptor interaction also exists in this RM by which NMDA receptor activation can recruit D_1 receptors to the plasma membrane, thereby leading to an increase in D_1 signaling and cAMP accumulation. It is of substantial interest to note that the NR1-1 C1 cassette contains an arginine-rich epitope, whereas the interacting D_1 C-terminal portion contains an acidic epitope with an adjacent glutamic acid and a serine residue that is susceptible to phosphorylation by casein kinase 1 (CK1) [92]. In fact, mass spectrometry demonstrated that these two peptide epitopes could interact via an electrostatic interaction. A similar electrostatic interaction is involved in producing the A_{2A}/D_2 heteromerization [89] and may be a general mechanism for direct physical receptor–receptor interactions [92].

In the striatum, the direct D_1/NMDA receptor interaction probably takes place in the direct D_1 receptor-enriched GABA pathway. The D_1 receptors are predominantly located extrasynaptically, and this direct receptor interaction probably occurs

with extrajunctional located NMDA receptors in the perisynaptic zones surrounding the glutamate boutons on the head of the dendritic spines. This is of special interest since the extrasynaptic NMDA receptor signaling easily leads to excitotoxic consequences involving the cyclic-AMP response element binding protein (CREB) dephosphorylation, Ca^{2+} loading, and depolarization of mitochondria producing the activation of cell death pathways. These can be counteracted by D_1 activation.

Instead, the synaptic NMDA receptor signaling may be mainly regulated via intracellular molecular networks involving PKA-dependent phosphorylation (see [136]; for review, see [11]). DARPP-32 plays a role in this cAMP-mediated process that leads to increased synaptic NMDA receptor signaling [137].

Still another site of D_1/NMDA interaction in the direct GABA pathway could be the endoplasmic reticulum (ER) [92], where PKA- and PKC-induced phosphorylation of sites close to the arginine-rich epitope of the NR1-1 subunit counteracts ER retention favoring its membrane surface recruitment [138]. This may be one of the mechanisms underlying D_1-induced increases of synaptic NMDA receptor expression at the plasma membrane [139]. It should be mentioned that the D_1/NR1-1 subunit complex only translocates to the surface membrane when bound to the NR2 subunit [135]. Furthermore, the D_1/NMDA receptor heteromerization may be strengthened by NMDA receptor-induced D_1 phosphorylation by activation of CK1 [92].

10.4.2.11 The D_2/NMDA Receptor Mosaic

This receptor mosaic is formed by a direct interaction between the C-terminal portion of the NR2B subunit and the N-terminal portion (within the first 32 residues adjacent to the calmodulin-binding domain) of the third intracellular loop (IC3) of the D_2 receptor as demonstrated by blot overlay assays [140]. Through coimmunoprecipitation, this receptor mosaic is found to be located within microdomains of postsynaptic densities of striatal glutamate synapses. It was found to be constitutively present but enhanced through an increased D_2 activation produced by cocaine administration. The functional consequences are an interference with the binding of Ca^{2+}/calmodulin-dependent protein kinase II to NR2B that leads to the diminished phosphorylation of NR2B and a reduction of NMDA receptor signaling (an inhibition of NMDA-mediated currents). Thus, D_2 receptors by participating in different receptor mosaics in the striato-pallidal GABA neurons and in the glutamate terminals that innervate them can exert powerful inhibitory actions on these neurons. Multiple actions of D_2 receptors in separate RMs in discrete local circuits lead to inhibition of glutamate release (A_{2A}/D_2/mGluR5 RM in glutamate afferents), reduction of synaptic NMDA signaling (D_2/NMDA RMs in glutamate synapses), and inhibition of L-type voltage-dependent calcium channels (perisynaptic A_{2A}/D_2/mGluR5 RMs). Such a panorama of actions allows D_2 receptors to effectively silence striato-pallidal GABA neurons and remove motor inhibition. Electrostatic epitope–epitope interactions play a critical role in the formation of such RMs as shown in the pioneering work of Dr. Amina

Woods and collaborators where the arginine–phosphate interaction represents a general mechanism for protein–protein interactions [141]. This work also emphasizes that phosphorylation–dephosphorylation processes play an important role in these receptor–receptor interactions, since phosphate stabilizes these intermolecular interactions [141].

10.4.2.12 The D_5/GABA-A Receptor Mosaic

The D_5/GABA-A receptor heteromerization was discovered by Dr. Fang Liu et al. [142] and was the first demonstration of a GPCR/ligand-gated ion channel receptor mosaic. Along with this discovery came the first evidence that GPCRs can modulate synaptic strength via direct receptor–receptor interactions in the membrane (see [7, 8, 10, 143]). There exists an interaction between the C-terminal tail of the D_5 receptor and the $\gamma 2$ (short) subunit of the GABA-A receptor. The results indicated that agonist coactivation of D_5 and GABA-A receptors was required for the formation of the RM. This appears to allow a direct bidirectional cross talk within the RM resulting in a reduction of the synaptic strength of GABA-A signaling and reduced D_5 signaling via reduced D_5/G_s protein coupling. This receptor mosaic may also allow a cotrafficking of the D_5 receptors. It seems that these receptor mosaics may exist not only in hippocampal neurons but also in striatal medium-sized neurons and striatal interneurons in view of the existence of both D_5 and GABA-A receptors in these neuronal systems [144]. However, so far the decrease of GABA-A receptor signaling in striatum via D_1-like receptors has mainly been found to be a result of changes in phosphorylation involving the PKA/DARPP-32 cascade [145]. Regarding the possible existence of D_2/GABA-A receptor mosaics, it may be commented that there exists evidence for striatal GABA-A/D_2 receptor–receptor interactions in membrane preparations, as studied at the level of D_2 recognition (see [146]). In the prefrontal cortex there exists evidence that D_2 activation can reduce GABA-A receptor signaling that may include transactivation of the platelet-derived growth factor receptors [147]. However, it is unknown if this receptor interaction involves a direct D_2/GABA-A receptor–receptor interaction.

10.4.2.13 Putative D_2-Receptor Tyrosine Kinase Receptor Mosaics

It has recently been discovered that the DA D_2 receptor stimulation of mitogen-activated protein kinases is mediated by a cell type-dependent transactivation of receptor tyrosine kinases (RTK) [148]. There may also exist RTK ligand-independent mechanisms for RTK transactivation with participation of both GPCRs and the RTK in a multi-receptor signaling complex [149, 150]. In fact, D_2 receptor agonist increases the coimmunoprecipitation of D_2 and epidermal growth factor (EGF) receptors in neuroblastoma cells, thereby suggesting that D_2 receptor activation induces the formation of a macromolecular signaling complex [148] as a D_2/RTK type 2 receptor mosaic.

10.5 General Comments on Receptor Mosaics

A dimeric and high-order RM is a quaternary structure in which networks of electrostatic interactions, hydrogen bonds and van der Waals forces shape the geometry of the single receptors and of the entire RM. This aspect has been discussed in the frame of the free energy landscape and, in particular, of intravalley and intervalley motion in this landscape which can be driven by the actions of several factors such as pH, temperature, and chemical composition of the medium.

This concept allows one to analyze protein geometry and protein function. As a matter of fact, a protein can assume a large number of similar conformations that are close to but differ in some details from its average conformation. Thus, there is a landscape for single receptors that can influence the formation of RMs. The landscape of the entire RM determines the possible global conformations (quaternary structure) in which each conformational state can be associated with a particular integrative function of the RM. When discussing the electrostatic epitope–epitope interactions between receptors in RMs, like those occurring in the A_{2A}/D_2 receptor–receptor interaction, it will be of interest to evaluate the influence of the slaved ("random") motions which are proportional to the fluctuation rate of the solvent versus the nonslaved (e.g., "agonist-induced") motions which are independent of the solvent fluctuations [151]. It has been proposed that if an agonist causes a signal associated with an increased stability of a RM, there may develop a tightening of the receptor–receptor interactions in the oligomer [152].

10.6 Conclusions

Unique functional DA receptor dimeric and high-order RMs exist in plasma membrane microdomains of different nerve cell populations of the basal ganglia and represent a crucial mechanism for the integration of signals especially in the dorsal and ventral striatum. By being part of horizontal and vertical molecular networks of the cell surface membrane, these DA RMs can make possible a proper tuning of multiple effector systems. These systems include GIRK channels, AC, PLC, and DAT activity in which scaffolding and adapter proteins can play an important role. Synaptic strength of ligand-gated ion channels such as GABA-A and NMDA receptors can be modulated by DA-R, e.g., in the striatal GABA output neurons through the formation of receptor mosaics mainly built up of such ion channel receptors and DA receptor subtypes and that are located both synaptically and extrasynaptically. Therefore, receptor–receptor interactions in a multitude of DA receptor-containing RMs play a major role in the "information handling" in the basal ganglia [153, 154]. In this way, the integration of information in the DA receptor-containing RMs is optimized leading to an appropriate control of ion channel activity and excitability as well as gene expression within neuronal systems regulated by DA transmission. High-order DA receptor-containing RMs could have a special role in motor learning and memory in the striatum and newly formed RMs of this type may represent

the molecular basis for motor engrams (see [38]). It is clear that the DA receptor-containing RMs represent novel molecular targets for drug development against mental and neurological diseases [20].

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References

1. Bouvier M. Oligomerization of G-protein-coupled transmitter receptors. Nat Rev Neurosci 2001;2:274–86.
2. Fuxe K, Agnati LF, Jacobsen K, et al. Receptor heteromerization in adenosine A2A receptor signaling: relevance for striatal function and Parkinson's disease. Neurology 2003;61: S19–23.
3. Marshall FH. Heterodimerization of G-protein-coupled receptors in the CNS. Curr Opin Pharmacol 2001;1:40–4.
4. Milligan G, White JH. Protein-protein interactions at G-protein-coupled receptors. Trends Pharmacol Sci 2001;22:513–8.
5. Prinster SC, Hague C, Hall RA. Heterodimerization of g protein-coupled receptors: specificity and functional significance. Pharmacol Rev 2005;57:289–98.
6. Terrillon S, Bouvier M. Roles of G-protein-coupled receptor dimerization. EMBO Rep 2004;5:30–4.
7. Agnati LF, Ferre S, Lluis C, Franco R, Fuxe K. Molecular mechanisms and therapeutical implications of intramembrane receptor/receptor interactions among heptahelical receptors with examples from the striatopallidal GABA neurons. Pharmacol Rev 2003;55:509–50.
8. Fuxe K, Canals M, Torvinen M, et al. Intramembrane receptor-receptor interactions: a novel principle in molecular medicine. J Neural Transm 2007;114:49–75.
9. Fuxe K, Ferre S, Canals M, et al. Adenosine A2A and dopamine D2 heteromeric receptor complexes and their function. J Mol Neurosci 2005;26:209–20.
10. Lee FJ, Wang YT, Liu F. Direct receptor cross-talk can mediate the modulation of excitatory and inhibitory neurotransmission by dopamine. J Mol Neurosci 2005;26:245–52.
11. Neve KA, Seamans JK, Trantham-Davidson H. Dopamine receptor signaling. J Recept Signal Transduct Res 2004;24:165–205.
12. Fowler CE, Aryal P, Suen KF, Slesinger PA. Evidence for association of GABA(B) receptors with Kir3 channels and regulators of G protein signalling (RGS4) proteins. J Physiol 2007;580:51–65.
13. Lee FJ, Liu F, Pristupa Z, Niznik H. Direct binding and functional coupling of the dopamine transporter with the dopamine D2 receptor. EMBO J 2007;26:2127–36.
14. Antonelli T, Tomasini MC, Fuxe K, Agnati LF, Tanganelli S, Ferraro L. Receptor-receptor interactions as studied with microdialysis. Focus on NTR/D2 interactions in the basal ganglia. J Neural Transm 2007;114:105–13.
15. Ferre S, Agnati LF, Ciruela F, et al. Neurotransmitter receptor heteromers and their integrative role in ′local modules′: the striatal spine module. Brain Res Rev 2007;55: 55–67.
16. Li XM, Zoli M, Finnman UB, Le Novere N, Changeux JP, Fuxe K. A single (-)-nicotine injection causes change with a time delay in the affinity of striatal D2 receptors for antagonist, but not for agonist, nor in the D2 receptor mRNA levels in the rat substantia nigra. Brain Res 1995;679:157–67.
17. Quarta D, Ciruela F, Patkar K, et al. Heteromeric nicotinic acetylcholine-dopamine autoreceptor complexes modulate striatal dopamine release. Neuropsychopharmacology 2007;32:35–42.

18. Carlsson ML, Carlsson A, Nilsson M. Schizophrenia: from dopamine to glutamate and back. Curr Med Chem 2004;11:267–77.
19. Fuxe K, Marcellino D, Genedani S, Agnati L. Adenosine A(2A) receptors, dopamine D(2) receptors and their interactions in Parkinson's disease. Mov Disord 2007;22: 1990–2017.
20. Fuxe K, Marcellino D, Rivera A, Diaz-Cabiale Z, Filip M. Receptor-receptor interactions within receptor mosaics.impact on neuropsychopharmacology. Brain Res Rev 2008; 58:415–52.
21. Agnati LF, Fuxe K, Zini I, Lenzi P, Hokfelt T. Aspects on receptor regulation and isoreceptor identification. Med Biol 1980;58:182–7.
22. Franco R, Ferre S, Agnati L, et al. Evidence for adenosine/dopamine receptor interactions: indications for heteromerization. Neuropsychopharmacology 2000;23:S50–9.
23. Fuxe K, Agnati LF. Receptor-receptor interactions in the central nervous system. A new integrative mechanism in synapses. Med Res Rev 1985;5:441–82.
24. Fuxe K, Agnati LF, Benfenati F, et al. Evidence for the existence of receptor—receptor interactions in the central nervous system. Studies on the regulation of monoamine receptors by neuropeptides. J Neural Transm Suppl 1983;18:165–79.
25. Fuxe K, Agnati LF, Benfenati F, et al. Modulation by cholecystokinins of 3H-spiroperidol binding in rat striatum: evidence for increased affinity and reduction in the number of binding sites. Acta Physiol Scand 1981;113:567–9.
26. Fuxe K, Ferre S, Zoli M, Agnati LF. Integrated events in central dopamine transmission as analyzed at multiple levels. Evidence for intramembrane adenosine A2A/dopamine D2 and adenosine A1/dopamine D1 receptor interactions in the basal ganglia. Brain Res Brain Res Rev 1998;26:258–73.
27. Zoli M, Agnati LF, Hedlund PB, Li XM, Ferre S, Fuxe K. Receptor-receptor interactions as an integrative mechanism in nerve cells. Mol Neurobiol 1993;7:293–334.
28. Milligan G. G protein-coupled receptor dimerization: function and ligand pharmacology. Mol Pharmacol 2004;66:1–7.
29. Milligan G. G protein-coupled receptor dimerization: molecular basis and relevance to function. Biochim Biophys Acta 2007;1768:825–35.
30. George SR, O'Dowd BF, Lee SP. G-protein-coupled receptor oligomerization and its potential for drug discovery. Nat Rev Drug Discov 2002;1:808–20.
31. Gonzalez-Maeso J, Ang RL, Yuen T, et al. Identification of a serotonin/glutamate receptor complex implicated in psychosis. Nature 2008;452:93–7.
32. Gouldson PR, Higgs C, Smith RE, Dean MK, Gkoutos GV, Reynolds CA. Dimerization and domain swapping in G-protein-coupled receptors: a computational study. Neuropsychopharmacology 2000;23:S60–77.
33. Gouldson PR, Snell CR, Bywater RP, Higgs C, Reynolds CA. Domain swapping in G-protein coupled receptor dimers. Protein Eng 1998;11:1181–93.
34. Kortemme T, Baker D. A simple physical model for binding energy hot spots in protein-protein complexes. Proc Natl Acad Sci USA 2002;99:14116–21.
35. Agnati LF, Leo G, Genedani S, et al. Structural plasticity in G-protein coupled receptors as demonstrated by the allosteric actions of homocysteine and computer-assisted analysis of disordered domains. Brain Res Rev 2008;58:459–74.
36. Frauenfelder H, McMahon B. Dynamics and function of proteins: the search for general concepts. Proc Natl Acad Sci USA 1998;95:4795–7.
37. Frauenfelder H, Sligar SG, Wolynes PG. The energy landscapes and motions of proteins. Science 1991;254:1598–603.
38. Agnati LF, Franzen O, Ferre S, Leo G, Franco R, Fuxe K. Possible role of intramembrane receptor-receptor interactions in memory and learning via formation of long-lived heteromeric complexes: focus on motor learning in the basal ganglia. J Neural Transm Suppl 2003;65:1–28.
39. Agnati LF, Fuxe K, Zoli M, Rondanini C, Ogren SO. New vistas on synaptic plasticity: the receptor mosaic hypothesis of the engram. Med Biol 1982;60:183–90.

40. Koshland DE, Jr., Hamadani K. Proteomics and models for enzyme cooperativity. J Biol Chem 2002;277:46841–4.
41. Agnati LF, Guidolin D, Leo G, Fuxe K. A boolean network modelling of receptor mosaics relevance of topology and cooperativity. J Neural Transm 2007;114:77–92.
42. Nimchinsky EA, Hof PR, Janssen WG, Morrison JH, Schmauss C. Expression of dopamine D3 receptor dimers and tetramers in brain and in transfected cells. J Biol Chem 1997;272:29229–37.
43. Elmhurst JL, Xie Z, O'Dowd BF, George SR. The splice variant D3nf reduces ligand binding to the D3 dopamine receptor: evidence for heterooligomerization. Brain Res Mol Brain Res 2000;80:63–74.
44. Limbird LE, Meyts PD, Lefkowitz RJ. Beta-adrenergic receptors: evidence for negative cooperativity. Biochem Biophys Res Commun 1975;64:1160–8.
45. Armstrong D, Strange PG. Dopamine D2 receptor dimer formation: evidence from ligand binding. J Biol Chem 2001;276:22621–9.
46. Strange PG. Oligomers of D2 dopamine receptors: evidence from ligand binding. J Mol Neurosci 2005;26:155–60.
47. Ng GY, Mouillac B, George SR, et al. Desensitization, phosphorylation and palmitoylation of the human dopamine D1 receptor. Eur J Pharmacol 1994;267:7–19.
48. Ng GY, O'Dowd BF, Caron M, Dennis M, Brann MR, George SR. Phosphorylation and palmitoylation of the human D2L dopamine receptor in Sf9 cells. J Neurochem 1994;63:1589–95.
49. Lee SP, Xie Z, Varghese G, Nguyen T, O'Dowd BF, George SR. Oligomerization of dopamine and serotonin receptors. Neuropsychopharmacology 2000;23:S32–40.
50. Fotiadis D, Liang Y, Filipek S, Saperstein DA, Engel A, Palczewski K. Atomic-force microscopy: rhodopsin dimers in native disc membranes. Nature 2003;421:127–8.
51. Lee SP, O'Dowd BF, George SR. Homo- and hetero-oligomerization of G protein-coupled receptors. Life Sci 2003;74:173–80.
52. Lee SP, O'Dowd BF, Rajaram RD, Nguyen T, George SR. D2 dopamine receptor homodimerization is mediated by multiple sites of interaction, including an intermolecular interaction involving transmembrane domain 4. Biochemistry 2003;42:11023–31.
53. Guo W, Shi L, Javitch JA. The fourth transmembrane segment forms the interface of the dopamine D2 receptor homodimer. J Biol Chem 2003;278:4385–8.
54. Scarselli M, Novi F, Schallmach E, et al. D2/D3 dopamine receptor heterodimers exhibit unique functional properties. J Biol Chem 2001;276:30308–14.
55. Maggio R, Vogel Z, Wess J. Coexpression studies with mutant muscarinic/adrenergic receptors provide evidence for intermolecular "cross-talk" between G-protein-linked receptors. Proc Natl Acad Sci USA 1993;90:3103–7.
56. Maggio R, Scarselli M, Novi F, Millan MJ, Corsini GU. Potent activation of dopamine D3/D2 heterodimers by the antiparkinsonian agents, S32504, pramipexole and ropinirole. J Neurochem 2003;87:631–41.
57. Le Moine C, Bloch B. Expression of the D3 dopamine receptor in peptidergic neurons of the nucleus accumbens: comparison with the D1 and D2 dopamine receptors. Neuroscience 1996;73:131–43.
58. Rashid AJ, So CH, Kong MM, et al. D1-D2 dopamine receptor heterooligomers with unique pharmacology are coupled to rapid activation of Gq/11 in the striatum. Proc Natl Acad Sci USA 2007;104:654–9.
59. So CH, Varghese G, Curley KJ, et al. D1 and D2 dopamine receptors form heterooligomers and cointernalize after selective activation of either receptor. Mol Pharmacol 2005;68:568–78.
60. Lee SP, So CH, Rashid AJ, et al. Dopamine D1 and D2 receptor Co-activation generates a novel phospholipase C-mediated calcium signal. J Biol Chem 2004;279:35671–8.
61. Wong AC, Shetreat ME, Clarke JO, Rayport S. D1- and D2-like dopamine receptors are co-localized on the presynaptic varicosities of striatal and nucleus accumbens neurons in vitro. Neuroscience 1999;89:221–33.

62. Rashid AJ, O'Dowd BF, Verma V, George SR. Neuronal Gq/11-coupled dopamine receptors: an uncharted role for dopamine. Trends Pharmacol Sci 2007;28:551–5.
63. Seeman P, Niznik HB, Guan HC, Booth G, Ulpian C. Link between D1 and D2 dopamine receptors is reduced in schizophrenia and Huntington diseased brain. Proc Natl Acad Sci USA 1989;86:10156–60.
64. Bordet R, Ridray S, Schwartz JC, Sokoloff P. Involvement of the direct striatonigral pathway in levodopa-induced sensitization in 6-hydroxydopamine-lesioned rats. Eur J Neurosci 2000;12:2117–23.
65. Fuxe K, Ferre S, Woods A, et al. Novel strategies for the treatment of Parkinson's disease. Focus on receptor-receptor interactions in the basal ganglia. In: Kehr J, Fuxe K, Ungerstedt U, Svensson T, eds. Monitoring Molecules in Neuroscience; Stockholm: Karolinska University Press; 2003b, pp. 199–202.
66. Marcellino D, Ferre S, Casado V, et al. Identification of dopamine D1-D3 receptor heteromers:indications for a role of synergistic D1-D3 receptor interactions in the striatum. J Biol Chem 2008;283:26016–25.
67. Fiorentini C, Busi C, Gorruso E, Gotti C, Spano P, Missale C. Reciprocal regulation of dopamine D1 and D3 receptor function and trafficking by heterodimerization. Mol Pharmacol 2008;74(1):59–69.
68. Rocheville M, Lange DC, Kumar U, Patel SC, Patel RC, Patel YC. Receptors for dopamine and somatostatin: formation of hetero-oligomers with enhanced functional activity. Science 2000;288:154–7.
69. Rocheville M, Lange DC, Kumar U, Sasi R, Patel RC, Patel YC. Subtypes of the somatostatin receptor assemble as functional homo- and heterodimers. J Biol Chem 2000;275:7862–9.
70. Agnati LF, Fuxe K, Benfenati F, et al. Differential modulation by CCK-8 and CCK-4 of [3H]spiperone binding sites linked to dopamine and 5-hydroxytryptamine receptors in the brain of the rat. Neurosci Lett 1983;35:179–83.
71. Li XM, Hedlund PB, Fuxe K. Cholecystokinin octapeptide in vitro and ex vivo strongly modulates striatal dopamine D2 receptors in rat forebrain sections. Eur J Neurosci 1995;7:962–71.
72. Li XM, Hedlund PB, Agnati LF, Fuxe K. Dopamine D1 receptors are involved in the modulation of D2 receptors induced by cholecystokinin receptor subtypes in rat neostriatal membranes. Brain Res 1994;650:289–98.
73. Dasgupta S, Li XM, Jansson A, et al. Regulation of dopamine D2 receptor affinity by cholecystokinin octapeptide in fibroblast cells cotransfected with human CCKB and D2L receptor cDNAs. Brain Res Mol Brain Res 1996;36:292–9.
74. Antonelli T, Fuxe K, Tomasini MC, et al. Neurotensin receptor mechanisms and its modulation of glutamate transmission in the brain: relevance for neurodegenerative diseases and their treatment. Prog Neurobiol 2007;83:92–109.
75. Ferraro L, Tomasini MC, Mazza R, et al. Neurotensin receptors as modulators of glutamatergic transmission. Brain Res Rev 2008;58:365–73.
76. Deutch AY, Zahm DS. The current status of neurotensin-dopamine interactions. Issues and speculations. Ann N Y Acad Sci 1992;668:232–52.
77. Agnati LF, Fuxe K, Benfenati F, Battistini N. Neurotensin in vitro markedly reduces the affinity in subcortical limbic 3H-N-propylnorapomorphine binding sites. Acta Physiol Scand 1983;119:459–61.
78. Diaz-Cabiale Z, Fuxe K, Narvaez JA, et al. Neurotensin-induced modulation of dopamine D2 receptors and their function in rat striatum: counteraction by a NTR1-like receptor antagonist. Neuroreport 2002;13:763–6.
79. Fuxe K, O'Connor WT, Antonelli T, et al. Evidence for a substrate of neuronal plasticity based on pre- and postsynaptic neurotensin-dopamine receptor interactions in the neostriatum. Proc Natl Acad Sci USA 1992;89:5591–5.

80. Fuxe K, Von Euler G, Agnati LF, et al. Intramembrane interactions between neurotensin receptors and dopamine D2 receptors as a major mechanism for the neuroleptic-like action of neurotensin. Ann N Y Acad Sci 1992;668:186–204.
81. Tanganelli S, Li XM, Ferraro L, et al. Neurotensin and cholecystokinin octapeptide control synergistically dopamine release and dopamine D2 receptor affinity in rat neostriatum. Eur J Pharmacol 1993;230:159–66.
82. von Euler G. Biochemical characterization of the intramembrane interaction between neurotensin and dopamine D2 receptors in the rat brain. Brain Res 1991;561:93–8.
83. Von Euler G, Fuxe K. Neurotensin reduces the affinity of D-2 dopamine receptors in rat striatal membranes. Acta Physiol Scand 1987;131:625–6.
84. von Euler G, van der Ploeg I, Fredholm BB, Fuxe K. Neurotensin decreases the affinity of dopamine D2 agonist binding by a G protein-independent mechanism. J Neurochem 1991;56:178–83.
85. Li XM, Hedlund PB, Fuxe K. Strong effects of NT/NN peptides on DA D2 receptors in rat neostriatal sections. Neuroreport 1994;5:1621–4.
86. Li XM, Ferraro L, Tanganelli S, et al. Neurotensin peptides antagonistically regulate postsynaptic dopamine D2 receptors in rat nucleus accumbens: a receptor binding and microdialysis study. J Neural Transm Gen Sect 1995;102:125–37.
87. Tanganelli S, von Euler G, Fuxe K, Agnati LF, Ungerstedt U. Neurotensin counteracts apomorphine-induced inhibition of dopamine release as studied by microdialysis in rat neostriatum. Brain Res 1989;502:319–24.
88. Canals M, Marcellino D, Fanelli F, et al. Adenosine A2A-dopamine D2 receptor-receptor heteromerization: qualitative and quantitative assessment by fluorescence and bioluminescence energy transfer. J Biol Chem 2003;278:46741–9.
89. Ciruela F, Burgueno J, Casado V, et al. Combining mass spectrometry and pull-down techniques for the study of receptor heteromerization. Direct epitope-epitope electrostatic interactions between adenosine A2A and dopamine D2 receptors. Anal Chem 2004;76:5354–63.
90. Hillion J, Canals M, Torvinen M, et al. Coaggregation, cointernalization, and codesensitization of adenosine A2A receptors and dopamine D2 receptors. J Biol Chem 2002;277:18091–7.
91. Kamiya T, Saitoh O, Yoshioka K, Nakata H. Oligomerization of adenosine A2A and dopamine D2 receptors in living cells. Biochem Biophys Res Commun 2003;306:544–9.
92. Woods AS, Ciruela F, Fuxe K, et al. Role of electrostatic interaction in receptor-receptor heteromerization. J Mol Neurosci 2005;26:125–32.
93. Torvinen M, Kozell LB, Neve KA, Agnati LF, Fuxe K. Biochemical identification of the dopamine D2 receptor domains interacting with the adenosine A2A receptor. J Mol Neurosci 2004;24:173–80.
94. Ferre S, Fuxe K. Dopamine denervation leads to an increase in the intramembrane interaction between adenosine A2 and dopamine D2 receptors in the neostriatum. Brain Res 1992;594:124–30.
95. Ferre S, von Euler G, Johansson B, Fredholm BB, Fuxe K. Stimulation of high-affinity adenosine A2 receptors decreases the affinity of dopamine D2 receptors in rat striatal membranes. Proc Natl Acad Sci USA 1991;88:7238–41.
96. Ferre S, O'Connor WT, Snaprud P, Ungerstedt U, Fuxe K. Antagonistic interaction between adenosine A2A receptors and dopamine D2 receptors in the ventral striopallidal system. Implications for the treatment of schizophrenia. Neuroscience 1994;63:765–73.
97. Filip M, Frankowska M, Zaniewska M, et al. Involvement of adenosine A2A and dopamine receptors in the locomotor and sensitizing effects of cocaine. Brain Res 2006;1077:67–80.
98. Marcellino D, Roberts DC, Navarro G, et al. Increase in A2A receptors in the nucleus accumbens after extended cocaine self-administration and its disappearance after cocaine withdrawal. Brain Res 2007;1143:208–20.

99. Bara-Jimenez W, Sherzai A, Dimitrova T, et al. Adenosine A(2A) receptor antagonist treatment of Parkinson's disease. Neurology 2003;61:293–6.
100. Fuxe K, Dahlstrom A, Hoistad M, et al. From the Golgi-Cajal mapping to the transmitter-based characterization of the neuronal networks leading to two modes of brain communication: wiring and volume transmission. Brain Res Rev 2007;55:17–54.
101. Rimondini R, Ferre S, Gimenez-Llort L, Ogren SO, Fuxe K. Differential effects of selective adenosine A1 and A2A receptor agonists on dopamine receptor agonist-induced behavioural responses in rats. Eur J Pharmacol 1998;347:153–8.
102. Rimondini R, Ferre S, Ogren SO, Fuxe K. Adenosine A2A agonists: a potential new type of atypical antipsychotic. Neuropsychopharmacology 1997;17:82–91.
103. Tanganelli S, Sandager Nielsen K, Ferraro L, et al. Striatal plasticity at the network level. Focus on adenosine A2A and D2 interactions in models of Parkinson's Disease. Parkinsonism Relat Disord 2004;10:273–80.
104. Ferre S, Karcz-Kubicha M, Hope BT, et al. Synergistic interaction between adenosine A2A and glutamate mGlu5 receptors: implications for striatal neuronal function. Proc Natl Acad Sci USA 2002;99:11940–5.
105. Ferre S, Popoli P, Rimondini R, Reggio R, Kehr J, Fuxe K. Adenosine A2A and group I metabotropic glutamate receptors synergistically modulate the binding characteristics of dopamine D2 receptors in the rat striatum. Neuropharmacology 1999;38:129–40.
106. Fuxe K, Celani MF, Martire M, Zini I, Zoli M, Agnati LF. l-Glutamate reduces the affinity of [3H]N-propylnorapomorphine binding sites in striatal membranes. Eur J Pharmacol 1984;100:127–30.
107. Popoli P, Pezzola A, Torvinen M, et al. The selective mGlu(5) receptor agonist CHPG inhibits quinpirole-induced turning in 6-hydroxydopamine-lesioned rats and modulates the binding characteristics of dopamine D(2) receptors in the rat striatum: interactions with adenosine A(2a) receptors. Neuropsychopharmacology 2001;25:505–13.
108. Binda AV, Kabbani N, Lin R, Levenson R. D2 and D3 dopamine receptor cell surface localization mediated by interaction with protein 4.1 N. Mol Pharmacol 2002;62:507–13.
109. Li M, Bermak JC, Wang ZW, Zhou QY. Modulation of dopamine D(2) receptor signaling by actin-binding protein (ABP-280). Mol Pharmacol 2000;57:446–52.
110. Lin R, Karpa K, Kabbani N, Goldman-Rakic P, Levenson R. Dopamine D2 and D3 receptors are linked to the actin cytoskeleton via interaction with filamin A. Proc Natl Acad Sci USA 2001;98:5258–63.
111. Smith FD, Oxford GS, Milgram SL. Association of the D2 dopamine receptor third cytoplasmic loop with spinophilin, a protein phosphatase-1-interacting protein. J Biol Chem 1999;274:19894–900.
112. Bockaert J, Roussignol G, Becamel C, et al. GPCR-interacting proteins (GIPs): nature and functions. Biochem Soc Trans 2004;32:851–5.
113. Yao WD, Gainetdinov RR, Arbuckle MI, et al. Identification of PSD-95 as a regulator of dopamine-mediated synaptic and behavioral plasticity. Neuron 2004;41:625–38.
114. Aarts M, Liu Y, Liu L, et al. Treatment of ischemic brain damage by perturbing NMDA receptor- PSD-95 protein interactions. Science 2002;298:846–50.
115. Torvinen M, Marcellino D, Canals M, et al. Adenosine A2A receptor and dopamine D3 receptor interactions: evidence of functional A2A/D3 heteromeric complexes. Mol Pharmacol 2005;67:400–7.
116. Schwartz JC, Diaz J, Pilon C, Sokoloff P. Possible implications of the dopamine D(3) receptor in schizophrenia and in antipsychotic drug actions. Brain Res Brain Res Rev 2000;31:277–87.
117. Kearn CS, Blake-Palmer K, Daniel E, Mackie K, Glass M. Concurrent stimulation of cannabinoid CB1 and dopamine D2 receptors enhances heterodimer formation: a mechanism for receptor cross-talk? Mol Pharmacol 2005;67:1697–704.
118. Marcellino D, Carriba P, Filip M, et al. Antagonistic cannabinoid CB(1)/dopamine D(2) receptor interactions in striatal CB(1)/D(2) heteromers. A combined neurochemical and behavioral analysis. Neuropharmacology 2008;54(5):815–23.

119. Pickel VM, Chan J, Kearn CS, Mackie K. Targeting dopamine D2 and cannabinoid-1 (CB1) receptors in rat nucleus accumbens. J Comp Neurol 2006;495:299–313.
120. Uchigashima M, Narushima M, Fukaya M, Katona I, Kano M, Watanabe M. Subcellular arrangement of molecules for 2-arachidonoyl-glycerol-mediated retrograde signaling and its physiological contribution to synaptic modulation in the striatum. J Neurosci 2007;27: 3663–76.
121. Carriba P, Ortiz O, Patkar K, et al. Striatal adenosine A2A and cannabinoid CB1 receptors form functional heteromeric complexes that mediate the motor effects of cannabinoids. Neuropsychopharmacology 2007;32:2249–59.
122. Andersson M, Usiello A, Borgkvist A, et al. Cannabinoid action depends on phosphorylation of dopamine- and cAMP-regulated phosphoprotein of 32 kDa at the protein kinase A site in striatal projection neurons. J Neurosci 2005;25:8432–8.
123. Borgkvist A, Marcellino D, Fuxe K, Greengard P, Fisone G. Regulation of DARPP-32 phosphorylation by Delta9-tetrahydrocannabinol. Neuropharmacology 2008;54:31–5.
124. Mukhopadhyay S, McIntosh HH, Houston DB, Howlett AC. The CB(1) cannabinoid receptor juxtamembrane C-terminal peptide confers activation to specific G proteins in brain. Mol Pharmacol 2000;57:162–70.
125. Franco R, Lluis C, Canela EI, et al. Receptor-receptor interactions involving adenosine A1 or dopamine D1 receptors and accessory proteins. J Neural Transm 2007;114: 93–104.
126. Fuxe K, Ferre S, Genedani S, Franco R, Agnati LF. Adenosine receptor-dopamine receptor interactions in the basal ganglia and their relevance for brain function. Physiol Behav 2007;92:210–7.
127. Gines S, Hillion J, Torvinen M, et al. Dopamine D1 and adenosine A1 receptors form functionally interacting heteromeric complexes. Proc Natl Acad Sci USA 2000;97: 8606–11.
128. Torvinen M, Gines S, Hillion J, et al. Interactions among adenosine deaminase, adenosine A(1) receptors and dopamine D(1) receptors in stably cotransfected fibroblast cells and neurons. Neuroscience 2002;113:709–19.
129. Ferre S, Popoli P, Gimenez-Llort L, et al. Postsynaptic antagonistic interaction between adenosine A1 and dopamine D1 receptors. Neuroreport 1994;6:73–6.
130. Ferre S, Rimondini R, Popoli P, et al. Stimulation of adenosine A1 receptors attenuates dopamine D1 receptor-mediated increase of NGFI-A, c-fos and jun-B mRNA levels in the dopamine-denervated striatum and dopamine D1 receptor-mediated turning behaviour. Eur J Neurosci 1999;11:3884–92.
131. Ferre S, Fredholm BB, Morelli M, Popoli P, Fuxe K. Adenosine-dopamine receptor-receptor interactions as an integrative mechanism in the basal ganglia. Trends Neurosci 1997;20: 482–7.
132. Juhasz JR, Hasbi A, Rashid AJ, So CH, George SR, O'Dowd BF. Mu-opioid receptor heterooligomer formation with the dopamine D(1) receptor as directly visualized in living cells. Eur J Pharmacol 2008;581:235–43.
133. Lee FJ, Xue S, Pei L, et al. Dual regulation of NMDA receptor functions by direct protein-protein interactions with the dopamine D1 receptor. Cell 2002;111:219–30.
134. Pei L, Lee FJ, Moszczynska A, Vukusic B, Liu F. Regulation of dopamine D1 receptor function by physical interaction with the NMDA receptors. J Neurosci 2004;24:1149–58.
135. Fiorentini C, Gardoni F, Spano P, Di Luca M, Missale C. Regulation of dopamine D1 receptor trafficking and desensitization by oligomerization with glutamate N-methyl-D-aspartate receptors. J Biol Chem 2003;278:20196–202.
136. Flores-Hernandez J, Cepeda C, Hernandez-Echeagaray E, et al. Dopamine enhancement of NMDA currents in dissociated medium-sized striatal neurons: role of D1 receptors and DARPP-32. J Neurophysiol 2002;88:3010–20.
137. Snyder GL, Fienberg AA, Huganir RL, Greengard P. A dopamine/D1 receptor/protein kinase A/dopamine- and cAMP-regulated phosphoprotein (Mr 32 kDa)/protein phosphatase-1 pathway regulates dephosphorylation of the NMDA receptor. J Neurosci 1998;18:10297–303.

138. Scott DB, Blanpied TA, Swanson GT, Zhang C, Ehlers MD. An NMDA receptor ER retention signal regulated by phosphorylation and alternative splicing. J Neurosci 2001;21: 3063–72.
139. Dunah AW, Standaert DG. Dopamine D1 receptor-dependent trafficking of striatal NMDA glutamate receptors to the postsynaptic membrane. J Neurosci 2001;21:5546–58.
140. Liu XY, Chu XP, Mao LM, et al. Modulation of D2R-NR2B interactions in response to cocaine. Neuron 2006;52:897–909.
141. Jackson SN, Wang HY, Yergey A, Woods AS. Phosphate stabilization of intermolecular interactions. J Proteome Res 2006;5:122–6.
142. Liu F, Wan Q, Pristupa ZB, Yu XM, Wang YT, Niznik HB. Direct protein-protein coupling enables cross-talk between dopamine D5 and gamma-aminobutyric acid A receptors. Nature 2000;403:274–80.
143. Wang Q, Liu L, Pei L, et al. Control of synaptic strength, a novel function of Akt. Neuron 2003;38:915–28.
144. Rivera A, Alberti I, Martin AB, Narvaez JA, de la Calle A, Moratalla R. Molecular phenotype of rat striatal neurons expressing the dopamine D5 receptor subtype. Eur J Neurosci 2002;16:2049–58.
145. Flores-Hernandez J, Hernandez S, Snyder GL, et al. D(1) dopamine receptor activation reduces GABA(A) receptor currents in neostriatal neurons through a PKA/DARPP-32/PP1 signaling cascade. J Neurophysiol 2000;83:2996–3004.
146. Perez de la Mora M, Ferre S, Fuxe K. GABA-dopamine receptor-receptor interactions in neostriatal membranes of the rat. Neurochem Res 1997;22:1051–4.
147. Seamans JK, Gorelova N, Durstewitz D, Yang CR. Bidirectional dopamine modulation of GABAergic inhibition in prefrontal cortical pyramidal neurons. J Neurosci 2001;21: 3628–38.
148. Wang C, Buck DC, Yang R, Macey TA, Neve KA. Dopamine D2 receptor stimulation of mitogen-activated protein kinases mediated by cell type-dependent transactivation of receptor tyrosine kinases. J Neurochem 2005;93:899–909.
149. Maudsley S, Pierce KL, Zamah AM, et al. The beta(2)-adrenergic receptor mediates extracellular signal-regulated kinase activation via assembly of a multi-receptor complex with the epidermal growth factor receptor. J Biol Chem 2000;275:9572–80.
150. Wetzker R, Bohmer FD. Transactivation joins multiple tracks to the ERK/MAPK cascade. Nat Rev Mol Cell Biol 2003;4:651–7.
151. Fenimore PW, Frauenfelder H, McMahon BH, Parak FG. Slaving: solvent fluctuations dominate protein dynamics and functions. Proc Natl Acad Sci USA 2002;99:16047–51.
152. Williams DH, O'Brien DP, Sandercock AM, Stephens E. Order changes within receptor systems upon ligand binding: receptor tightening/oligomerisation and the interpretation of binding parameters. J Mol Biol 2004;340:373–83.
153. Girault JA, Greengard P. The neurobiology of dopamine signaling. Arch Neurol 2004;61:641–4.
154. Greengard P, Nairn AC, Girault JA, et al. The DARPP-32/protein phosphatase-1 cascade: a model for signal integration. Brain Res Brain Res Rev 1998;26:274–84.

Chapter 11
Dopamine Receptor Modulation of Glutamatergic Neurotransmission

Carlos Cepeda, Véronique M. André, Emily L. Jocoy, and Michael S. Levine

Abstract Dopamine (DA), a prominent neuromodulator in the brain, regulates neuronal excitability and synaptic transmission. These actions are effected through diverse DA receptor subtypes whose effects vary as a function of a number of factors including pre- or postsynaptic localization and the intracellular signaling cascades they activate. We have chosen the corticostriatal synapse as a model to study the interactions between DA and glutamate, the major excitatory neurotransmitter for striatal inputs. In the striatum, DA receptors modulate glutamate release via presynaptic mechanisms and synaptic responses mediated by activation of postsynaptic glutamate receptors through alterations in voltage-gated channels, phosphorylation of glutamate receptor subunits, as well as physical interactions with other receptors. The outcomes of these actions are diverse and can lead to opposite or synergistic effects. These multiple effects are important to keep the balance between striatal output pathways to coordinate sensorimotor integration.

Keywords Dopamine receptors · Glutamate · Interactions · NMDA

11.1 Introduction

Dopamine (DA) and glutamate receptor interactions provide a necessary and important substrate for numerous brain functions. Ionotropic glutamate receptors mediate fast excitatory neurotransmission and DA receptors modulate neuronal excitability and the actions of multiple neurotransmitter systems. Alterations in the density and/or sensitivity of these receptors occur in pathological conditions such as Parkinson's (PD) and Huntington's diseases (HD), schizophrenia, and attention deficit hyperactivity disorder, to name a few.

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DA and glutamate receptor interactions are complex and their outcomes depend on multiple factors including receptor subtype, site of action (i.e., pre- or postsynaptic), timing of inputs, and concentration of neurotransmitter. After the discovery of different subtypes of glutamate and DA receptors, the number of potential interactions and their mechanisms has multiplied because glutamate and DA receptor subtypes elicit their actions by using multiple signaling pathways. Thus, the outcomes of interactions between these receptor families can be very diverse.

Recently, exciting findings have added new levels of complexity to these already intricate systems. For example, in addition to intracellular interactions via second messenger transduction pathways, the presence of physical interactions between glutamate and DA receptors at the membrane and cytoplasm levels has been revealed. Furthermore, the generation of mice deficient in specific DA receptors or glutamate receptor subunits and also of mice expressing enhanced green fluorescent protein (EGFP) under the control of specific DA receptor subtype promoters has provided new tools for the study of the relationships between DA and glutamate receptors. In this chapter we have chosen the corticostriatal synapse as a model to study glutamate and DA receptor interactions because of their relative abundance and because of their important implications in normal function and in pathological states involving the basal ganglia.

11.2 Classification of DA and Glutamate Receptors

There is diversity among DA receptors. Five different receptor subtypes have been cloned. These have been classified into two main families: the D1-like (D_1 and D_5 receptor subtypes) and the D2-like (D_2, D_3, and D_4 receptor subtypes) families [1, 2]. All DA receptors are G protein coupled and primarily alter the production of 3′-5′-cyclic adenosine monophosphate (cAMP) in the cell when activated but also affect other transduction systems [3].

Glutamate receptors have been classified into two principal groups: ionotropic and metabotropic receptors. Ionotropic glutamate [α-amino-3-hydroxy-5-methyl-4-propionate (AMPA), kainate (KA), and NMDA] receptors are ligand-gated cation channels, whereas metabotropic glutamate receptors are coupled to various signal transduction systems [4–6]. NMDA receptors are unique in that their activation is governed by a strong voltage dependence due to receptor-channel blockade by Mg^{2+} at hyperpolarized membrane potentials [7]. Mg^{2+} block gives NMDA receptors their characteristic negative slope conductance. Also, NMDA receptors allow more Ca^{2+} influx compared to non-NMDA receptors.

11.3 Morphological Basis for DA and Glutamate Receptor Interactions in Striatum

The striatum is the main input structure of the basal ganglia. It is the nucleus where afferents from the cerebral cortex, thalamus, and substantia nigra converge and interact. Glutamate is released from cortical and thalamic terminals [8, 9]. DA is released

from nigrostriatal terminals [10]. Because DA and glutamate inputs terminate on the same spines of striatal medium-sized spiny neurons (MSSNs), these sites offer the potential for physiological interactions between the DA and glutamate transmitter systems [11]. Morphological evidence demonstrates the presence of synaptic complexes formed by axospinous contacts in which the dendritic spine is the target of both an asymmetric (glutamatergic) bouton and a DA-positive symmetric synapse in striatal MSSNs [12]. This arrangement provides a morphological basis for DA–glutamate receptor interactions at the synapse. These interactions in the striatum support major sensory, motor, cognitive, and motivational functions [13–16].

Although all striatal MSSNs are GABAergic, they form separate populations based on distinct anatomic projections and neuropeptide expression. There are two major output pathways. The direct pathway consists of MSSNs that predominantly express D_1 DA receptors [17], substance P [18], and dynorphin [19]. These neurons project to the substantia nigra pars reticulata and the internal segment of the globus pallidus [17, 20]. The indirect pathway is comprised of striatal neurons that express predominantly D_2 receptors [17], met-enkephalin, and neurotensin [18, 21]. These neurons project to the external segment of the globus pallidus [20, 22]. DA inputs from the substantia nigra modulate the activity of these pathways, exerting a net excitatory effect on the direct pathway and a net inhibitory effect on the indirect pathway [23]. Assuming that D_1 and D_2 receptor-expressing MSSNs are segregated, the balanced opposition of these output systems is required to produce correlated and balanced activity. The prevailing hypothesis is that direct pathway promotes desired movements [24] while the indirect pathway inhibits unwanted movements [25].

The degree of DA receptor colocalization in the striatum remains an unresolved question. In particular, the percentage of MSSNs colocalizing D_1 and D_2 receptors varies from a low of 5% to a high of 80% depending on the experimental conditions [17, 26–30]. The recent generation of mice expressing enhanced green fluorescent protein (EGFP) as a marker of D_1 and D_2 receptor-containing striatal MSSNs [31] supports the idea that these receptors are segregated to different populations. In our hands, using single-cell RT-PCR in EGFP-expressing MSSNs, colocalization occurs only in about 10% of neurons from dorsal striatum [32]. However, a recent report demonstrated that EGFP probably underestimates the numbers of D_1- or D_2-expressing neurons because of difficulties detecting neurons that express low titers of receptors [33]. With regard to colocalization of DA receptors with various glutamate receptor subunits, evidence has shown that a high degree of coexpression occurs in striatal neurons [29]. This provides a morphological framework for physiological interactions.

11.4 DA Receptors Modulate Neuronal Excitability by Altering Voltage-Gated Conductances

For a more exhaustive examination of how DA modulates voltage-gated channels please see Chapter 7. Here we only summarize aspects that are directly relevant for DA–glutamate receptor interactions. Electrophysiological experiments have shown

that DA reduces most of the voltage-activated inward and outward currents in striatal neurons [34–36] and these reductions have complex effects on cell excitability. For example, D_1-like receptor agonists reduce the amplitude of evoked Na^+ currents in the vast majority of cells but they also reduce a slowly inactivating K^+ current (favoring the transition to a depolarized membrane potential). In contrast, D_2-like agonists enhance this current. In addition, D1 receptor activation can produce differential effects on high-voltage-activated (HVA) Ca^{2+} currents depending on the specific type of current activated. D_1-like receptor agonists reversibly reduce N- and P-type HVA currents, probably via the cAMP–protein kinase A (PKA) transduction cascade. However, in a subset of neurons, D_1-like receptor-mediated activation enhances L-type HVA current [37]. These observations imply that DA produces differential effects on D_1 and D_2 receptor-containing MSSNs, hence affecting their excitability and readiness to respond to excitatory and inhibitory inputs. MSSNs containing D_1 receptors will respond to phasic release of DA with depolarization and increased Ca^{2+} through L-type channels. In contrast, D_2 receptor-containing neurons will remain hyperpolarized and Ca^{2+} influx will be reduced.

11.5 DA Modulation of Glutamate Release

DA receptors are present on presynaptic terminals where they can modulate neurotransmitter release [38]. In the dorsal striatum, D_2 receptors have been found on corticostriatal terminal endings and function to decrease glutamate release by presynaptic mechanisms [39]. While early pharmacological studies provided evidence that DA is capable of altering glutamate release, probably via D_2-like receptors [40–44], more recent evidence for presynaptic mechanisms was obtained using electrophysiological methods [45–48]. Studies in mice lacking D_2 receptors also provide compelling evidence that presynaptic D_2 receptors can function as gatekeepers of glutamate release, i.e., primarily preventing excessive excitation in the striatum [39]. Further, optical techniques visualizing neurotransmitter release via destaining of terminals after incorporation of FM1-43, a styryl dye, have provided definite confirmation of D_2 receptor modulation of glutamate release at corticostriatal synapses [49]. Interestingly, DA inhibition of glutamate release appears to be frequency dependent as it increases as the rates of stimulation of corticostriatal inputs increase. DA inhibition is minimal with low-frequency stimulation (1 Hz) but increases with higher rates of stimulation (20 Hz). Thus, DA acts as a low-pass filter selective for terminals with low probability of release [50]. In this way, DA released by salient stimuli can directly regulate striatal neurotransmission by selecting specific sets of corticostriatal projections [49].

DA–NMDA receptor interactions can also modulate glutamate release indirectly through retrograde mechanisms. For example, enhancement of NMDA receptor function by D_1-like receptor activation increases adenosine release which then acts as a retrograde messenger to reduce glutamate release in nucleus accumbens neurons [51]. DA also modulates excitatory transmission by means of retrograde endocannabinoid signaling in dorsal striatum [52]. Thus, confirming previous

results [49], activation of D_2 receptors reduces glutamatergic transmission only when receptor activation is combined with stimulation of cortical afferents at high frequencies (20 Hz). However, for this low-pass filtering function the authors provided evidence for a retrograde signal resulting from convergent DA and glutamate inputs that cooperate to produce endocannabinoid synthesis and release from the postsynaptic cell [52]. This retrograde signal reduces glutamate release by activation of CB_1 receptors on presynaptic terminals.

11.6 DA Modulation of Glutamate Receptor-Mediated Responses

The question whether or not DA is able to modulate directly excitatory postsynaptic responses, at least in dorsal striatum, remains controversial. While we and others observed clear DA modulation, other studies found no effect [53, 54]. We have provided possible explanations about why modulation was not observed [55–57]. One possibility is that the K^+ concentration was very low and the resting membrane potential of striatal neurons was extremely hyperpolarized. At these potentials, a number of voltage-dependent conductances may not be available for modulation. In addition, Nicola and Malenka used very young animals (<3 weeks). DA modulation at this age is minimal in the dorsal striatum. Finally, they did not use selective D_1- or D_2-like receptor agonists and they did not stimulate the white matter–cerebral cortex junction, the location which is more likely to produce glutamate receptor-mediated responses.

One problem involved in understanding the modulation of ligand-gated currents is how to tease apart indirect effects on voltage-gated conductances from those that are independent of these conductances [36]. This question may be difficult to resolve as DA activates multiple intracellular cascades that lead to phosphorylation of both glutamate receptor subunits and voltage-gated channels [58]. In the past we postulated that the most parsimonious explanation of DA's effects is the existence of redundant and cooperative actions involving both voltage- and ligand-gated conductances [56]. Regardless of the mechanisms, it has become clear that DA can alter glutamate receptor-mediated inputs as demonstrated in Sections 11.6.1, 11.6.2, 11.6.3, 11.6.4, 11.6.5, and 11.6.6. The main DA–glutamate receptor interactions are illustrated in Fig. 11.1.

11.6.1 DA and D_2-like Receptors Decrease AMPA Receptor-Mediated Responses

Early studies using extracellular recordings and iontophoresis showed that DA decreased spontaneous or glutamate-induced firing in anesthetized animals [59]. However, excitatory or mixed effects of DA were also reported [60], and these effects were hypothesized to be mediated by different DA receptors [61]. Intracellular studies in vivo demonstrated that iontophoretic application of DA inhibits spontaneous or glutamate-induced cell firing but concomitantly depolarizes

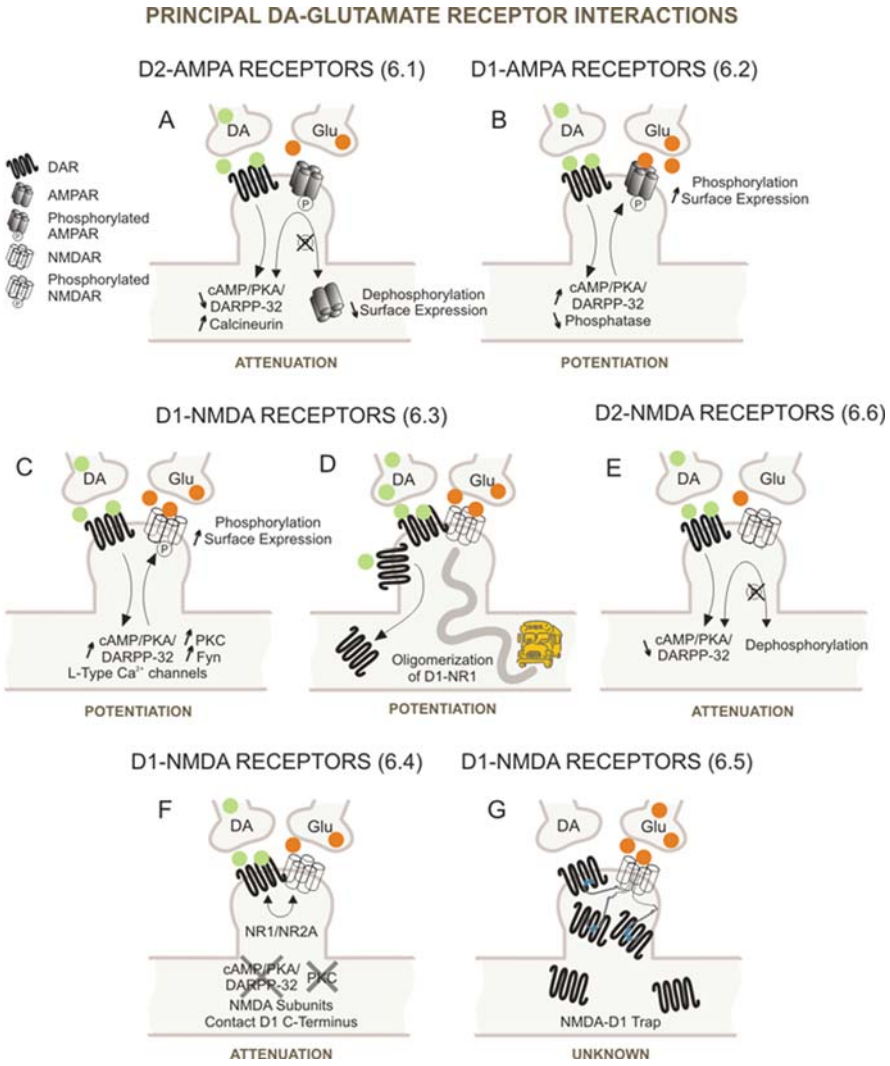

Fig. 11.1 This schematic illustrates the principal DA–glutamate receptor interactions in striatum and cerebral cortex. The numbers in parenthesis indicate the section of the chapter where each interaction is explained. The *lower part* of each diagram represents a dendritic shaft and a spine, the *upper part* represents the DA- and glutamate-releasing terminals. **A–E** are interactions primarily mediated by a number of intracellular signaling cascades. **F** and **G** are physical receptor interactions. In **D**, a D1–NR1 protein complex is formed in the cytoplasm and shuttled to the membrane surface

striatal neurons, providing evidence for both excitatory and inhibitory actions of DA [62].

Results from in vitro preparations showed that most actions of DA were inhibitory [63]. To explain the reduction in cell firing, DA was proposed to inhibit

action potentials by reducing inward rectification mediated by sodium channels. DA also reduced excitatory postsynaptic potentials but this effect was dependent on the membrane potential [63], suggesting that some actions of DA rely on alterations in voltage-gated conductances only present at depolarized membrane potentials [64].

It remained unclear whether DA could also act on postsynaptic D_2-like receptors to modulate AMPA receptor-mediated responses. More recently, activation of these receptors was indeed shown to exert inhibitory effects on AMPA responses [65, 66]. Further confirmation of direct postsynaptic modulation was obtained using an isolated cell preparation [67]. Interestingly, in these experiments the magnitude of the inhibition appeared to depend on the morphological integrity of the dendritic arbor of MSSNs and/or the amplitude of the current. Isolated cells with few and short dendrites produced small AMPA currents and modulation by the D_2-like receptor agonist quinpirole was small and inconsistent. In contrast, in cells with a more intact dendritic arbor, AMPA produced larger currents and the decrement in the current in the presence of quinpirole also was greater. Cyclothiazide, by preventing AMPA receptor desensitization, increased the amplitude of the current, and the degree of modulation by the D_2-like agonist was increased. In slices where most of the dendrites are present, the inhibitory effects of quinpirole are very consistent [67]. These findings suggest that the topography and/or subcellular distribution of glutamate and DA receptors plays an important role in the modulation of AMPA currents [29]. For example, it is possible that AMPA receptor density and subunit composition of somatic receptors differ from those found on the dendrites and spines. Morphological studies have shown that the density of AMPA and D_2 receptors is higher on spines and dendrites than on the soma [68, 69], reflecting the preferential mode of termination of cortical afferents [12].

What is the mechanism underlying D_2-like receptor modulation of AMPA currents? AMPA receptor properties result from the combination of distinct subunits (GluR1–GluR4) and their state of phosphorylation [70, 71]. A balance between kinase and phosphatase activity is also an important determinant in the regulation of AMPA receptor function [72]. In MSSNs there is evidence that D_2-like receptor activation affects intracellular Ca^{2+} concentrations [73, 74] and therefore kinase and phosphatase activity. GluR1–3 subunits of the AMPA receptor are expressed in MSSNs [75–77]. The GluR1 subunit is mainly located on dendrites where asymmetrical synapses are established [75] and is phosphorylated at Ser845 by PKA following DA application [78], enhancing AMPA currents [79, 80]. Therefore, any disruption in PKA function which favors phosphatase activity may reduce AMPA currents. D_2-like receptor activation reduces cAMP production through a G protein-mediated mechanism that also reduces the phosphorylation of the dopamine- and 3′,5′-cyclic adenosine monophosphate (cAMP)-regulated phosphoprotein of 32 kDa (DARPP-32) [81, 82], a protein involved in AMPA receptor phosphorylation in the striatum [83]. D_2-like receptor activation also could increase the activity of the protein phosphatase calcineurin, which dephosphorylates DARPP-32. Both of these processes lead to a decrease in PKA activity [82] and GluR1 phosphorylation which, in turn, could reduce AMPA currents.

11.6.2 D_1-Like Receptors Can Increase AMPA Receptor-Mediated Responses

Modulation of AMPA channels by D1-like receptors is relevant to striatal function because it is another mechanism by which the efficacy of glutamatergic neurotransmission can be regulated. In our original study on DA–glutamate receptor interactions in slices, we demonstrated that AMPA receptor-mediated responses are generally potentiated by concurrent activation of D_1-like receptors [65]. Activation of D_1-like receptors in cultured striatal neurons also promoted phosphorylation of AMPA receptors by PKA as well as potentiation of current amplitude [84]. Similarly, in acutely isolated MSSNs, activation of D_1-like receptors stabilized AMPA currents by preventing the rundown that is observed during repeated applications of the agonist [83]. This effect was explained by increased phosphorylation and decreased dephosphorylation of AMPA channels that required inhibition of PP-1 activity by phosphorylated DARPP-32 [83].

In MSSNs from nucleus accumbens, incubation with a D_1-like receptor agonist increased surface expression of GluR1 subunits [85, 86]. This effect has important functional consequences as increased surface expression of AMPA receptors would enhance AMPA receptor transmission, a finding supported by in vivo experiments recorded from MSSNs [87].

11.6.3 DA and D_1-Like Receptor Activation Enhances NMDA Receptor-Mediated Responses

DA and D_1-like receptor-mediated potentiation of NMDA responses was first described in human cortex and rodent striatum 15 years ago [65, 88]. Since then, with only a few notable exceptions [53, 54], this enhancement has been verified in these and other brain structures [51, 55–57, 65, 66, 84, 88–99]. D_1-like receptor enhancement of NMDA responses can be mediated by a number of redundant and cooperative signaling cascades in the striatum [55, 100]. The most prominent involve PKA and DARPP-32 [56, 89, 94], phosphorylation of NMDA receptor NR1 subunits [101], and activation of voltage-gated Ca^{2+} channels, particularly L-type channels [90, 97]. In other cerebral regions different mechanisms may be in place. For example, in nucleus accumbens NMDA receptor potentiation by phospholipase C-coupled D_1-like receptors occurs via protein kinase C (PKC) activation [93]. Similarly, in cortical pyramidal neurons intracellular application of the Ca^{2+} chelator, calmodulin, or inhibition of PKC activity significantly reduces the potentiation of NMDA currents, indicating that this interaction can occur independent of PKA [92].

Activation of D_1 receptors can alter the surface distribution of NMDA receptors [78, 102]. For example, D_1 receptor activation produces an increase in NR1, NR2A, and NR2B proteins in the synaptosomal membrane fraction [103] that is dependent on Fyn protein tyrosine kinase but not DARPP-32 [104]. Based on the fact

that NMDA and D_1 receptors partially overlap in dendritic spines, protein–protein interactions might direct the trafficking of D_1 and NMDA receptors to the same subcellular domain.

The mechanism by which D_1 receptors are delivered to different spine domains has been examined in co-immunoprecipitation studies [105]. In the striatal postsynaptic density, the D_1 receptor selectively complexes with the NR1 subunit of the NMDA channel through its C-terminus. The physical proximity between D_1 receptors and NR1 subunits can be explained by the formation of constitutive protein dimers. Oligomerization with the NMDA receptor thus regulates D_1 receptor targeting to the plasma membrane. When the D_1 receptor and the NR1 subunit are coexpressed in HEK293 cells, the D_1 receptor is only partially targeted to the cell membrane, with the majority of D_1 receptor staining retained in cytoplasmic structures where it is colocalized with NR1. Coexpression of the D_1 receptor with both the NR1 and NR2B subunits relieves the cytoplasmic retention of the complex, allowing insertion of both the NR1 subunit and the D_1 receptor at the plasma membrane, where they are completely colocalized. These data suggest that D_1 and NMDA receptors are assembled as oligomeric units in the endoplasmic reticulum and transported to the cell surface as a preformed complex [105]. This implies that a direct protein–protein interaction with the NMDA receptor is one of the mechanisms directing the trafficking of D_1 receptors to specific subcellular compartments. Furthermore, this direct interaction may be crucial to recruit the D_1 receptor to the place where synaptic activity is occurring and to keep it in close proximity with the NMDA receptor to allow rapid cAMP–PKA–DARPP-32-mediated potentiation of NMDA transmission [105].

11.6.4 D1-Like Receptor Activation Can Depress NMDA Responses by Physical Receptor Interactions

In specific circumstances D_1-like receptor activation can lead to reduction of NMDA responses. In addition to regulating subcellular localization, the physical interactions between these receptors also allow cross talk via receptor linkages. The C-terminus of the D_1 receptor physically interacts with both NR1 and NR2A NMDA receptor subunits [106]. This protein–protein interaction has functional relevance because D_1-like receptor activation decreases NMDA currents when PKA and PKC activations are blocked. The reduction of NMDA currents is caused by the interaction of D_1 receptors with the NR2A subunit and occurs through a decrease in the number of cell surface receptors [106]. The D_1 interaction with the NR1 subunit has been implicated in the attenuation of NMDA receptor-mediated excitotoxicity through a phosphatidylinositol 3-kinase-dependent pathway. The D_1–NR1 interaction also enables NMDA receptor activation to increase membrane insertion of D_1 receptors [107].

The observation that physical receptor–receptor interactions reduce NMDA currents when second messenger pathways are blocked has been complicated by the demonstration that other mechanisms independent of D_1-like receptor activation

can produce similar effects. A recent study revealed that one mechanism underlying reduction of NMDA currents is direct channel pore block of NMDA receptors by DA and several D_1-like receptor ligands [108]. Thus, without excluding the possibility that receptor–receptor interactions may lead to functional modulation, the inhibitory effects of DA or its agonists and antagonists require further examination since they may also directly occlude the channel.

11.6.5 The NMDA–D_1 Receptor Trap

In primary cultures of striatal neurons, activation of NMDA receptors increases the recruitment of D_1 but not D_2 receptors into the plasma membrane [109]. This translocation is abolished in the presence of an NMDA receptor antagonist or by removing Ca^{2+}. In addition, after NMDA treatment, a dramatic increase in the number of D_1 receptor-containing spines occurs. The translocation of D_1 receptors to the plasma membrane has been confirmed in subcellular fractionation experiments using slices of adult rat striatum. Furthermore, in organotypic cultures from rat striatum application of NMDA causes an increase in D_1 receptor-positive spines [110]. Surprisingly, under these conditions, this effect is independent of Ca^{2+} and also occurs in the presence of Mg^{2+} indicating that, in addition to the Ca^{2+}-dependent recruitment of D_1 receptors by activation of NMDA receptors seen in primary cultures, other NMDA receptor-dependent mechanisms can cause redistribution of D_1 receptors to spines. This, according to the authors, is achieved by a diffusion-trap mechanism in which subsets of D_1 receptors that typically move by lateral diffusion in the plasma membrane get trapped in the spines when NMDA binds to its receptor. Exposure to NMDA reduces the diffusion rate of D_1 receptors and allows the formation of D_1–NMDA heteroreceptor complexes. This process could be explained by the allosteric theory of receptor activation [111]. After ligand binding, one conformation of the receptor is stabilized, shifting the equilibrium toward this state, so that occupation of the binding site of the NMDA receptor favors a conformation that will bind to D_1 receptors and stabilizes them in spines. Thus, D_1 and NMDA receptor heteromers can be formed both constitutively prior to insertion of the receptors in the membrane [105] and in a NMDA receptor-regulated manner within the membranes of dendritic spines [110].

11.6.6 DA, via D_2-Like Receptors, Reduces NMDA Receptor-Mediated Responses

In contrast to the enhancing effects of D_1-like receptors on NMDA receptor-mediated responses, D_2-like receptor activation leads to inhibitory effects [65, 66]. This may be relevant to preventing excessive activation of NMDA receptors and its consequent Ca^{2+} accumulation which could be deleterious for the neuron. For example, DA and the D_1-like receptor agonist SKF38393 increase the magnitude of

NMDA-induced cell swelling, an index of excitotoxicity [112, 113]. This effect is reduced in the presence of the D_1-like receptor antagonist SCH23390 demonstrating specificity. In contrast, activation of D_2-like receptors with quinpirole results in decreased cell swelling [112]. These results provide evidence that DA receptors have the potential to modulate excitotoxicity in the striatum, a process that has been suggested to be responsible for cell dysfunction and, ultimately, cell death as occurs in HD.

Compared to D_1-like–NMDA receptor interactions, much less is known about the mechanisms by which D_2-like receptor activation leads to reduction of NMDA currents. Decreased cAMP production and PKA activity are certainly potential mechanisms. D_2-like receptors can also modulate neuronal excitability by activating the PLC–IP_3–Ca^{2+} cascade [73]. However, at least in cortical pyramidal neurons, D_2-like receptor attenuation of NMDA responses does not require intracellular Ca^{2+} or PKA inhibition but requires activation of $GABA_A$ receptors, suggesting that this effect is mediated through excitation of GABA interneurons [97].

D_4 receptors are abundant in the prefrontal cortex [114] and may play an important role in schizophrenia and other psychiatric disorders [115]. Mice without D_4 receptors show signs of hyperexcitability [116]. Application of a D_4 receptor agonist produces a decrease of NMDA currents via inhibition of PKA, activation of PP1, and the consequent inhibition of Ca^{2+}–calmodulin-dependent kinase II (CaMKII) [117]. In CA1 pyramidal neurons quinpirole depresses excitatory transmission mediated by NMDA receptors by increasing release of intracellular Ca^{2+}. This depression is dependent on transactivation of platelet-derived growth factor β by D_4 receptors [118]. In prefrontal cortical neurons similar effects were found but they were mediated by $D_{2/3}$ receptors [119].

Physical coupling between D_2 receptors and NR2B subunits can also reduce NMDA currents [120]. The mechanism underlying this effect involves disruption of the association between NR2B and CaMKII, thereby reducing subunit phosphorylation. It is believed that the D_2–NR2B interaction plays a critical role in the stimulative effect of cocaine [120].

11.7 Genetic Manipulations of DA–Glutamate Receptor Interactions

The generation of mice lacking specific receptors or receptor subunits using genetic engineering marked a new era in the study of receptor function. These techniques have permitted the development of mice deficient in selective DA receptors or NMDA receptor subunits. Previous studies demonstrated that in D_1 receptor-deficient mice DA potentiation of striatal NMDA responses was greatly reduced [66]. Similarly, glutamate release along the corticostriatal pathway was enhanced in D_2 receptor knockout animals [39]. We are examining the enhancement of NMDA currents in mice lacking NR2A subunits [121]. In preliminary studies, D_1-like receptor modulation of these currents is similar in MSSNs from NR2A knockout mice and their controls. Additionally we examined D_2-like receptor attenuation of

NMDA responses in these mice and again found no statistically significant differences in modulation. Taken together, these results suggest that the presence or absence of the NR2A subunit does not affect D1-like or D2-like receptor modulation of NMDA receptor-mediated currents. These studies are relevant to DA–NMDA interactions as modulation of NMDA currents by DA receptors may be mediated by phosphorylation of specific receptor subunits or by physical coupling. Further, recent evidence indicates that specific NMDA receptor subunits may play different roles in synaptic plasticity and excitotoxicity [122–124].

Recently, mice that express EGFP reporter genes in a variety of cells have been generated [31]. In particular, mice that express specific DA receptor subtypes represent an important tool to differentiate neuronal subpopulations within the striatum [32]. Although in MSSNs DA or its agonists almost always modulate responses induced by NMDA receptor activation, the magnitude of this modulation varies from cell to cell. This is likely due to the fact that D_1 and D_2 receptors are largely segregated in different populations of MSSNs.

We currently are examining DA–NMDA receptor interactions in acutely dissociated D_1 and D_2 EGFP-positive MSSNs. Application of the D1-like receptor agonist SKF81297 dose-dependently and reversibly increased NMDA currents in D_1 but not in D_2 cells. NMDA current enhancement was prevented by the D1-like receptor antagonist SCH23390 (data not published). In contrast, quinpirole dose-dependently and reversibly decreased NMDA currents in D_2 but not in D_1 cells and the effect was blocked by the D2-like receptor antagonist remoxipride. At the highest concentration quinpirole also induced decreases of NMDA currents in about 25% of D_1 cells, suggesting colocalization of D_1 and D_2 receptors in a subset of MSSNs [125].

11.8 A Model of Striatal DA–Glutamate Receptor Interactions

According to the classical model, the basal ganglia in conjunction with thalamocortical circuits can be viewed as components of multiple parallel, segregated circuits [24]. Behavioral coordination is achieved by balanced activity within the direct and indirect pathway MSSNs that is regulated by differential actions of DA [23]. DA acting on D1-like receptors increases activity along the direct pathway but acting on D2-like receptors it reduces activity along the indirect pathway [23]. However, strong electrophysiological evidence for these actions of DA has been missing. Our findings demonstrating differential effects of DA based on the DA and glutamate receptor subtypes preferentially activated have provided a framework to begin to explain several assumptions of the model. In this framework, some interactions are highly predictable whereas others are less predictable. Inhibitory effects of DA are very predictable when high-affinity D2-like receptors are activated, as during tonic release of DA. In these conditions glutamate release is reduced and AMPA receptor-mediated responses are depressed. Such effects could induce elimination of specific behaviors. In contrast, facilitatory actions are very predictable when low-affinity D1-like receptors are activated, as occurs during phasic release of DA [126]. Under these conditions NMDA receptor-mediated responses are enhanced

and would promote synaptic plasticity and production of specific behaviors. A caveat of this model is that the segregation of D1- and D2-like receptors is not absolute and a number of MSSNs colocalize both receptors and/or send projections to the direct and indirect pathways [28, 127]. However, since the proportion of DA receptor colocalization may be very low, these caveats do not jeopardize the general validity of the model.

11.9 Functional Relevance of DA–Glutamate Receptor Interactions

The ultimate outcome of DA–glutamate receptor interactions will depend on a number of factors including temporal and topographic aspects [55, 100]. The outcome of activation of interacting receptors may depend heavily on the temporal sequence of neurotransmitter release. For example, activation of D1-like receptors due to DA release caused by unexpected reward can prime particular corticostriatal synapses and recruit D_1–NMDA receptor complexes in a more regulated manner [105] to induce rapid potentiation of glutamatergic corticostriatal synapses. Furthermore, timing is an important requirement for this type of synaptic plasticity because DA release should occur before excitatory afferents are activated in order to induce the potentiation [128]. Massive DA release due to unexpected reward enhances the relevance of the stimulus by potentiating NMDA responses. This process is particularly important in MSSNs enriched with D_1 receptors that promote approach behaviors.

DA concentration and the mode of release are also important. Phasic release may produce different effects than tonic release. MSSNs are constantly bombarded by cortical and thalamic inputs, and tonic release of DA filters a sizable percentage of these glutamatergic inputs through D_2 receptors located on presynaptic terminals [45, 49]. Higher local concentrations of DA, which occur when it is phasically released [126], are likely to activate D1 receptors and enhance selected corticostriatal synapses.

Activation of D2-like receptors reduces glutamate release and the amplitude of the excitatory postsynaptic potential by pre- and postsynaptic mechanisms [39, 45, 46, 67]. It is plausible that D2-like receptor stimulation could counter or prevent exacerbated excitation of MSSNs induced by activation of D1-like receptors. This is important in terms of the physiological and pathological role of glutamatergic inputs in the striatum [112]. There are several lines of evidence showing that antagonists of NMDA and AMPA receptors have anti-parkinsonian effects, essentially by attenuating the imbalance between the DA and glutamate pathways within the basal ganglia network [129]. A possible function of D2-like receptor activation, both pre- and postsynaptically, could be to prevent a surge of glutamatergic activity that would otherwise be deleterious to MSSNs.

In neurodegenerative diseases DA–NMDA interactions also play an important role as unregulated enhancement of excitation, particularly excitation mediated by NMDA receptors, will cause neuronal dysfunction and disturb structural neuronal integrity. For example, the excitotoxicity hypothesis of HD posits that excessive

glutamate release at the corticostriatal terminal or altered sensitivity of postsynaptic NMDA receptors and their signaling systems can induce cell death [130]. Studies in genetic mouse models of HD have confirmed increased sensitivity of NMDA receptors in MSSNs [131–133]. However, the precise location of NMDA receptors in synaptic or extrasynaptic compartments determines the outcome of receptor activation. In hippocampal neurons, activation of synaptic NMDA receptors triggers an anti-apoptotic pathway, whereas activation of extrasynaptic NMDA receptors may cause cell death [134].

Assuming that activation of NMDA receptors recruits more functional D_1 receptors into the plasma membrane [107, 109, 110] and that these D_1 receptors in turn recruit more NMDA receptors [103], a positive feedback mechanism can be created and the outcome of these interactions can be deleterious for the neuron if it is not stopped [135]. Both D_1 and NMDA receptors independently exert toxic effects on striatal neurons. In addition, D1-like receptor activation also potentiates NMDA toxicity [112]. Thus, a number of protective mechanisms need to be present to prevent excessive D1-like–NMDA receptor stimulation. Activation of D2-like receptors could be neuroprotective as it reduces NMDA responses [112, 136]. Other mechanisms can also be considered. For example, the physical interaction between D_1 and NMDA receptors, independent of cAMP production, and the D_2–NR2B interaction both reduce NMDA currents and excitotoxicity [106]. The diffusion-trap system could also represent a fast and efficient way to prevent excessive potentiation of NMDA responses if it makes the D_1 receptor less functional, a conclusion that remains to be verified.

Finally, DA–glutamate receptor interactions also could be important to gain a better understanding of PD. For example, a recent report indicated that loss of spines in models of PD selectively affects D_2 EGFP-positive MSSNs [137]. Although strong evidence was presented that this effect is due to dysregulation of postsynaptic L-type Ca^{2+} channels, there may also be a presynaptic contribution. Previous studies had shown that DA-depleting lesions increase spontaneous glutamate-mediated synaptic activity [138, 139]. As this increase selectively affects D_2 receptor-containing MSSNs [32], it is likely that excessive glutamate release becomes neurotoxic and induces spine elimination. Membrane loss can then induce increases in input resistance [139], making these cells even more excitable and susceptible to deleterious effects of increased glutamate release. Supporting experimental evidence for this idea was obtained recently by the demonstration that dendritic remodeling of MSSNs seen in models of PD occurs only secondary to increases in corticostriatal glutamatergic drive [140].

11.10 Conclusions

It has been 15 years since a differential effect of DA on glutamate receptor-mediated responses was first observed [88]. As generally occurs with any scientific observation or hypothesis, explanations become more complex than initially assumed. The

potential mechanisms and even the outcomes of DA–glutamate receptor interactions continue to multiply. One might speculate that various interactions accomplish different functions. Some might be intended to enhance, whereas others might be designed to inhibit the outcome of receptor interactions. In the case of D1-like–NMDA receptor interactions, the traditional pathway involving D1-like receptor activation and the cAMP–PKA–DARPP-32 cascade produces various effects that enhance NMDA receptor function [141, 142].

Physical interactions between these receptors, either in the cytoplasm or in the membrane, have added new levels of complexity. In these interactions, two pathways in the formation of D_1–NMDA heteroreceptor complexes are envisaged. One is G protein and Ca^{2+} dependent, occurs in the cytoplasm, and delivers the complex to the plasma membrane, in particular the postsynaptic density [105]. The other is G protein and Ca^{2+} independent, is membrane delimited, and could function as an inhibitory mechanism or brake to prevent and dampen continuous positive feedback [106, 109, 110]. These interactions, in conjunction with the more traditional interactions through signaling pathways, fine-tune neuronal function. Alterations in these interactions, as occur in pathological states, jeopardize functional and structural neuronal integrity. A better understanding of DA–glutamate interactions will thus provide more rational therapeutic targets in numerous diseases where these interactions are altered.

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References

1. Civelli O, Bunzow JR, Grandy DK. Molecular diversity of the dopamine receptors. Annu Rev Pharmacol Toxicol 1993;33:281–307.
2. Sibley DR, Monsma FJ, Jr. Molecular biology of dopamine receptors. Trends Pharmacol Sci 1992;13(2):61–9.
3. Neve KA, Seamans JK, Trantham-Davidson H. Dopamine receptor signaling. J Recept Signal Transduct Res 2004;24(3):165–205.
4. Hollmann M, Heinemann S. Cloned glutamate receptors. Annu Rev Neurosci 1994; 17:31–108.
5. Monaghan DT, Bridges RJ, Cotman CW. The excitatory amino acid receptors: their classes, pharmacology, and distinct properties in the function of the central nervous system. Annu Rev Pharmacol Toxicol 1989;29:365–402.
6. Nakanishi S. Metabotropic glutamate receptors: synaptic transmission, modulation, and plasticity. Neuron 1994;13(5):1031–7.
7. Nowak L, Bregestovski P, Ascher P, Herbet A, Prochiantz A. Magnesium gates glutamate-activated channels in mouse central neurones. Nature 1984;307(5950):462–5.
8. Fonnum F, Storm-Mathisen J, Divac I. Biochemical evidence for glutamate as neurotransmitter in corticostriatal and corticothalamic fibres in rat brain. Neuroscience 1981;6(5):863–73.
9. McGeer PL, McGeer EG, Scherer U, Singh K. A glutamatergic corticostriatal path? Brain Res 1977;128(2):369–73.
10. Lindvall O, Bjorklund A, Skagerberg G. Selective histochemical demonstration of dopamine terminal systems in rat di- and telencephalon: new evidence for dopaminergic innervation of hypothalamic neurosecretory nuclei. Brain Res 1984;306(1–2):19–30.

11. Freund TF, Powell JF, Smith AD. Tyrosine hydroxylase-immunoreactive boutons in synaptic contact with identified striatonigral neurons, with particular reference to dendritic spines. Neuroscience 1984;13(4):1189–215.
12. Smith AD, Bolam JP. The neural network of the basal ganglia as revealed by the study of synaptic connections of identified neurones. Trends Neurosci 1990;13(7):259–65.
13. Chesselet MF, Delfs JM. Basal ganglia and movement disorders: an update. Trends Neurosci 1996;19(10):417–22.
14. Graybiel AM. Building action repertoires: memory and learning functions of the basal ganglia. Curr Opin Neurobiol 1995;5(6):733–41.
15. Rolls ET. Neurophysiology and cognitive functions of the striatum. Rev Neurol (Paris) 1994;150(8–9):648–60.
16. Schultz W. Dopamine neurons and their role in reward mechanisms. Curr Opin Neurobiol 1997;7(2):191–7.
17. Gerfen CR, Engber TM, Mahan LC, et al. D1 and D2 dopamine receptor-regulated gene expression of striatonigral and striatopallidal neurons. Science 1990;250(4986):1429–32.
18. Haber SN, Nauta WJ. Ramifications of the globus pallidus in the rat as indicated by patterns of immunohistochemistry. Neuroscience 1983;9(2):245–60.
19. Vincent S, Hokfelt T, Christensson I, Terenius L. Immunohistochemical evidence for a dynorphin immunoreactive striato-nigral pathway. Eur J Pharmacol 1982;85(2):251–2.
20. Albin RL, Young AB, Penney JB. The functional anatomy of basal ganglia disorders. Trends Neurosci 1989;12(10):366–75.
21. Steiner H, Gerfen CR. Enkephalin regulates acute D2 dopamine receptor antagonist-induced immediate-early gene expression in striatal neurons. Neuroscience 1999;88(3):795–810.
22. Kawaguchi Y, Wilson CJ, Emson PC. Projection subtypes of rat neostriatal matrix cells revealed by intracellular injection of biocytin. J Neurosci 1990;10(10):3421–38.
23. DeLong MR, Wichmann T. Circuits and circuit disorders of the basal ganglia. Arch Neurol 2007;64(1):20–4.
24. Alexander GE, DeLong MR, Strick PL. Parallel organization of functionally segregated circuits linking basal ganglia and cortex. Annu Rev Neurosci 1986;9:357–81.
25. Alexander GE, Crutcher MD. Functional architecture of basal ganglia circuits: neural substrates of parallel processing. Trends Neurosci 1990;13(7):266–71.
26. Le Moine C, Normand E, Guitteny AF, Fouque B, Teoule R, Bloch B. Dopamine receptor gene expression by enkephalin neurons in rat forebrain. Proc Natl Acad Sci USA 1990;87(1):230–4.
27. Surmeier DJ, Eberwine J, Wilson CJ, Cao Y, Stefani A, Kitai ST. Dopamine receptor subtypes colocalize in rat striatonigral neurons. Proc Natl Acad Sci USA 1992;89(21): 10178–82.
28. Surmeier DJ, Song WJ, Yan Z. Coordinated expression of dopamine receptors in neostriatal medium spiny neurons. J Neurosci 1996;16(20):6579–91.
29. Ariano MA, Larson ER, Noblett KL, Sibley DR, Levine MS. Coexpression of striatal dopamine receptor subtypes and excitatory amino acid subunits. Synapse 1997;26(4): 400–14.
30. Aizman O, Brismar H, Uhlen P, et al. Anatomical and physiological evidence for D1 and D2 dopamine receptor colocalization in neostriatal neurons. Nat Neurosci 2000;3(3):226–30.
31. Gong S, Zheng C, Doughty ML, et al. A gene expression atlas of the central nervous system based on bacterial artificial chromosomes. Nature 2003;425(6961):917–25.
32. Cepeda C, Andre VM, Yamazaki I, Wu N, Kleiman-Weiner M, Levine MS. Differential electrophysiological properties of dopamine D1 and D2 receptor-containing striatal medium-sized spiny neurons. Eur J Neurosci 2008;27(3):671–82.
33. Shuen JA, Chen M, Gloss B, Calakos N. Drd1a-tdTomato BAC transgenic mice for simultaneous visualization of medium spiny neurons in the direct and indirect pathways of the basal ganglia. J Neurosci 2008;28(11):2681–5.

34. Cepeda C, Chandler SH, Shumate LW, Levine MS. Persistent Na^+ conductance in medium-sized neostriatal neurons: characterization using infrared videomicroscopy and whole cell patch-clamp recordings. J Neurophysiol 1995;74(3):1343–8.
35. Surmeier DJ, Kitai ST. D1 and D2 dopamine receptor modulation of sodium and potassium currents in rat neostriatal neurons. Prog Brain Res 1993;99:309–24.
36. Surmeier DJ, Ding J, Day M, Wang Z, Shen W. D1 and D2 dopamine-receptor modulation of striatal glutamatergic signaling in striatal medium spiny neurons. Trends Neurosci 2007;30(5):228–35.
37. Surmeier DJ, Bargas J, Hemmings HC, Jr., Nairn AC, Greengard P. Modulation of calcium currents by a D1 dopaminergic protein kinase/phosphatase cascade in rat neostriatal neurons. Neuron 1995;14(2):385–97.
38. Wang H, Pickel VM. Dopamine D2 receptors are present in prefrontal cortical afferents and their targets in patches of the rat caudate-putamen nucleus. J Comp Neurol 2002;442(4): 392–404.
39. Cepeda C, Hurst RS, Altemus KL, et al. Facilitated glutamatergic transmission in the striatum of D2 dopamine receptor-deficient mice. J Neurophysiol 2001;85(2):659–70.
40. Kornhuber J, Kornhuber ME. Presynaptic dopaminergic modulation of cortical input to the striatum. Life Sci 1986;39(8):699–74.
41. Maura G, Giardi A, Raiteri M. Release-regulating D-2 dopamine receptors are located on striatal glutamatergic nerve terminals. J Pharmacol Exp Ther 1988;247(2):680–4.
42. Mitchell PR, Doggett NS. Modulation of striatal [3H]-glutamic acid release by dopaminergic drugs. Life Sci 1980;26(24):2073–81.
43. Rowlands GF, Roberts PJ. Activation of dopamine receptors inhibits calcium-dependent glutamate release from cortico-striatal terminals in vitro. Eur J Pharmacol 1980;62(2–3): 241–2.
44. Yamamoto BK, Davy S. Dopaminergic modulation of glutamate release in striatum as measured by microdialysis. J Neurochem 1992;58(5):1736–42.
45. Flores-Hernandez J, Galarraga E, Bargas J. Dopamine selects glutamatergic inputs to neostriatal neurons. Synapse 1997;25(2):185–95.
46. Hsu KS, Huang CC, Yang CH, Gean PW. Presynaptic D2 dopaminergic receptors mediate inhibition of excitatory synaptic transmission in rat neostriatum. Brain Res 1995;690(2):264–8.
47. Mercuri N, Bernardi G, Calabresi P, Cotugno A, Levi G, Stanzione P. Dopamine decreases cell excitability in rat striatal neurons by pre- and postsynaptic mechanisms. Brain Res 1985;358(1–2):110–21.
48. Umemiya M, Raymond LA. Dopaminergic modulation of excitatory postsynaptic currents in rat neostriatal neurons. J Neurophysiol 1997;78(3):1248–55.
49. Bamford NS, Zhang H, Schmitz Y, et al. Heterosynaptic dopamine neurotransmission selects sets of corticostriatal terminals. Neuron 2004;42(4):653–63.
50. Dani JA, Zhou FM. Selective dopamine filter of glutamate striatal afferents. Neuron 2004;42(4):522–4.
51. Harvey J, Lacey MG. A postsynaptic interaction between dopamine D1 and NMDA receptors promotes presynaptic inhibition in the rat nucleus accumbens via adenosine release. J Neurosci 1997;17(14):5271–80.
52. Yin HH, Lovinger DM. Frequency-specific and D2 receptor-mediated inhibition of glutamate release by retrograde endocannabinoid signaling. Proc Natl Acad Sci USA 2006;103(21):8251–6.
53. Calabresi P, De Murtas M, Pisani A, et al. Vulnerability of medium spiny striatal neurons to glutamate: role of Na+/K+ ATPase. Eur J Neurosci 1995;7(8):1674–83.
54. Nicola SM, Malenka RC. Modulation of synaptic transmission by dopamine and norepinephrine in ventral but not dorsal striatum. J Neurophysiol 1998;79(4):1768–76.
55. Cepeda C, Levine MS. Dopamine and N-methyl-D-aspartate receptor interactions in the neostriatum. Dev Neurosci 1998;20(1):1–18.

56. Flores-Hernandez J, Cepeda C, Hernandez-Echeagaray E, et al. Dopamine enhancement of NMDA currents in dissociated medium-sized striatal neurons: role of D1 receptors and DARPP-32. J Neurophysiol 2002;88(6):3010–20.
57. Liu JC, DeFazio RA, Espinosa-Jeffrey A, Cepeda C, de Vellis J, Levine MS. Calcium modulates dopamine potentiation of N-methyl-D-aspartate responses: electrophysiological and imaging evidence. J Neurosci Res 2004;76(3):315–22.
58. Greengard P. The neurobiology of slow synaptic transmission. Science 2001;294 (5544):1024–30.
59. Bloom FE, Costa E, Salmoiraghi GC. Anesthesia and the responsiveness of individual neurons of the caudate nucleus of the cat to acetylcholine, norepinephrine and dopamine administered by microelectrophoresis. J Pharmacol Exp Ther 1965;150(2): 244–52.
60. Spencer HJ, Havlicek V. Alterations by anesthetic agents of the responses of rat striatal neurons to iontophoretically applied amphetamine, acetylcholine, noradrenaline, and dopamine. Can J Physiol Pharmacol 1974;52(4):808–13.
61. Norcross K, Spehlmann R. A quantitative analysis of the excitatory and depressant effects of dopamine on the firing of caudatal neurons: electrophysiological support for the existence of two distinct dopamine-sensitive receptors. Brain Res 1978;156(1):168–74.
62. Herrling PL, Hull CD. Iontophoretically applied dopamine depolarizes and hyperpolarizes the membrane of cat caudate neurons. Brain Res 1980;192(2):441–62.
63. Calabresi P, Mercuri N, Stanzione P, Stefani A, Bernardi G. Intracellular studies on the dopamine-induced firing inhibition of neostriatal neurons in vitro: evidence for D1 receptor involvement. Neuroscience 1987;20(3):757–71.
64. Hernandez-Lopez S, Bargas J, Surmeier DJ, Reyes A, Galarraga E. D1 receptor activation enhances evoked discharge in neostriatal medium spiny neurons by modulating an L-type Ca2+ conductance. J Neurosci 1997;17(9):3334–42.
65. Cepeda C, Buchwald NA, Levine MS. Neuromodulatory actions of dopamine in the neostriatum are dependent upon the excitatory amino acid receptor subtypes activated. Proc Natl Acad Sci USA 1993;90(20):9576–80.
66. Levine MS, Altemus KL, Cepeda C, et al. Modulatory actions of dopamine on NMDA receptor-mediated responses are reduced in D1A-deficient mutant mice. J Neurosci 1996;16(18):5870–82.
67. Hernandez-Echeagaray E, Starling AJ, Cepeda C, Levine MS. Modulation of AMPA currents by D2 dopamine receptors in striatal medium-sized spiny neurons: are dendrites necessary? Eur J Neurosci 2004;19(9):2455–63.
68. Delle Donne KT, Sesack SR, Pickel VM. Ultrastructural immunocytochemical localization of the dopamine D2 receptor within GABAergic neurons of the rat striatum. Brain Res 1997;746(1–2):239–55.
69. Hersch SM, Ciliax BJ, Gutekunst CA, et al. Electron microscopic analysis of D1 and D2 dopamine receptor proteins in the dorsal striatum and their synaptic relationships with motor corticostriatal afferents. J Neurosci 1995;15(7 Pt 2):5222–37.
70. Bettler B, Mulle C. Review: neurotransmitter receptors. II. AMPA and kainate receptors. Neuropharmacology 1995;34(2):123–39.
71. Bleakman D, Lodge D. Neuropharmacology of AMPA and kainate receptors. Neuropharmacology 1998;37(10–11):1187–204.
72. Tavalin SJ, Colledge M, Hell JW, Langeberg LK, Huganir RL, Scott JD. Regulation of GluR1 by the A-kinase anchoring protein 79 (AKAP79) signaling complex shares properties with long-term depression. J Neurosci 2002;22(8):3044–51.
73. Hernandez-Lopez S, Tkatch T, Perez-Garci E, et al. D2 dopamine receptors in striatal medium spiny neurons reduce L-type Ca2+ currents and excitability via a novel PLC[beta]1-IP3-calcineurin-signaling cascade. J Neurosci 2000;20(24):8987–95.
74. Nicola SM, Surmeier J, Malenka RC. Dopaminergic modulation of neuronal excitability in the striatum and nucleus accumbens. Annu Rev Neurosci 2000;23:185–215.

75. Bernard V, Somogyi P, Bolam JP. Cellular, subcellular, and subsynaptic distribution of AMPA-type glutamate receptor subunits in the neostriatum of the rat. J Neurosci 1997;17(2):819–33.
76. Ghasemzadeh MB, Sharma S, Surmeier DJ, Eberwine JH, Chesselet MF. Multiplicity of glutamate receptor subunits in single striatal neurons: an RNA amplification study. Mol Pharmacol 1996;49(5):852–9.
77. Stefani A, Chen Q, Flores-Hernandez J, Jiao Y, Reiner A, Surmeier DJ. Physiological and molecular properties of AMPA/Kainate receptors expressed by striatal medium spiny neurons. Dev Neurosci 1998;20(2–3):242–52.
78. Snyder GL, Allen PB, Fienberg AA, et al. Regulation of phosphorylation of the GluR1 AMPA receptor in the neostriatum by dopamine and psychostimulants in vivo. J Neurosci 2000;20(12):4480–8.
79. Banke TG, Bowie D, Lee H, Huganir RL, Schousboe A, Traynelis SF. Control of GluR1 AMPA receptor function by cAMP-dependent protein kinase. J Neurosci 2000;20(1): 89–102.
80. Roche KW, O'Brien RJ, Mammen AL, Bernhardt J, Huganir RL. Characterization of multiple phosphorylation sites on the AMPA receptor GluR1 subunit. Neuron 1996;16(6): 1179–88.
81. Lindgren N, Usiello A, Goiny M, et al. Distinct roles of dopamine D2L and D2S receptor isoforms in the regulation of protein phosphorylation at presynaptic and postsynaptic sites. Proc Natl Acad Sci USA 2003;100(7):4305–9.
82. Nishi A, Snyder GL, Greengard P. Bidirectional regulation of DARPP-32 phosphorylation by dopamine. J Neurosci 1997;17(21):8147–55.
83. Yan Z, Hsieh-Wilson L, Feng J, et al. Protein phosphatase 1 modulation of neostriatal AMPA channels: regulation by DARPP-32 and spinophilin. Nat Neurosci 1999;2(1):13–7.
84. Price CJ, Kim P, Raymond LA. D1 dopamine receptor-induced cyclic AMP-dependent protein kinase phosphorylation and potentiation of striatal glutamate receptors. J Neurochem 1999;73(6):2441–6.
85. Chao SZ, Ariano MA, Peterson DA, Wolf ME. D1 dopamine receptor stimulation increases GluR1 surface expression in nucleus accumbens neurons. J Neurochem 2002;83(3): 704–12.
86. Sun X, Milovanovic M, Zhao Y, Wolf ME. Acute and chronic dopamine receptor stimulation modulates AMPA receptor trafficking in nucleus accumbens neurons cocultured with prefrontal cortex neurons. J Neurosci 2008;28(16):4216–30.
87. West AR, Grace AA. Opposite influences of endogenous dopamine D1 and D2 receptor activation on activity states and electrophysiological properties of striatal neurons: studies combining in vivo intracellular recordings and reverse microdialysis. J Neurosci 2002;22(1):294–304.
88. Cepeda C, Radisavljevic Z, Peacock W, Levine MS, Buchwald NA. Differential modulation by dopamine of responses evoked by excitatory amino acids in human cortex. Synapse 1992;11(4):330–41.
89. Blank T, Nijholt I, Teichert U, et al. The phosphoprotein DARPP-32 mediates cAMP-dependent potentiation of striatal N-methyl-D-aspartate responses. Proc Natl Acad Sci USA 1997;94(26):14859–64.
90. Cepeda C, Colwell CS, Itri JN, Chandler SH, Levine MS. Dopaminergic modulation of NMDA-induced whole cell currents in neostriatal neurons in slices: contribution of calcium conductances. J Neurophysiol 1998;79(1):82–94.
91. Cepeda C, Itri JN, Flores-Hernandez J, Hurst RS, Calvert CR, Levine MS. Differential sensitivity of medium- and large-sized striatal neurons to NMDA but not kainate receptor activation in the rat. Eur J Neurosci 2001;14(10):1577–89.
92. Chen G, Greengard P, Yan Z. Potentiation of NMDA receptor currents by dopamine D1 receptors in prefrontal cortex. Proc Natl Acad Sci USA 2004;101(8):2596–600.

93. Chergui K, Lacey MG. Modulation by dopamine D1-like receptors of synaptic transmission and NMDA receptors in rat nucleus accumbens is attenuated by the protein kinase C inhibitor Ro 32-0432. Neuropharmacology 1999;38(2):223–31.
94. Colwell CS, Levine MS. Excitatory synaptic transmission in neostriatal neurons: regulation by cyclic AMP-dependent mechanisms. J Neurosci 1995;15(3 Pt 1):1704–13.
95. Levine MS, Li Z, Cepeda C, Cromwell HC, Altemus KL. Neuromodulatory actions of dopamine on synaptically-evoked neostriatal responses in slices. Synapse 1996;24(1):65–78.
96. Seamans JK, Durstewitz D, Christie BR, Stevens CF, Sejnowski TJ. Dopamine D1/D5 receptor modulation of excitatory synaptic inputs to layer V prefrontal cortex neurons. Proc Natl Acad Sci USA 2001;98(1):301–6.
97. Tseng KY, O'Donnell P. Dopamine-glutamate interactions controlling prefrontal cortical pyramidal cell excitability involve multiple signaling mechanisms. J Neurosci 2004;24(22):5131–9.
98. Wang J, O'Donnell P. D(1) dopamine receptors potentiate nmda-mediated excitability increase in layer V prefrontal cortical pyramidal neurons. Cereb Cortex 2001;11(5):452–62.
99. Zheng P, Zhang XX, Bunney BS, Shi WX. Opposite modulation of cortical N-methyl-D-aspartate receptor-mediated responses by low and high concentrations of dopamine. Neuroscience 1999;91(2):527–35.
100. Seamans JK, Yang CR. The principal features and mechanisms of dopamine modulation in the prefrontal cortex. Prog Neurobiol 2004;74(1):1–58.
101. Snyder GL, Fienberg AA, Huganir RL, Greengard P. A dopamine/D1 receptor/protein kinase A/dopamine- and cAMP-regulated phosphoprotein (Mr 32 kDa)/protein phosphatase-1 pathway regulates dephosphorylation of the NMDA receptor. J Neurosci 1998;18(24):10297–303.
102. Hallett PJ, Spoelgen R, Hyman BT, Standaert DG, Dunah AW. Dopamine D1 activation potentiates striatal NMDA receptors by tyrosine phosphorylation-dependent subunit trafficking. J Neurosci 2006;26(17):4690–700.
103. Dunah AW, Standaert DG. Dopamine D1 receptor-dependent trafficking of striatal NMDA glutamate receptors to the postsynaptic membrane. J Neurosci 2001;21(15):5546–58.
104. Dunah AW, Sirianni AC, Fienberg AA, Bastia E, Schwarzschild MA, Standaert DG. Dopamine D1-dependent trafficking of striatal N-methyl-D-aspartate glutamate receptors requires Fyn protein tyrosine kinase but not DARPP-32. Mol Pharmacol 2004;65(1):121–9.
105. Fiorentini C, Gardoni F, Spano P, Di Luca M, Missale C. Regulation of dopamine D1 receptor trafficking and desensitization by oligomerization with glutamate N-methyl-D-aspartate receptors. J Biol Chem 2003;278(22):20196–202.
106. Lee FJ, Xue S, Pei L, et al. Dual regulation of NMDA receptor functions by direct protein-protein interactions with the dopamine D1 receptor. Cell 2002;111(2):219–30.
107. Pei L, Lee FJ, Moszczynska A, Vukusic B, Liu F. Regulation of dopamine D1 receptor function by physical interaction with the NMDA receptors. J Neurosci 2004;24(5):1149–58.
108. Cui C, Xu M, Atzori M. Voltage-dependent block of N-methyl-D-aspartate receptors by dopamine D1 receptor ligands. Mol Pharmacol 2006;70(5):1761–70.
109. Scott L, Kruse MS, Forssberg H, Brismar H, Greengard P, Aperia A. Selective up-regulation of dopamine D1 receptors in dendritic spines by NMDA receptor activation. Proc Natl Acad Sci USA 2002;99(3):1661–4.
110. Scott L, Zelenin S, Malmersjo S, et al. Allosteric changes of the NMDA receptor trap diffusible dopamine 1 receptors in spines. Proc Natl Acad Sci USA 2006;103(3):762–7.
111. Changeux JP, Edelstein SJ. Allosteric mechanisms of signal transduction. Science 2005;308(5727):1424–8.
112. Cepeda C, Colwell CS, Itri JN, Gruen E, Levine MS. Dopaminergic modulation of early signs of excitotoxicity in visualized rat neostriatal neurons. Eur J Neurosci 1998;10(11):3491–7.

113. Colwell CS, Levine MS. Glutamate receptor-induced toxicity in neostriatal cells. Brain Res 1996;724(2):205–12.
114. Ariano MA, Wang J, Noblett KL, Larson ER, Sibley DR. Cellular distribution of the rat D4 dopamine receptor protein in the CNS using anti-receptor antisera. Brain Res 1997;752 (1–2):26–34.
115. Tarazi FI, Zhang K, Baldessarini RJ. Dopamine D4 receptors: beyond schizophrenia. J Recept Signal Transduct Res 2004;24(3):131–47.
116. Rubinstein M, Cepeda C, Hurst RS, et al. Dopamine D4 receptor-deficient mice display cortical hyperexcitability. J Neurosci 2001;21(11):3756–63.
117. Wang X, Zhong P, Gu Z, Yan Z. Regulation of NMDA receptors by dopamine D4 signaling in prefrontal cortex. J Neurosci 2003;23(30):9852–61.
118. Kotecha SA, Oak JN, Jackson MF, et al. A D2 class dopamine receptor transactivates a receptor tyrosine kinase to inhibit NMDA receptor transmission. Neuron 2002;35(6): 1111–22.
119. Beazely MA, Tong A, Wei WL, Van Tol H, Sidhu B, MacDonald JF. D2-class dopamine receptor inhibition of NMDA currents in prefrontal cortical neurons is platelet-derived growth factor receptor-dependent. J Neurochem 2006;98(5):1657–63.
120. Liu XY, Chu XP, Mao LM, et al. Modulation of D2R-NR2B interactions in response to cocaine. Neuron 2006;52(5):897–909.
121. Sakimura K, Kutsuwada T, Ito I, et al. Reduced hippocampal LTP and spatial learning in mice lacking NMDA receptor epsilon 1 subunit. Nature 1995;373(6510):151–5.
122. Li R, Huang FS, Abbas AK, Wigstrom H. Role of NMDA receptor subtypes in different forms of NMDA-dependent synaptic plasticity. BMC Neurosci 2007;8(1):55.
123. Liu Y, Wong TP, Aarts M, et al. NMDA receptor subunits have differential roles in mediating excitotoxic neuronal death both in vitro and in vivo. J Neurosci 2007;27(11): 2846–57.
124. von Engelhardt J, Coserea I, Pawlak V, et al. Excitotoxicity in vitro by NR2A- and NR2B-containing NMDA receptors. Neuropharmacology 2007;53(1):10–7.
125. Surmeier DJ, Reiner A, Levine MS, Ariano MA. Are neostriatal dopamine receptors co-localized? Trends Neurosci 1993;16(8):299–305.
126. Gonon F. Prolonged and extrasynaptic excitatory action of dopamine mediated by D1 receptors in the rat striatum in vivo. J Neurosci 1997;17(15):5972–8.
127. Levesque M, Parent A. The striatofugal fiber system in primates: a reevaluation of its organization based on single-axon tracing studies. Proc Natl Acad Sci USA 2005;102(33): 11888–93.
128. Wickens JR, Reynolds JN, Hyland BI. Neural mechanisms of reward-related motor learning. Curr Opin Neurobiol 2003;13(6):685–90.
129. Schmidt WJ, Kretschmer BD. Behavioural pharmacology of glutamate receptors in the basal ganglia. Neurosci Biobehav Rev 1997;21(4):381–92.
130. DiFiglia M. Excitotoxic injury of the neostriatum: a model for Huntington's disease. Trends Neurosci 1990;13(7):286–9.
131. Cepeda C, Ariano MA, Calvert CR, et al. NMDA receptor function in mouse models of Huntington disease. J Neurosci Res 2001;66(4):525–39.
132. Levine MS, Klapstein GJ, Koppel A, et al. Enhanced sensitivity to N-methyl-D-aspartate receptor activation in transgenic and knockin mouse models of Huntington's disease. J Neurosci Res 1999;58(4):515–32.
133. Zeron MM, Hansson O, Chen N, et al. Increased sensitivity to N-methyl-D-aspartate receptor-mediated excitotoxicity in a mouse model of Huntington's disease. Neuron 2002;33(6):849–60.
134. Hardingham GE, Fukunaga Y, Bading H. Extrasynaptic NMDARs oppose synaptic NMDARs by triggering CREB shut-off and cell death pathways. Nat Neurosci 2002;5(5):405–14.

135. Yang CR, Chen L. Targeting prefrontal cortical dopamine D1 and N-methyl-D-aspartate receptor interactions in schizophrenia treatment. Neuroscientist 2005;11(5):452–70.
136. Bozzi Y, Borrelli E. Dopamine in neurotoxicity and neuroprotection: what do D2 receptors have to do with it? Trends Neurosci 2006;29(3):167–74.
137. Day M, Wang Z, Ding J, et al. Selective elimination of glutamatergic synapses on striatopallidal neurons in Parkinson disease models. Nat Neurosci 2006;9(2):251–9.
138. Cepeda C, Walsh JP, Hull CD, Howard SG, Buchwald NA, Levine MS. Dye-coupling in the neostriatum of the rat: I. Modulation by dopamine-depleting lesions. Synapse 1989;4(3):229–37.
139. Galarraga E, Bargas J, Martinez-Fong D, Aceves J. Spontaneous synaptic potentials in dopamine-denervated neostriatal neurons. Neurosci Lett 1987;81(3):351–5.
140. Neely MD, Schmidt DE, Deutch AY. Cortical regulation of dopamine depletion-induced dendritic spine loss in striatal medium spiny neurons. Neuroscience 2007;149(2):457–64.
141. Cepeda C, Levine MS. Where do you think you are going? The NMDA-D1 receptor trap. Sci STKE 2006;2006(333):20.
142. Greengard P, Allen PB, Nairn AC. Beyond the dopamine receptor: the DARPP-32/protein phosphatase-1 cascade. Neuron 1999;23(3):435–47.

Chapter 12
Unraveling the Role of Dopamine Receptors In Vivo: Lessons from Knockout Mice

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Abstract Dopamine exerts its action through membrane receptors that belong to the seven transmembrane domains (7TM) G protein-coupled receptor family. The dopamine receptor family is composed of five members, which have been divided into two subgroups: the D_1-like family, which contains the D_1 receptor (D_1R) and D_5R, and the D_2-like family containing D_2R, D_3R, and D_4R. This subdivision is based on pharmacological, biochemical, and structural properties. Nevertheless, the close pharmacological properties together with the common anatomical site of expression of these receptors have induced the interest for generating animal models with which to assess the function of each individual dopamine receptor in vivo. To date, there exist mutants for all five receptors, in particular using the knockout technology each dopamine receptor has been independently knocked out. In this chapter we will summarize major findings related to the contribution of each dopamine receptor in the control of physiological functions regulated by dopamine. Analyses of these mutants clearly show a preponderant role for dopamine D_1R and D_2R receptors in most dopamine-mediated effects. At the same time these mutants are also revealing more hidden functions for D_3R, D_4R, and D_5R very likely in the modulation of D_1R- and D_2R-mediated signaling.

Keywords Dopamine receptors · Knockout technology · Animal models · Behavior · Locomotion · Reward

12.1 Introduction

Understanding the mechanisms by which dopamine modulates functions as diverse as locomotion and reward or pituitary hormone production is a great scientific challenge. Since its discovery as a neurotransmitter in the 1950s, dopamine has attracted

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the attention of scientists from different disciplines with the common aim to unravel its mode of action in vivo. The presence of five dopamine receptors (DAR) [1, 2] together with their overlapping pattern of expression [3, 4] and shared signaling pathways [5] has not facilitated this task. In addition, members of the D_2-like receptor subfamily are expressed into multiple isoforms generated by alternative splicing events in vivo; of these the best characterized are the long (D_{2L}) and short (D_{2S}) isoforms of the dopamine D_2 receptor (D_2R) [5–8], although splice variants have also been reported for the D_3R and D_4R [9, 10]. Similarly, while it is possible pharmacologically to distinguish between D_1-like and D_2-like receptors, the presence of multiple members in each subfamily with similar pharmacological properties does not allow a full characterization of each receptor [1]. This promiscuity arises from the high molecular, biochemical, and pharmacological similarity existing between members of the D_1-like (D_1R and D_5R) and D_2-like (D_{2L}, D_{2S}, D_3R, and D_4R) receptor subfamilies [5], not to mention the possibility that each receptor might be able to form homo- and heterodimers/polymers and that these different conformations might modify in vivo responses [11], further enhancing the complexity of studying these proteins.

Thus, the possibility to genetically engineer mice [12, 13] and generate mutants lacking one particular DAR has been welcome for assessing the specific role of each receptor in vivo.

12.2 Advantages and Drawbacks of the Knockout Technology

Before discussing the results originated with DAR knockout (KO) mice, a premise needs to be made. First, KO models represent a great tool for investigating the role of specific proteins in vivo; however, as any other existent model, they have advantages as well as drawbacks. The advantages in the studies of the dopaminergic system have been multiple and we will mention only a few: (a) DAR KO mice have, indeed, allowed analyzing in vivo how absence of a specific receptor impairs well-defined dopamine-dependent functions. This would have not been possible using only pharmacological tools, due to the shared affinity for the same compound of members of each dopaminergic subfamily. In this respect, KO mice have also been used to assess the specificity of defined compounds in vivo [14, 15], which would not have been otherwise possible. (b) In contrast to transient analyses, KO models also enable to study the impact of loss of a specific receptor throughout the animal's life. This aspect is particularly important when analyzing proteins belonging to systems involved in neurodegenerative disorders such as the dopamine system. It has been possible, for example, to show that absence of D_2-mediated signaling in D_2R KO females leads to development of pituitary tumors, but only as they age [16, 17].

On the other hand, there are drawbacks of the constitutive KO technology that should not be overlooked. (a) Animals are deprived of the specific receptor as soon as the gene becomes transcriptionally active and thus even during embryogenesis (if it has an early expression pattern). This underlies the possible establishment of

homeostatic responses from the organism to adjust/cope with that loss of function/s. Therefore, phenotypes of DAR KO mice might partly result from compensatory mechanisms more than from the ablation of a specific receptor. Although this is a reasonable eventuality, results obtained from studies of the different DAR mutants suggest that it might have had very limited effects, at least based on parallels between acute pharmacological studies and phenotypes of KO mice. (b) A second debated issue is technical: most commonly KOs are generated from embryonal stem cells of a different genetic background than that of the recipient embryos. This leads to animals with mixed genetic backgrounds and the possibility that some behavioral phenotypes can be influenced by specific background contributions and not by deletion of the gene in itself. For this, littermates from heterozygote mating are used in order to have comparable wild-type (WT) and mutated mice. When possible, backcrossing KOs in the genetic background desired, for at least five generations, is advised [18, 19].

More importantly, the outcome of behavioral analyses performed with DAR KO mice has generated some controversies; this is a major issue since the behavioral settings differ in different laboratories. This is a more difficult point to address, as it would require the development of well-standardized behavioral procedures and settings common to every laboratory or at least those analyzing the same KO line.

Nevertheless, most published phenotypes have been subsequently confirmed in different laboratories using independently generated KO lines for the same receptor. Generally, the amplitude of particular impairments might increase/decrease in mixed backgrounds, but robust phenotypes are maintained whether in mixed or pure backgrounds. Despite these drawbacks, DAR KOs have contributed and will, in the future, generate important information on the physiology of these receptors that would have not been otherwise gathered.

12.3 Lessons from KO Mice

Since 1994 a large series of reports have been published in which DAR knockout mice were used to establish the role of individual receptors in vivo. Multiple KO lines have been made in particular for D_1R, D_2R, and D_3R, while only one KO line has been generated for D_4R and D_5R. In this paragraph, we will highlight some of the major findings obtained through the analysis of DAR KO mice, and in particular from D_2R KO mice, which well illustrate the relevance of KO studies. For instance, genetic studies have shown the importance of dopaminergic signaling not only for brain functions but also overall for the survival of the organism. Indeed, while single KO of each of the five DARs does not impact birth, growth, or reproduction of KO mice, the simultaneous KO of the two major DARs, D_1R and D_2R, results in a striking phenotype, which leads to death of double KO mice during the second postnatal week [20]. Notably, this phenotype strongly resembles that of tyrosine hydroxylase KO mice, in which dopamine levels are extremely reduced [21, 22]. Interestingly, lethality is observed only in the D_1R/D_2R double KO [20], while D_2R/D_3R [23, 24]

or D_1R/D_3R [25, 26] KOs are born normally, reach adulthood, and reproduce. These findings strongly point to a preponderant physiological role of D_1Rs and D_2Rs in the regulation of basal body functions. At the same time, we might speculate that the principal function of D_3R, D_4R, and D_5R is to act as sensors of dopamine activity and to modulate D_1R- and D_2R-dependent signaling. This view is supported by the higher affinity of the D_3R, D_4R, and D_5R for dopamine [27] as well as by their co-localization with either D_1R or D_2R, for example, in striatal medium spiny neurons (MSNs) [4, 28].

A second important finding has been the identification of D_2Rs as the bona fide dopamine autoreceptor [29, 30]. This function has a critical role in the regulation and physiology of the dopaminergic system since it is responsible for the control of the synthesis and release of dopamine. Alteration or malfunction of autoreceptors strongly impairs the response of any DAR by changing inappropriately the physiological levels of the ligand in response to stimuli. Two D_2-like receptors, D_2R and D_3R, could be anatomically responsible for such functions due to their localization in dopaminergic neurons of the substantia nigra (SN) and ventral tegmental area (VTA). Because these receptors have similar pharmacological characteristics, assessing autoreceptor function only with pharmacological tools has produced ambiguous results. The use of D_2R and D_3R KOs has clarified that despite the presence of D_2R and D_3R on dopaminergic neurons, absence of D_2R completely abolishes the autoreceptor regulation of dopamine release from dopaminergic neurons [30]. This indicates that D_3Rs, in the absence of D_2Rs, cannot compensate for the regulation of dopamine release upon challenge. Nevertheless, D_3Rs might have a role in regulating dopamine levels under phasic but not tonic conditions [31] or in cooperation with D_2Rs.

The third example concerns the target of antipsychotics normally used in the treatment of schizophrenia. Haloperidol, a commonly used antipsychotic, is known to exert its beneficial effect in the treatment of schizophrenia through blockade of D_2-like receptors [32]. Importantly, knocking out the long isoform of the D_2R, D_{2L}, strongly impairs the property of this drug to induce catalepsy in D_{2L} KO mice, thus identifying D_{2L} as the major target for the postsynaptic effects of this antipsychotic [7, 8]. Furthermore, studies in which D_1R, D_2R, D_3R, and D_4R KO mice were compared to assess their involvement in modulating pre-pulse inhibition (PPI) of startle reflex have shown that only the absence of D_2Rs results in disruption of PPI after amphetamine treatment [33], while D_1Rs, D_2Rs, and D_3Rs are all involved in the disruption of PPI induced by cocaine [34].

12.4 Dopamine Receptors in the Control of Motor Behavior

One key role of dopamine in the CNS is the control of locomotion, as evidenced by degeneration of dopaminergic neurons in Parkinson's disease. The dopaminergic pathway involved in this control is the nigro-striatal pathway formed by fibers arising from dopaminergic neurons located in the SN, which project to the striatum.

The striatum represents the site of integration of the motor circuitry, receiving the dopaminergic fibers as well as stimuli from the cortex and thalamus [35]. The complex nature of this circuitry and co-expression of several different DARs in the same neurons make it very difficult to define the role of each receptor in this function. Indeed, DARs are present not only on striatal MSNs but also on cortical and thalamic neurons, as well as on interneurons (Fig. 12.1). It is thus very arduous to analyze how the different DARs regulate the physiology of neurons in this circuitry. Nevertheless, the development of KO mice and technical tools formed either by specific antibodies, tracers [36], or mouse models in which BAC constructs expressing fluorescent proteins, driven by the D_1R and D_2R [35, 37, 38] promoters, have been inserted in the mouse genome is starting to give useful insights into the rules that govern this circuitry.

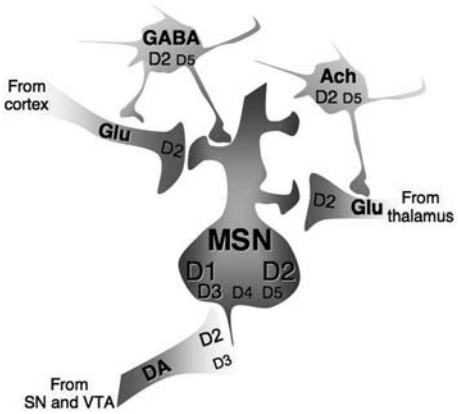

Fig. 12.1 This figure schematically illustrates how dopamine elicits its effects on striatal medium spiny neurons (MSNs). We aimed at representing the sites of expression of dopamine receptors on most striatal neurons and afferents. D_1Rs and D_2Rs are the most highly expressed neurons by MSNs, followed by D_3Rs and then D_4Rs and D_5Rs (font size is indicative of the expression level). MSNs receive inputs from neurons originating in the SN, VTA, cortex, and thalamus as well as from striatal interneurons (i.e., striatal GABAergic and cholinergic interneurons). D_2Rs have a very intricate expression pattern. Indeed, they are present on MSNs, but also presynaptically on dopaminergic fibers, as well as on cortical, thalamic, and interneuron terminals. D_3Rs are both pre- and postsynaptic; D_1R, D_4R, and D_5R expression is as indicated

Depending on which cell/region of the striatum is analyzed, different combinations of dopamine receptors are found. For example, most dorsal regions express almost exclusively D_1Rs and D_2Rs and are devoid of D_3R expression, while the ventral striatum expresses higher levels of D_3Rs [31]. Similarly, D_5Rs have very low expression levels overall in the striatum, but they are robustly expressed in cholinergic interneurons [3]. Furthermore, co-segregation of some members of the D_1-like and D_2-like receptor subfamilies in the same cells has been observed; this together with the possibility that they could physically associate in vivo [39, 40] increases

the complexity of this system. Behavioral results obtained by the study of KO mice should be interpreted keeping in mind this intricacy.

12.4.1 Motor Behavior: D_1R KO

D_1R KO mice were first reported in 1994 by two independent laboratories; for clarity we will call them D_1R KO-A [41] and D_1R KO-B [42]. In D_1R KO-A, the targeting construct contained a deletion of 95% of the D_1R coding sequence, while D_1R KO-B was created by deleting the region of the D_1R gene encoding the putative third intracytoplasmic loop of the receptor. D_1R KO-A mice were reported to present a hyperactive phenotype [41, 43, 44]; conversely, D_1R KO-B mice were first reported to have decreased stereotypies, but no difference in locomotion [42]. However, more recently the D_1R KO-B line has also been reported to be hyperactive in different behavioral settings [45]. Therefore, it can be concluded that loss of the D_1R results in a potentiation of horizontal locomotion.

12.4.2 Motor Behavior: D_2R KO

D_2R KO-A mice were first reported in 1995 [46]; in this line exon 2 of the D_2R gene was deleted leading to the absence of D_2Rs in vivo. These mice presented impairments of posture and motor activity when analyzed in an open field. In particular, a significant reduction of horizontal locomotion, stereotypies, and coordination of movements were reported [46]. A second KO line, D_2R KO-B [23], was generated using a similar KO strategy as for D_2R KO-A. Analyses of D_2R KO-B mice supported findings obtained with D_2R KO-A and confirmed motor impairments in the absence of the D_2R. A third line, D_2R KO-C mice, was generated using a different KO strategy, in which the 3′-region of the D_2R gene containing exons 6 and 7 was deleted [47]. These mice were also reported to have motor impairments, but to a lower extent than D_2R KO-A and B mice. Interestingly, ethological analyses performed by the same laboratory using D_2R KO-A and C lines revealed an absence of difference in the different parameters tested between these two independent lines [48, 49]. Ethological analyses identified, in both lines, specific reductions of one type of stereotypy (total rearing) and of horizontal locomotion, although the deficits were much milder than those found when D_2R KO mice were tested in the open field and rotarod. It is perhaps not surprising to obtain results from ethological analyses that differ from other behavioral tests (open field, rotarod); the first are performed in non-stressful settings (home cage), while the latter are performed under more stressful/challenging conditions. Overall, it can be concluded that loss of D_2R-mediated signaling results in reduction of motor behaviors. Interestingly, deletion of the long and most abundant isoform of D_2R, D_{2L}, does not result in modifications of motor activity or coordination of movements under basal conditions [7, 8]. This indicates that the presence of only D_{2S} is able to insure a normal D_2R-dependent basal activity, very likely dependent on the still persistent control of dopamine release [7]

mediated by D_{2S}. However, challenge by D_1R-specific agonists of D_{2L} KO mice resulted in a greatly attenuated motor response as compared to their WT littermates [7]. This suggests that D_{2L} is the D_2R isoform cooperating/synergizing with the D_1R in the control of motor behavior [50].

12.4.3 Motor Behavior: D_3R KO

The first reported D_3R KO-A line [51] showed increased locomotor activity. Hyperactivity in a novel environment, in the absence of the D_3R, was confirmed in a second KO line, D_3R KO-B [52]. However, the increased motor behavior was observable only during the first several minutes of assessment, but not thereafter, as D_3R KO mice habituated faster than WT to the new environment. D_3R KO-A mice were later reported to show an increase of activity only during the night phase of the light/dark cycle, but not consistently during the day [53]. The mechanism leading to hyperactivity in mice lacking D_3R was correlated with an increased dopaminergic tone [54]. Indeed, increased basal extracellular levels of dopamine, as compared to WT mice, were reported in several D_3R KO lines [54–56]. This contrasts with D_2R KO mice [57, 58] in which basal extracellular dopamine levels are normal, but dopamine release upon stimulation by drugs of abuse leads to an outstanding elevation of dopamine levels [30]. This suggests that the D_3R might regulate dopamine levels under tonic conditions.

Other groups failed to confirm the hyperactive phenotype of D_3R KO-A mice [59, 60], as well as of two independently generated lines, the D_3R KO-C [23] and D_3R KO-D [55]. The reason for this discrepancy is not clear, but very likely depends on the conditions used to perform the experiments in different laboratories. Indeed, D_3R KO-A mice also revealed reduced thigmotaxis in the open field and increased time spent in the open arms of the elevated plus maze with respect to WT mice [61], indicating a reduced level of anxiety in these mice, which might well affect other behavioral measures. Thus, absence of D_3R signaling might facilitate locomotion in conditions yet to be completely defined.

12.4.4 Motor Behavior: D_4R KO

D_4R KO mice have been shown to exhibit less spontaneous locomotion and rearing activity than WT in novel and familiar environments [62]. These mice, in a mixed genetic background (129SV/C57BL/6), outperformed WT mice in the rotarod test, experiencing 50% fewer falls and remaining on the rotating rod 2.5 times longer than WT mice [62], suggesting that D_4Rs participate in the modulation of motor functions.

Interestingly, polymorphisms of the D_4R gene in humans have been correlated with variations in personality traits associated with higher novelty seeking [63, 64]. In line with these findings, D_4R KO mice show reduced exploration in the open field, emergence, and novel object tests despite an overall activity level similar to

that of WT mice. This suggests that D_4R polymorphisms in humans might induce a gain of function for this receptor, which could be correlated with the decrease in novelty-related behaviors observed in mice in which the receptor is deleted [65]. However, recent studies performed in different behavioral conditions, but with the same KO line although in a pure C57BL/6 background, do not support the previously observed decrease of novelty-seeking behaviors in the absence of D_4R [66]. Additional studies are requested to clarify whether or not the D_4R plays a key role in motor functions and novelty seeking by testing D_4R KO (C57BL/6 background) in the same experimental setting used to characterize these mutants in the mixed (F2) genetic background. To date it is possible to conclude that absence of the D_4R does not appear to negatively affect locomotion and actually could even strengthen coordination of movement [62], possibly indicating an inhibitory role of D_4Rs on this function.

12.4.5 Motor Behavior: D_5R KO

Generation of D_5R KO mice has shown that the influence of D_5R-mediated signaling on motor behavior is very subtle. D_5R KO mice responded as WT littermates in a series of behavioral tests aimed at checking most of the functions activated by dopamine signaling. Indeed, when tested in locomotor activity tests, the rotarod test, acoustic startle response, PPI [67], elevated plus maze, light/dark exploration, Morris water maze, and cued and contextual fear conditioning, no statistically relevant differences with WT mice were noted [68]. Nevertheless, more recent analyses are starting to shed some light on the function of this receptor, which appears to have facilitatory functions on motor behavior as established by pharmacological, electrophysiological [43], and ethological analyses [69].

The motor phenotype of DAR KO mice is summarized in Table 12.1.

Table 12.1 Summary of motor phenotypes observed in DAR KO mice

DAR KO	Motor phenotype: basal conditions
D_1 KO	↑ [41, 43, 44]
D_2R KO	↓ [23, 46, 47]
D_{2L} KO	↔ [7, 8]
D_3R KO	↑ [51, 52]
	↔ [59, 60]
D_4R KO	↑ [62]
D_5R KO	↔ [68]
D_1R/D_2R KO	↓↓ [24]
D_1R/D_3R KO	↔ [25, 26]
D_2R/D_3R KO	↓ [23]

References to the phenotype are indicated. The direction of changes is indicated with respect to WT animals exposed to similar conditions. ↑: increase, ↓: decrease, ↔: no difference.

12.5 Dopamine Receptors and Drugs of Abuse

Converging evidence supports the implication of the mesolimbic dopaminergic pathway in the regulation of the rewarding/reinforcing properties of natural stimuli, such as food or sex [70]. This pathway is hijacked by drugs of abuse, which by different mechanisms elevate dopamine levels in the limbic system. As for other dopamine-regulated functions, pharmacological studies have shown that D_1R- and D_2R-mediated signaling have a major impact on the acute and chronic responses to drugs. In this respect, the behavior of KO mutants for D_1R and D_2R strongly supports a preponderant role of signaling mediated by these two receptors in the behavioral and cellular response to abused drugs.

12.5.1 The D_1R and Drugs of Abuse

Interestingly, the D_1R KO-A and KO-B mutant mice failed to exhibit the psychomotor stimulant effect of cocaine on motor and stereotyped behaviors as compared to wild-type littermates [71–73]. Conversely, D_1R KO mice show a significant dose-dependent locomotor decrease after cocaine administration [71]. Investigations at the electrophysiological and gene expression levels of the striatum of the D_1R KO mouse lines showed that this receptor plays an essential role in cocaine [71, 72], amphetamine [74], and thereby dopamine-mediated effects of drugs of abuse. Electrophysiological studies of nucleus accumbens neurons showed that the inhibitory effects of dopamine as well as those of D_1R and D_2R agonists were strongly reduced, whereas those of serotonin were unaffected [71]. In addition, it was later demonstrated that the cellular and behavioral effects of cocaine require intact D_1R- and glutamate receptor-mediated signaling, in vivo, for the activation of the ERK pathway and downstream immediate early genes [75–78]. On the contrary, D_2R agonists and antagonists induced functional effects in the D_1R KO mice at least with respect to induction of immediate early genes (c-fos and jun-b). These results hint that D_1R–D_2R synergism is not obligatory for D_2R function and that D_1-like/D_2-like receptors may interact synergistically or in opposition in striatal neurons, depending on the neural subpopulations engaged [38, 79, 80].

The rewarding properties of drugs can be evaluated by performing conditioned place preference (CPP) experiments. The D_1R KO-B mouse line was used to investigate cocaine reward using this paradigm [81]. While these experiments confirmed that the D_1R is involved in the stimulatory locomotor effects of cocaine, they showed that this receptor does not play a major role in the rewarding effects of this drug [81]. The reinforcing effects of food and drugs of abuse have also been analyzed in D_1R KO-A. Food and opioid agonists in these mice function as positive reinforcers, whereas the effect of cocaine is affected [82]. In addition, D_1R- or D_2R-selective agonists do not work as positive reinforcers in D_1R KO-A mice, whereas these drugs are self-administered by WT littermates [82]. Accordingly, electrophysiological studies showed that KO of the D_1R abolished all DAR-mediated effects, within the nucleus accumbens, including those produced by the D_2-like agonist quinpirole

[71]. These results suggest that D_1Rs participate in the reinforcing effects of cocaine [82] and acquisition of self-administration of this drug. The contradictory results between CPP and self-administration studies might be due to the use of two different paradigms that measure qualitatively different aspects of cocaine effects. More recently, additional experiments have confirmed lack of motor activation upon acute cocaine administration in D_1R KO mice, but a mild sensitization of these mice to the same drug after repeated administration of specific cocaine concentrations [83], indicating that absence of D_1Rs does not fully prevent sensitization to this drug. It is worth mentioning that cocaine is, however, a drug affecting multiple receptor systems and thereby the contribution of other neurotransmitters/modulators in the behavioral response of D_1R KO mice cannot be completely excluded.

Similar results were obtained after amphetamine administration, which, although it failed to induce locomotor behavior in D_1R KO-B mice with respect to WT mice upon acute treatments, did induce a sensitized response upon chronic administration. However, the amplitude of sensitization was less pronounced in the D_1R KO-B than in WT mice in agreement with their reduced acute motor response to the drug [84]. Similar results were also obtained with D_1R KO-A mice [73, 74]. These results confirm earlier pharmacological studies using mice and rats that described the effects of D_1R antagonists on locomotion, behavioral sensitization, and stereotyped responses to cocaine and amphetamine [85, 86]. In addition to the reduced response to cocaine and amphetamine, D_1R KO-B consumed ethanol by self-administration or under forced conditions in a significantly lower amount than the WT control animals [87]. Thus, D_1Rs are clearly central in signaling the rewarding/reinforcing properties of drugs of abuse.

12.5.2 The D_2R and Drugs of Abuse

The contribution of D_2R signaling in the motivational response to drugs of abuse has produced very interesting although complex findings. Indeed, D_2R KO mice increase their locomotion upon treatment with opioids (i.e., morphine) [88], while in response to psychostimulants and alcohol they exhibit a blunted motor response [89–92]. In addition, the blunted motor response to cocaine of D_2R KO-A mice was accompanied at the cellular level by absence of activation of the immediate early gene, c-fos, in the striatum [91]. Interestingly, absence of cellular and behavioral effects in D_2R KO-A mice was specifically observed in response to cocaine, but not to D_1R-specific agonists, which elicited an increased motor response and sustained c-fos induction in these animals [7, 91], thus indicating that the D_1R-mediated signaling is overactivated in D_2R KO mice, as expected by absence of D_2R signaling. Consistent with this observation, cocaine in D_2R KO-A mice induced increased stereotyped behaviors, which very likely contribute to the reduced forward motor response to the drug. However, loss of induction of c-fos expression is at odds with an overactivation of the D_1R-mediated signaling. It was hypothesized that absence of c-fos induction in response to cocaine might be generated by lack of

D_2R heteroreceptors on striatal interneurons and possibly on regulation of GABA release [93]. Increased GABA release might well explain the blunted response of immediate early gene responses to cocaine in animals lacking D_2R. Alternatively, loss of the D_2R might prevent the formation of heteromers with other receptors, which would be required for the induction of pathways leading to c-fos activation.

The rewarding properties of morphine were tested in D_2R KO-A mice by performing CPP analyses. Strikingly, the results of these experiments showed absence of CPP to morphine in these mutants as compared to their WT siblings [88]. These results were recently confirmed and supported by absence of morphine self-administration in D_2R KO-C mice [94, 95]. In addition to loss of the reinforcing properties of morphine, D_2R KO mice do not self-administer ethanol [89, 96, 97]. Conversely, D_2R KO-A mice performed CPP in response to cocaine [91], although the CPP response was attenuated with respect to that of WT controls. D_2R KO-A mice also self-administer more cocaine than WT littermates [98], indicating that D_2R-mediated signaling might be involved in mechanisms that limit rates of high-dose cocaine self-administration. Keeping in mind that the mechanism of action of psychostimulants involves the activation of multiple neurotransmitters/modulators, the possibility exists that in D_2R KO mice absence of reward/reinforcement to morphine and ethanol, but presence of response to cocaine and amphetamine (although attenuated), might be generated by molecules other than dopamine. These results indicate a general role for D_2R-mediated signaling in motivated responding, more than a specific effect of a particular drug in D_2R KO mice. Thereby, D_2R-mediated signaling appears to play an essential role in the response to drugs and ultimately in addiction [99]. Importantly, D_{2L} KO mice have a normal motor response to cocaine and also perform as WT animals in the CPP paradigm to this drug ([91], and our unpublished data). This suggests that the presence of only D_{2S} is sufficient to mediate responses to addictive drugs, possibly through normal modulation of dopamine release [7, 30].

12.5.3 The D_3R and Drugs of Abuse

The D_3R, although expressed at much lower levels with respect to the D_1R and the D_2R, shows a highly restricted expression in limbic regions involved in the regulation of reward, particularly in the nucleus accumbens [100–102]. This suggests that this receptor may play a prominent role in mediating reward-related behaviors [54]. Indeed, drugs acting at the D_3R are potent modulators of the reinforcing effects of psychostimulants, and changes in D_3R function are associated with altered responses to these drugs [54, 103].

The analysis of the D_3R KO mice has provided further evidence by showing that D_3R KO mice are more sensitive to the motor stimulant effects of cocaine at low doses of the drug, as compared to WT animals [52]. However, at high doses the degree of stimulation is similar in both genotypes. As for cocaine, CPP analyses in response to amphetamine demonstrated that D_3R KO mice exhibited an increased response to amphetamine relative to control mice at low doses of the drug [52].

Similar results were also obtained when the D_3R KO mice were assessed for presence of morphine-induced rewarding effects. Indeed, a significant CPP for the drug-associated compartment was observed in D_3R KO mice at low doses of morphine (0.1–0.56 mg/kg), whereas no preference was observed in WT mice at these doses [104].

A different study [105] also reported a higher dose-dependent CPP response to morphine in D_3R KO versus WT animals. D_3R KO mice were more responsive than their WT littermates at the lowest dose of morphine tested while morphine-induced CPP was attenuated in response to higher concentrations of the drug.

The implication of the D_3R in regulating the rewarding/reinforcing properties of drugs has also been assessed pharmacologically, using a D_3R-specific partial agonist BP897 [106–108]. It has been concluded that D_3R is not directly implicated in the reinforcing effects of drugs of abuse, but appears to be involved in the motivation to self-administer drugs in specific conditions (high-requirement self-administration schedules) [109, 110]. These data suggest that the D_3R plays a modulatory role over dopamine-mediated responses to drugs of abuse and, in particular, that this receptor functions as a sensor in the system. This is very likely dependent on the high affinity of the D_3R for dopamine with respect to other D_2-like receptors.

12.5.4 The D_4R and Drugs of Abuse

Polymorphisms of the dopamine D_4R have been associated with novelty seeking in humans [63, 64]; this trait has been associated with risk-taking behaviors and thereby drug abuse. This hypothesis is consistent with studies showing avoidance of novel objects placed in a familiar environment and supersensitivity to the locomotor-stimulating effects of alcohol, amphetamine, and cocaine in D_4R KO mice [62, 65]. More recently, it has been reported that cocaine's potency in producing discriminative stimulus and psychomotor stimulant effects is stronger in D_4R KO than in WT mice. This despite the absence of appreciable novelty seeking in the testing environments in D_4R KO mice, thus suggesting that the response to a novel environment plays only a marginal role in the different sensitivity to cocaine [111]. In addition, D_4R KO mice demonstrated an enhanced and dose-dependent increase in amphetamine-stimulated activity; they also showed enhanced dose-dependent sensitized response to repeated amphetamine administration compared to the WT mice [112].

12.5.5 The D_5R and Drugs of Abuse

In agreement with a very limited effect of absence of D_5Rs on motor functions, responses to drugs of abuse and in particular to cocaine are quite similar in WT and D_5R KO mice. The acute response to cocaine was found mildly attenuated in an original report [113], which has not been subsequently supported using

D_5R KO in a congenic background [83]. This well illustrates the point discussed in the first paragraph of this chapter, namely that small differences found when testing KO animals in mixed backgrounds (F2) might not be maintained in pure backgrounds. Congenic D_5R KO mice were also found to perform as WT littermates in cocaine discrimination stimulus effect as well as in CPP for the same drug [83].

The response to drugs of abuse of DAR KO mice is summarized in Table 12.2.

Table 12.2 Response to drugs of abuse of DAR KO mice

	Responses to drugs of abuse		
DAR KO	Motor activity (acute doses)	CPP	Self-administration
D_1R KO	Cocaine: Ø [71–73]	Cocaine: + [81]	Cocaine: Ø [82] Ethanol: Ø [87]
D_2R KO	Cocaine: ↑ [91]	Cocaine: + [91] (only at high doses) Morphine: Ø[88]	Cocaine: + [98] Ethanol: Ø [89, 96, 97] Morphine: Ø [94, 95]
D_3R KO	Cocaine: ↑↑ [52] (more sensitive to low does than WT mice)	Morphine: + [104] Amphetamine: + [52] (more sensitive to low doses than WT mice)	Implicated: [109, 110]
D_4R O	Cocaine: ↑↑↑ [62, 65]	na	na
D_5R KO	Cocaine: ↑ [83]	Cocaine: + [83]	na

References to the phenotype are indicated. The direction of changes is indicated with respect to WT animals exposed to similar conditions. +: positive response, Ø: no response, ↑: hyperactivity, ↔: same as WT, ↑ : minimal response, na: not assessed.

12.6 Dopamine and Growth

The dopaminergic mesolimbic pathway is naturally involved in the motivational and rewarding mechanisms regulating food intake, thus interfering with growth and very likely with the development of obesity [114, 115]. The generation of KO mice has clarified some aspects of dopamine signaling in the control of this key function. Interestingly, KO mice for both the D_1R and the D_2R have shown that absence of each of the two receptors interferes with food intake and thereby with growth. Indeed, one of the principal features observed in D_1R KO-A and B animals is a reduction of body weight and smaller brain size as compared to their WT littermates [41, 42]. D_2R KO-A mice were also reported to eat less and have a reduction of body weight of 10–15% [23, 46, 47] when adult. These differences in food consumption and growth, however, although relevant do not impair the animal life and indeed both D_1R and D_2R mutants reach adulthood and have normal life span. Nevertheless,

when D_1R and D_2R genes are deleted simultaneously [20] the result is deadly and animals do not survive the second week after birth. These findings clearly show that D_1R- and D_2R-mediated signaling is crucial to regulate food intake, not only by regulating motivation but also by regulating gastrointestinal functions [20]. In agreement with these findings, KO of either D_3Rs, D_4Rs, or D_5Rs does not interfere with either food intake or growth. Importantly, this also shows that D_1R- and D_2R-specific functions cannot be compensated by the activity of any of the other DARs, at least with respect to food intake. Future analyses should be performed to evaluate the role of D_1R- and D_2R-mediated signaling in the escalating search for food, which leads to obesity [114].

12.7 Future Challenges

A decade and a half after the publication of the first DAR KO model, it is now time to move forward and generate additional animal models that would allow an even more detailed analysis of the function of these receptors in vivo. Novel technologies and strategies have been developed to create cell- and region-specific KOs; this surely will expand the types of questions that can be addressed in vivo, as well as produce further insights into the complexity of the dopaminergic system. In addition, recently developed models [35, 38] and others to come might further help the understanding of the dopaminergic system with hopeful positive outcomes not only at the basic science levels but also for the development of more specific and efficient human therapies for the treatment of dopaminergic dysfunctions.

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References

1. Tan S, Hermann B, Borrelli E. Dopaminergic mouse mutants: investigating the roles of the different dopamine receptor subtypes and the dopamine transporter. Int Rev Neurobiol 2003;54:145–97.
2. Missale C, Nash SR, Robinson SW, Jaber M, Caron MG. Dopamine receptors: from structure to function. Physiol Rev 1998;78(1):189–225.
3. Surmeier DJ, Song WJ, Yan Z. Coordinated expression of dopamine receptors in neostriatal medium spiny neurons. J Neurosci 1996;16(20):6579–91.
4. Pickel VM, Nirenberg MJ, Milner TA. Ultrastructural view of central catecholaminergic transmission: immunocytochemical localization of synthesizing enzymes, transporters and receptors. J Neurocytol 1996;25(12):843–56.
5. Picetti R, Saiardi A, Abdel Samad T, Bozzi Y, Baik JH, Borrelli E. Dopamine D2 receptors in signal transduction and behavior. Crit Rev Neurobiol 1997;11(2–3):121–42.
6. Montmayeur JP, Bausero P, Amlaiky N, Maroteaux L, Hen R, Borrelli E. Differential expression of the mouse D2 dopamine receptor isoforms. FEBS Lett 1991;278(2):239–43.
7. Usiello A, Baik JH, Rouge-Pont F, et al. Distinct functions of the two isoforms of dopamine D2 receptors. Nature 2000;408(6809):199–203.

8. Wang Y, Xu R, Sasaoka T, Tonegawa S, Kung MP, Sankoorikal EB. Dopamine D2 long receptor-deficient mice display alterations in striatum-dependent functions. J Neurosci 2000;20(22):8305–14.
9. Giros B, Martres MP, Pilon C, Sokoloff P, Schwartz JC. Shorter variants of the D3 dopamine receptor produced through various patterns of alternative splicing. Biochem Biophys Res Commun 1991;176(3):1584–92.
10. Van Tol HH, Wu CM, Guan HC, et al. Multiple dopamine D4 receptor variants in the human population. Nature 1992;358(6382):149–52.
11. Skrabanek L, Murcia M, Bouvier M, et al. Requirements and ontology for a G protein-coupled receptor oligomerization knowledge base. BMC Bioinformatics 2007;8:177.
12. Thomas KR, Capecchi MR. Site-directed mutagenesis by gene targeting in mouse embryo-derived stem cells. Cell 1987;51(3):503–12.
13. Capecchi MR. Altering the genome by homologous recombination. Science (New York, NY) 1989;244(4910):1288–92.
14. Seeman P, Guan HC, Nobrega J, et al. Dopamine D2-like sites in schizophrenia, but not in Alzheimer's, Huntington's, or control brains, for [3H]benzquinoline. Synapse 1997;25(2):137–46.
15. Boulay D, Depoortere R, Oblin A, Sanger DJ, Schoemaker H, Perrault G. Haloperidol-induced catalepsy is absent in dopamine D(2), but maintained in dopamine D(3) receptor knock-out mice. Eur J Pharmacol 2000;391(1–2):63–73.
16. Saiardi A, Bozzi Y, Baik JH, Borrelli E. Antiproliferative role of dopamine: loss of D2 receptors causes hormonal dysfunction and pituitary hyperplasia. Neuron 1997;19(1): 115–26.
17. Kelly MA, Rubinstein M, Asa SL, et al. Pituitary lactotroph hyperplasia and chronic hyperprolactinemia in dopamine D2 receptor-deficient mice. Neuron 1997;19(1): 103–13.
18. Silva AJ, Simpson EM, Takahashi JS, et al. Mutant mice and neuroscience: recommendations concerning genetic background. Neuron 1997;19(4):755–9.
19. Crawley JN. Behavioral phenotyping strategies for mutant mice. Neuron 2008;57(6): 809–18.
20. Kobayashi M, Iaccarino C, Saiardi A, et al. Simultaneous absence of dopamine D1 and D2 receptor-mediated signaling is lethal in mice. Proc Natl Acad Sci USA 2004;101(31): 11465–70.
21. Zhou QY, Quaife CJ, Palmiter RD. Targeted disruption of the tyrosine hydroxylase gene reveals that catecholamines are required for mouse fetal development. Nature 1995;374(6523):640–3.
22. Zhou QY, Palmiter RD. Dopamine-deficient mice are severely hypoactive, adipsic, and aphagic. Cell 1995;83(7):1197–209.
23. Jung MY, Skryabin BV, Arai M, et al. Potentiation of the D2 mutant motor phenotype in mice lacking dopamine D2 and D3 receptors. Neuroscience 1999;91(3):911–24.
24. Vallone D, Pignatelli M, Grammatikopoulos G, et al. Activity, non-selective attention and emotionality in dopamine D2/D3 receptor knock-out mice. Behav Brain Res 2002;130 (1–2):141–8.
25. Karasinska JM, George SR, El-Ghundi M, Fletcher PJ, O'Dowd BF. Modification of dopamine D(1) receptor knockout phenotype in mice lacking both dopamine D(1) and D(3) receptors. Eur J Pharmacol 2000;399(2–3):171–81.
26. Wong JY, Clifford JJ, Massalas JS, et al. Neurochemical changes in dopamine D1, D3 and D1/D3 receptor knockout mice. Eur J Pharmacol 2003;472(1–2):39–47.
27. Seeman P, Van Tol HH. Dopamine receptor pharmacology. Trends Pharmacol Sci 1994;15(7):264–70.
28. Nicola SM, Surmeier J, Malenka RC. Dopaminergic modulation of neuronal excitability in the striatum and nucleus accumbens. Annu Rev Neurosci 2000;23:185–215.
29. L'Hirondel M, Cheramy A, Godeheu G, et al. Lack of autoreceptor-mediated inhibitory control of dopamine release in striatal synaptosomes of D2 receptor-deficient mice. Brain Res 1998;792(2):253–62.

30. Rouge-Pont F, Usiello A, Benoit-Marand M, Gonon F, Piazza PV, Borrelli E. Changes in extracellular dopamine induced by morphine and cocaine: crucial control by D2 receptors. J Neurosci 2002;22(8):3293–301.
31. Sokoloff P, Diaz J, Le Foll B, et al. The dopamine D3 receptor: a therapeutic target for the treatment of neuropsychiatric disorders. CNS Neurol Disord Drug Targets 2006;5(1): 25–43.
32. Seeman P, Kapur S. Schizophrenia: more dopamine, more D2 receptors. Proc Natl Acad Sci USA 2000;97(14):7673–5.
33. Ralph RJ, Varty GB, Kelly MA, et al. The dopamine D2, but not D3 or D4, receptor subtype is essential for the disruption of prepulse inhibition produced by amphetamine in mice. J Neurosci 1999;19(11):4627–33.
34. Doherty JM, Masten VL, Powell SB, et al. Contributions of dopamine D1, D2, and D3 receptor subtypes to the disruptive effects of cocaine on prepulse inhibition in mice. Neuropsychopharmacology 2007.
35. Surmeier DJ, Ding J, Day M, Wang Z, Shen W. D1 and D2 dopamine-receptor modulation of striatal glutamatergic signaling in striatal medium spiny neurons. Trends Neurosci 2007;30(5):228–35.
36. Mallet N, Ballion B, Le Moine C, Gonon F. Cortical inputs and GABA interneurons imbalance projection neurons in the striatum of parkinsonian rats. J Neurosci 2006;26(14): 3875–84.
37. Shen W, Flajolet M, Greengard P, Surmeier DJ. Dichotomous dopaminergic control of striatal synaptic plasticity. Science (New York, NY) 2008;321(5890):848–51.
38. Bertran-Gonzalez J, Bosch C, Maroteaux M, et al. Opposing patterns of signaling activation in dopamine D1 and D2 receptor-expressing striatal neurons in response to cocaine and haloperidol. J Neurosci 2008;28(22):5671–85.
39. O'Dowd BF, Ji X, Alijaniaram M, et al. Dopamine receptor oligomerization visualized in living cells. J Biol Chem 2005;280(44):37225–35.
40. Rashid AJ, So CH, Kong MM, et al. D1-D2 dopamine receptor heterooligomers with unique pharmacology are coupled to rapid activation of Gq/11 in the striatum. Proc Natl Acad Sci USA 2007;104(2):654–9.
41. Xu M, Moratalla R, Gold LH, et al. Dopamine D1 receptor mutant mice are deficient in striatal expression of dynorphin and in dopamine-mediated behavioral responses. Cell 1994;79(4):729–42.
42. Drago J, Gerfen CR, Lachowicz JE, et al. Altered striatal function in a mutant mouse lacking D1A dopamine receptors. Proc Natl Acad Sci USA 1994;91(26):12564–8.
43. Centonze D, Grande C, Saulle E, et al. Distinct roles of D1 and D5 dopamine receptors in motor activity and striatal synaptic plasticity. J Neurosci 2003;23(24):8506–12.
44. Granado N, Ortiz O, Suarez LM, et al. D1 but not D5 dopamine receptors are critical for LTP, spatial learning, and LTP-Induced arc and zif268 expression in the hippocampus. Cereb Cortex 2008;18(1):1–12.
45. McNamara FN, Clifford JJ, Tighe O, et al. Congenic D1A dopamine receptor mutants: ethologically based resolution of behavioural topography indicates genetic background as a determinant of knockout phenotype. Neuropsychopharmacology 2003;28(1):86–99.
46. Baik JH, Picetti R, Saiardi A, et al. Parkinsonian-like locomotor impairment in mice lacking dopamine D2 receptors. Nature 1995;377(6548):424–8.
47. Kelly MA, Rubinstein M, Phillips TJ, et al. Locomotor activity in D2 dopamine receptor-deficient mice is determined by gene dosage, genetic background, and developmental adaptations. J Neurosci 1998;18(9):3470–9.
48. Clifford JJ, Usiello A, Vallone D, Kinsella A, Borrelli E, Waddington JL. Topographical evaluation of behavioural phenotype in a line of mice with targeted gene deletion of the D2 dopamine receptor. Neuropharmacology 2000;39(3):382–90.
49. Clifford JJ, Kinsella A, Tighe O, et al. Comparative, topographically-based evaluation of behavioural phenotype and specification of D(1)-like:D(2) interactions in a line of incipient congenic mice with D(2) dopamine receptor ′knockout′. Neuropsychopharmacology 2001;25(4):527–36.

50. Waddington JL, Daly SA, Downes RP, Deveney AM, McCauley PG, O'Boyle KM. Behavioural pharmacology of ′D-1-like′ dopamine receptors: further subtyping, new pharmacological probes and interactions with ′D-2-like′ receptors. Prog Neuropsychopharmacol Biol Psychiatry 1995;19(5):811–31.
51. Accili D, Fishburn CS, Drago J, et al. A targeted mutation of the D3 dopamine receptor gene is associated with hyperactivity in mice. Proc Natl Acad Sci USA 1996;93(5):1945–9.
52. Xu M, Koeltzow TE, Santiago GT, et al. Dopamine D3 receptor mutant mice exhibit increased behavioral sensitivity to concurrent stimulation of D1 and D2 receptors. Neuron 1997;19(4):837–48.
53. Betancur C, Lepee-Lorgeoux I, Cazillis M, Accili D, Fuchs S, Rostene W. Neurotensin gene expression and behavioral responses following administration of psychostimulants and antipsychotic drugs in dopamine D(3) receptor deficient mice. Neuropsychopharmacology 2001;24(2):170–82.
54. Le Foll B, Diaz J, Sokoloff P. Neuroadaptations to hyperdopaminergia in dopamine D3 receptor-deficient mice. Life Sci 2005;76(11):1281–96.
55. Joseph JD, Wang YM, Miles PR, et al. Dopamine autoreceptor regulation of release and uptake in mouse brain slices in the absence of D(3) receptors. Neuroscience 2002;112(1): 39–49.
56. Koeltzow TE, Xu M, Cooper DC, et al. Alterations in dopamine release but not dopamine autoreceptor function in dopamine D3 receptor mutant mice. J Neurosci 1998;18(6):2231–8.
57. Dickinson SD, Sabeti J, Larson GA, et al. Dopamine D2 receptor-deficient mice exhibit decreased dopamine transporter function but no changes in dopamine release in dorsal striatum. J Neurochem 1999;72(1):148–56.
58. Schmitz Y, Schmauss C, Sulzer D. Altered dopamine release and uptake kinetics in mice lacking D2 receptors. J Neurosci 2002;22(18):8002–9.
59. McNamara FN, Clifford JJ, Tighe O, et al. Phenotypic, ethologically based resolution of spontaneous and D(2)-like vs D(1)-like agonist-induced behavioural topography in mice with congenic D(3) dopamine receptor "knockout". Synapse 2002;46(1):19–31.
60. Boulay D, Depoortere R, Rostene W, Perrault G, Sanger DJ. Dopamine D3 receptor agonists produce similar decreases in body temperature and locomotor activity in D3 knock-out and wild-type mice. Neuropharmacology 1999;38(4):555–65.
61. Steiner H, Fuchs S, Accili D. D3 dopamine receptor-deficient mouse: evidence for reduced anxiety. Physiol Behav 1997;63(1):137–41.
62. Rubinstein M, Phillips TJ, Bunzow JR, et al. Mice lacking dopamine D4 receptors are supersensitive to ethanol, cocaine, and methamphetamine. Cell 1997;90(6):991–1001.
63. Benjamin J, Li L, Patterson C, Greenberg BD, Murphy DL, Hamer DH. Population and familial association between the D4 dopamine receptor gene and measures of Novelty Seeking. Nat Genet 1996;12(1):81–4.
64. Ebstein RP, Novick O, Umansky R, et al. Dopamine D4 receptor (D4DR) exon III polymorphism associated with the human personality trait of Novelty Seeking. Nat Genet 1996;12(1):78–80.
65. Dulawa SC, Grandy DK, Low MJ, Paulus MP, Geyer MA. Dopamine D4 receptor-knock-out mice exhibit reduced exploration of novel stimuli. J Neurosci 1999;19(21):9550–6.
66. Helms CM, Gubner NR, Wilhelm CJ, Mitchell SH, Grandy DK. D4 receptor deficiency in mice has limited effects on impulsivity and novelty seeking. Pharmacol Biochem Behav 2008;90(3):387–93.
67. Braff DL, Grillon C, Geyer MA. Gating and habituation of the startle reflex in schizophrenic patients. Arch Gen Psychiatry 1992;49(3):206–15.
68. Holmes A, Hollon TR, Gleason TC, et al. Behavioral characterization of dopamine D5 receptor null mutant mice. Behav Neurosci 2001;115(5):1129–44.
69. O'Sullivan GJ, Kinsella A, Sibley DR, Tighe O, Croke DT, Waddington JL. Ethological resolution of behavioural topography and D1-like versus D2-like agonist responses in congenic D5 dopamine receptor mutants: identification of D5:D2-like interactions. Synapse 2005;55(4):201–11.

70. Di Chiara G, Bassareo V. Reward system and addiction: what dopamine does and doesn't do. Curr Opin Pharmacol 2007;7(1):69–76.
71. Xu M, Hu XT, Cooper DC, et al. Elimination of cocaine-induced hyperactivity and dopamine-mediated neurophysiological effects in dopamine D1 receptor mutant mice. Cell 1994;79(6):945–55.
72. Drago J, Gerfen CR, Westphal H, Steiner H. D1 dopamine receptor-deficient mouse: cocaine-induced regulation of immediate-early gene and substance P expression in the striatum. Neuroscience 1996;74(3):813–23.
73. Zhang J, Walsh RR, Xu M. Probing the role of the dopamine D1 receptor in psychostimulant addiction. Ann N Y Acad Sci 2000;914:13–21.
74. Xu M, Guo Y, Vorhees CV, Zhang J. Behavioral responses to cocaine and amphetamine administration in mice lacking the dopamine D1 receptor. Brain Res 2000;852(1):198–207.
75. Konradi C. The molecular basis of dopamine and glutamate interactions in the striatum. Adv Pharmacol (San Diego, Calif) 1998;42:729–33.
76. Valjent E, Corvol JC, Pages C, Besson MJ, Maldonado R, Caboche J. Involvement of the extracellular signal-regulated kinase cascade for cocaine-rewarding properties. J Neurosci 2000;20(23):8701–9.
77. Valjent E, Pascoli V, Svenningsson P, et al. Regulation of a protein phosphatase cascade allows convergent dopamine and glutamate signals to activate ERK in the striatum. Proc Natl Acad Sci USA 2005;102(2):491–6.
78. Brami-Cherrier K, Valjent E, Herve D, et al. Parsing molecular and behavioral effects of cocaine in mitogen- and stress-activated protein kinase-1-deficient mice. J Neurosci 2005;25(49):11444–54.
79. Levine MS, Altemus KL, Cepeda C, et al. Modulatory actions of dopamine on NMDA receptor-mediated responses are reduced in D1A-deficient mutant mice. J Neurosci 1996;16(18):5870–82.
80. Moratalla R, Xu M, Tonegawa S, Graybiel AM. Cellular responses to psychomotor stimulant and neuroleptic drugs are abnormal in mice lacking the D1 dopamine receptor. Proc Natl Acad Sci USA 1996;93(25):14928–33.
81. Miner LL, Drago J, Chamberlain PM, Donovan D, Uhl GR. Retained cocaine conditioned place preference in D1 receptor deficient mice. Neuroreport 1995;6(17):2314–6.
82. Caine SB, Thomsen M, Gabriel KI, et al. Lack of self-administration of cocaine in dopamine D1 receptor knock-out mice. J Neurosci 2007;27(48):13140–50.
83. Karlsson RM, Hefner KR, Sibley DR, Holmes A. Comparison of dopamine D1 and D5 receptor knockout mice for cocaine locomotor sensitization. Psychopharmacology (Berl) 2008;200(1):117–27.
84. Crawford CA, Drago J, Watson JB, Levine MS. Effects of repeated amphetamine treatment on the locomotor activity of the dopamine D1A-deficient mouse. Neuroreport 1997;8(11):2523–7.
85. Koob GF, Caine B, Markou A, Pulvirenti L, Weiss F. Role for the mesocortical dopamine system in the motivating effects of cocaine. NIDA Res Monogr 1994;145:1–18.
86. Cabib S, Castellano C, Cestari V, Filibeck U, Puglisi-Allegra S. D1 and D2 receptor antagonists differently affect cocaine-induced locomotor hyperactivity in the mouse. Psychopharmacology (Berl) 1991;105(3):335–9.
87. El-Ghundi M, George SR, Drago J, et al. Disruption of dopamine D1 receptor gene expression attenuates alcohol-seeking behavior. Eur J Pharmacol 1998;353(2–3):149–58.
88. Maldonado R, Saiardi A, Valverde O, Samad TA, Roques BP, Borrelli E. Absence of opiate rewarding effects in mice lacking dopamine D2 receptors. Nature 1997;388(6642):586–9.
89. Phillips TJ, Brown KJ, Burkhart-Kasch S, et al. Alcohol preference and sensitivity are markedly reduced in mice lacking dopamine D2 receptors. Nat Neurosci 1998;1(7):610–5.
90. Parish CL, Stanic D, Drago J, Borrelli E, Finkelstein DI, Horne MK. Effects of long term treatment with dopamine receptor agonists and antagonists on terminal arbor size. Eur J Neurosci 2002;16(5):787–94.

91. Welter M, Vallone D, Samad TA, Meziane H, Usiello A, Borrelli E. Absence of dopamine D2 receptors unmasks an inhibitory control over the brain circuitries activated by cocaine. Proc Natl Acad Sci USA 2007;104(16):6840–5.
92. Kelly MA, Low MJ, Rubinstein M, Phillips TJ. Role of dopamine D1-like receptors in methamphetamine locomotor responses of D2 receptor knockout mice. Genes Brain Behav 2008;7(5):568–77.
93. Centonze D, Grande C, Usiello A, et al. Receptor subtypes involved in the presynaptic and postsynaptic actions of dopamine on striatal interneurons. J Neurosci 2003;23(15): 6245–54.
94. Elmer GI, Pieper JO, Rubinstein M, Low MJ, Grandy DK, Wise RA. Failure of intravenous morphine to serve as an effective instrumental reinforcer in dopamine D2 receptor knock-out mice. J Neurosci 2002;22(10):RC224.
95. Elmer GI, Pieper JO, Levy J, et al. Brain stimulation and morphine reward deficits in dopamine D2 receptor-deficient mice. Psychopharmacology (Berl) 2005;182(1):33–44.
96. Cunningham CL, Howard MA, Gill SJ, Rubinstein M, Low MJ, Grandy DK. Ethanol-conditioned place preference is reduced in dopamine D2 receptor-deficient mice. Pharmacol Biochem Behav 2000;67(4):693–9.
97. Risinger FO, Freeman PA, Rubinstein M, Low MJ, Grandy DK. Lack of operant ethanol self-administration in dopamine D2 receptor knockout mice. Psychopharmacology (Berl) 2000;152(3):343–50.
98. Caine SB, Negus SS, Mello NK, et al. Role of dopamine D2-like receptors in cocaine self-administration: studies with D2 receptor mutant mice and novel D2 receptor antagonists. J Neurosci 2002;22(7):2977–88.
99. Volkow ND, Fowler JS. Addiction, a disease of compulsion and drive: involvement of the orbitofrontal cortex. Cereb Cortex 2000;10(3):318–25.
100. Bouthenet ML, Souil E, Martres MP, Sokoloff P, Giros B, Schwartz JC. Localization of dopamine D3 receptor mRNA in the rat brain using in situ hybridization histochemistry: comparison with dopamine D2 receptor mRNA. Brain Res 1991;564(2):203–19.
101. Levesque D, Diaz J, Pilon C, et al. Identification, characterization, and localization of the dopamine D3 receptor in rat brain using 7-[3H]hydroxy-N,N-di-n-propyl-2-aminotetralin. Proc Natl Acad Sci USA 1992;89(17):8155–9.
102. Sokoloff P, Giros B, Martres MP, Bouthenet ML, Schwartz JC. Molecular cloning and characterization of a novel dopamine receptor (D3) as a target for neuroleptics. Nature 1990;347(6289):146–51.
103. Vorel SR, Ashby CR, Jr., Paul M, et al. Dopamine D3 receptor antagonism inhibits cocaine-seeking and cocaine-enhanced brain reward in rats. J Neurosci 2002;22(21):9595–603.
104. Narita M, Mizuo K, Mizoguchi H, et al. Molecular evidence for the functional role of dopamine D3 receptor in the morphine-induced rewarding effect and hyperlocomotion. J Neurosci 2003;23(3):1006–12.
105. Frances H, Le Foll B, Diaz J, Smirnova M, Sokoloff P. Role of DRD3 in morphine-induced conditioned place preference using drd3-knockout mice. Neuroreport 2004;15(14): 2245–9.
106. Pilla M, Perachon S, Sautel F, et al. Selective inhibition of cocaine-seeking behaviour by a partial dopamine D3 receptor agonist. Nature 1999;400(6742):371–5.
107. Le Foll B, Frances H, Diaz J, Schwartz JC, Sokoloff P. Role of the dopamine D3 receptor in reactivity to cocaine-associated cues in mice. Eur J Neurosci 2002;15(12):2016–26.
108. Le Foll B, Schwartz JC, Sokoloff P. Disruption of nicotine conditioning by dopamine D(3) receptor ligands. Mol Psychiatry 2003;8(2):225–30.
109. Holmes A, Lachowicz JE, Sibley DR. Phenotypic analysis of dopamine receptor knock-out mice; recent insights into the functional specificity of dopamine receptor subtypes. Neuropharmacology 2004;47(8):1117–34.
110. Le Foll B, Goldberg SR, Sokoloff P. The dopamine D3 receptor and drug dependence: effects on reward or beyond? Neuropharmacology 2005;49(4):525–41.

111. Katz JL, Chausmer AL, Elmer GI, Rubinstein M, Low MJ, Grandy DK. Cocaine-induced locomotor activity and cocaine discrimination in dopamine D4 receptor mutant mice. Psychopharmacology (Berl) 2003;170(1):108–14.
112. Kruzich PJ, Suchland KL, Grandy DK. Dopamine D4 receptor-deficient mice, congenic on the C57BL/6 J background, are hypersensitive to amphetamine. Synapse 2004;53(2):131–9.
113. Elliot EE, Sibley DR, Katz JL. Locomotor and discriminative-stimulus effects of cocaine in dopamine D5 receptor knockout mice. Psychopharmacology (Berl) 2003;169(2):161–8.
114. Wang GJ, Volkow ND, Logan J, et al. Brain dopamine and obesity. Lancet 2001;357(9253):354–7.
115. Volkow ND, Wise RA. How can drug addiction help us understand obesity? Nat Neurosci 2005;8(5):555–60.

Chapter 13
Dopamine Receptors and Behavior: From Psychopharmacology to Mutant Models

Gerard J. O'Sullivan, Colm O'Tuathaigh, Katsunori Tomiyama, Noriaki Koshikawa, and John L. Waddington

Abstract Elucidating the relative involvement of individual dopamine receptor subtypes in the regulation of behavior has been made difficult by anomalies at the psychopharmacology–molecular biology interface; specifically, the extent to which gene cloning has revealed greater diversity in dopamine receptor typology beyond the original D_1/D_2 classification, to include individual members of D1-like (D_1 and D_5) and D2-like (D_{2Short}, D_{2Long}, D_3, and D_4) families, has not been matched by similar progress in developing selective agonists and antagonists for these receptors. Thus, although classical psychopharmacological approaches have been instrumental in defining dopamine-dependent behaviors at the family level, more incisive molecular genetic techniques have been required to determine the functional roles of the individual members of these families. This chapter seeks to (a) summarize the classical psychopharmacology of dopamine receptor subtype function, (b) provide an overview of recent findings in dopamine receptor subtype knockouts across several domains of behavior, and (c) interpret new insights in the context of the limitations of these techniques and prior knowledge of the regulation of behavior by dopamine receptors.

Keywords Dopamine receptor subtypes · Behavior · Psychopharmacology · Selective agonists and antagonists · Molecular biology · Knockouts · Knockins · Transgenics · Mutant models

13.1 Introduction

Over the past 50 years it has been recognized that (a) dopaminergic neurons in the brain are topographically organized in four distinct populations, known as the nigrostriatal, mesolimbic, mesocortical, and tuberoinfundibular pathways and

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(b) these anatomical distinctions are, in broad terms, reflected in modulation of motoric, motivational, cognitive, and neuroendocrine functions, respectively [1, 2]. Furthermore, disruption of dopamine signaling is associated with a number of pathological conditions including schizophrenia, Parkinson's disease, and Huntington's disease, as detailed elsewhere in this book.

Elucidating the relative involvement of individual dopamine receptor subtypes in these processes and the identification of novel dopamine-mediated behaviors has been impeded by incongruence at the psychopharmacology–molecular biology interface [3]. Specifically, the extent to which gene cloning has revealed greater diversity in dopamine receptor typology beyond the original D_1/D_2 classification, to include individual members of D1-like (D_1 and D_5) and D2-like (D_{2Short}, D_{2Long}, D_3, and D_4) families, has not been matched by similar progress in developing selective agonists and antagonists for these receptors. Thus, although classical psychopharmacological approaches have been instrumental in defining dopamine-dependent behaviors at the family level, more incisive molecular genetic techniques have been required to determine the functional roles of the individual members of these families [4].

The profound impact of gene targeting on dopamine research is illustrated by the fact that within 10 years of its application to the field, knockout mice were created for dopamine [5], the dopamine transporter [6], each of the cloned mammalian dopamine receptor subtypes [1, 7–10], including splice variants of the D_2 receptor [11, 12], and discrete intracellular signal transduction coupling proteins [4].

During this period a number of reviews have described the evolution of findings in dopamine receptor knockouts at various levels of phenotypic investigation [1–4, 13–17]. With regard to dopaminergic regulation of behavior, recent reviews have evaluated findings in dopamine receptor knockouts in the context of a specific behavioral disorder [16] or a generalized perspective of behavioral roles for each receptor subtype [1]. We have offered comprehensive reviews on how studies in knockout mice have helped to elucidate the relative involvement of individual dopamine receptors in spontaneous and drug-induced unconditioned behavior [3, 4]. This chapter aims to elaborate upon these earlier reviews by (a) providing an update of recent behavioral findings in dopamine receptor knockouts, (b) describing additional findings from studies of conditioned and cognitive behavior in these mutants, and (c) placing these developments in the context of prior knowledge of dopamine receptor function in behavior.

13.2 Psychopharmacological Studies

A detailed account of findings obtained to date from behavioral studies using selective dopamine agonists and antagonists in intact vs. lesioned animals is voluminous and beyond the scope of this chapter. Reviews of basic concepts in the regulation of behavior by D1-like vs. D2-like receptors and by cooperative/synergistic and

oppositional D1-like:D2-like interactions are available [18–20]. Thus, we have chosen to focus primarily on behavioral modalities and psychopharmacological agents that have undergone complementary evaluation in dopamine receptor knockouts in an effort to facilitate the appraisal of behavioral findings using both approaches and the identification of dopamine receptor-specific functional roles. For example, we have previously investigated in rats the role of D1-like vs. D2-like receptors, their interactions and associated transduction mechanisms in the regulation of orofacial movements as a model system for studying these processes [21–24]. However, only by developing more incisive assessment systems applicable to mice [25–27] have we been able to exploit the potential of dopamine receptor subtype knockouts for elucidating the role of individual receptor subtypes in the regulation of specific topographies of orofacial movement.

13.2.1 D1-Like Receptors and Behavior

An early study found that the prototypical D1-like partial agonist SKF 38393 induced contralateral rotation in rats with unilateral lesions of substantia nigra [28]. Subsequent investigations in non-lesioned rodents demonstrated that SKF 38393 potently induced grooming behavior, including intense grooming syntax: a characteristic subset of generalized grooming where the animal grooms the snout and the face with the forepaws, followed immediately by vigorous grooming of the flank/anogenital area with the snout [29]. Syntactic, intense grooming has been well described in the ethological literature and appears to be modulated by a pattern generator site within the anterior dorsolateral striatum and expressed via the globus pallidus/ventral pallidum [30]. Induction of intense grooming behavior has since become a widely accepted behavioral index of D1-like receptor activation in rodents [18, 19, 31].

Low in vivo potency of SKF 38393 and only partial agonist activity at D1-like receptors in terms of stimulating dopamine-sensitive adenylate cyclase prompted the search for D1-like agonists with a more desirable pharmacological profile [18, 32]. Accordingly, several analogues of SKF 38393 were developed with varying efficacy to stimulate dopamine-sensitive adenylate cyclase [33, 34]. Behavioral studies using these agonists indicate that D1-like activation can, among others, alleviate motor deficits in animal models of Parkinson's disease [35], induce discrete topographies of orofacial movement [26], and reduce the reinforcing effects of cocaine [36]. Furthermore, studies using the selective D1-like antagonist SCH 23390 have identified functional roles for D1-like receptors in the cataleptic response to dopamine depletion [37] and in reward-related learning [38].

More recent work in our laboratories using two selective D1-like agonists with distinct biochemical effects indicates that subsets of D1-like-dependent behaviors are coupled to distinct intracellular signaling mechanisms [21–24, 39]. For example, SKF 83959, which fails to stimulate adenylate cyclase but stimulates phospholipase C-mediated intracellular calcium release via heterooligomerization with D_2 receptors [40], readily induces grooming and vacuous chewing that is sensitive to

antagonism by SCH 23390, together with other effects typical of D1-like receptor agonists [18]. Conversely, SKF 83822 which stimulates dopamine-sensitive adenylate cyclase, but not phospholipase C, induces prominent seizures in mice in the absence of any material effect on grooming behavior [39].

The high degree of sequence homology that exists between D_1 and D_5 receptors translates into substantial similarity in their respective pharmacological profiles. Accordingly, at present no ligands have been identified which can materially differentiate these cloned receptor subtypes in terms of ligand-binding affinity or potency to stimulate adenylate cyclase using recombinant cell lines. Ironically, dopamine itself is the most discriminating agent within the D1-like receptor family, demonstrating approximately tenfold higher affinity for the human D_5 receptor. Certain antagonists, including *cis* [Z]-flupentixol and [+]-butaclamol, exhibit seven- to tenfold lower affinity for D_5 than D_1 receptors. While these ligands can be used to differentiate D_1 and D_5 receptors in radioligand-binding studies and functional in vitro assays, none can be used reliably to determine subtype-specific functions in vivo [18].

13.2.2 D2-Like Receptors and Behavior

Early experiments with selective D2-like agonists, including quinpirole and quinelorane, found that activation of D2-like receptors induced circling behavior in rats with unilateral lesions in the basal ganglia [41]. Shortly thereafter, it was reported that the induction of locomotion and rearing by apomorphine in mice was mimicked by administration of RU 24213 alone [42], consistent with a fundamental role for D2-like receptors in facilitating movement-related behavior. Indeed, proven effectiveness of D2-like agonists in counteracting motor deficits in various animal models of Parkinson's disease initiated their use clinically, and D2-like agonists remain a primary strategy in treating the disease [43]. Other findings that several D2-like antagonists, including haloperidol and YM 09151-2, could attenuate apomorphine- or amphetamine-stimulated hyperactivity in rodents [44] provided indirect evidence for a fundamental role of D2-like receptors in the regulation of such behavior. Furthermore, reports that D2-like agonists and antagonists are, respectively, anticonvulsant and pro-convulsant, in epilepsy models [45] indicate an important role for D2-like receptors in modulating seizure threshold.

Despite extensive structural homology between D_2 and D_3 receptors, a number of compounds with high affinity and moderate selectivity for D_3 receptors have been developed. Low doses of the D_3-preferring agonists 7-OH-DPAT and PD 128907 have been reported to reduce spontaneous activity in rats, whereas higher doses stimulate non-stereotyped sniffing, locomotion, and chewing [46, 47]. More recent findings from studies with 7-OH-DPAT suggest that D_3 receptors are additionally involved in discrete aspects of social behavior [48], anxiety [49], and attention [50]. Furthermore, convergent evidence from studies with D_3 antagonists indicates an important functional role for D_3 receptors in facilitating drug-seeking behavior [51]. Localization of D_3 receptors in cortico-limbic brain regions associated with

cognitive and emotional functions has provoked considerable interest in selective D_3 receptor antagonists as potential antipsychotic agents devoid of the extrapyramidal side effects linked to striatal D_2 receptor blockade [52].

The initial observation that the second-generation antipsychotic clozapine has higher affinity for D_4 vs. D_2 receptors stimulated interest in developing D_4 antagonists as putative therapies in the treatment of schizophrenia. While some pre-clinical studies suggested antipsychotic effects for selective D_4 antagonists, others found little or no effect. Moreover, a number of clinical trials have found that selective D_4 receptor antagonism is ineffective in alleviating psychotic symptoms in patients with schizophrenia [53]. Studies in our own laboratory have demonstrated that the selective D_4 receptor antagonists L 745,870 and RO 61-6270 do not influence spontaneous or D2-like agonist-induced motor behavior [54], suggesting that D_4 receptors might be involved in more discrete social and cognitive processes. Indeed, D_4 receptor blockade has been found to improve cognitive impairments in both rodents [55] and non-human primates [56]. Furthermore, Zhang and colleagues have reported that D_4 antagonists are effective in an animal model of attention deficit hyperactivity disorder [57]. More recently, a role for D_4 receptors in emotion has been suggested by the report that infusion of L 745,870 into the medial prefrontal cortex increases the percentage of open-arm entries and open-arm time in the elevated plus maze test for anxiety [58]. Furthermore, studies with the selective D_4 agonists PD 168,077 and RO 10-5824 have suggested functional roles for the D_4 receptor in memory consolidation [59] and novelty-seeking behavior [60], respectively.

13.3 D1-like Receptor Family

Among the five cloned dopamine receptor subtypes, the D_1 receptor gene was the first to successfully undergo targeted deletion by homologous recombination [10, 61]. Despite normal gross anatomy, brain architecture, and primitive reflexes, homozygous D_1 knockouts are significantly growth retarded, weighing on average 20–30% less than heterozygous knockout and wild type littermates [10, 61–63]. Five years later, mice lacking functional D_5 receptors were reported [15]. Homozygous D_5 knockouts are indistinguishable from wild types on gross inspection and display intact sensory abilities and neurological reflexes [64].

13.3.1 D_1 Knockout: Spontaneous Behavior

Initial studies using D_1 knockouts maintained on a hybrid (129/Sv × C57BL/6) genetic background found that horizontal and vertical activity were markedly elevated in D_1 knockouts in both novel and familiar environments [10, 65]. In contrast, two subsequent studies using an independently created D_1 knockout line maintained on a similar hybrid genetic background observed reduced rearing, but normal locomotor activity, in D_1 knockouts relative to wild types [61, 66]. Conversely, a more

comprehensive behavioral evaluation of the same hybrid D_1 knockout line [63] and a third independently created D_1 knockout [67] demonstrated a modest reduction in the distance traveled by D_1 knockouts over a short period of assessment in a novel environment; however, a prominent delay in the initiation of movement was evident in D_1 knockouts upon initial exposure to the test arena in one of these studies, which likely contributed to the observed locomotor effect [63]. Another group has reported that hybrid D_1 knockouts exhibit reduced locomotion and rearing relative to wild types over 15 min in an open field but not over 60 min in an automated activity chamber [68]. In contrast, a more recent study by these investigators, using same knockout line backcrossed to C57BL/6 for two generations, demonstrated marked hyperactivity in D_1 knockouts in both novel and familiar environments [69]. Spontaneous hyperactivity has also been observed in hybrid D_1 knockouts over a longer time frame under habituated conditions [70], consistent with an inhibitory role for D_1 receptors in movement-related behavior. Studies in our own laboratory have confirmed and elaborated the hyperactive phenotype in mice lacking D_1 receptors. Specifically, using a comprehensive ethologically based behavioral assessment technique, hybrid D_1 knockouts were found to display increased sniffing and locomotion together with altered grooming during an initial period of exploration in a novel environment [71, 72]. Furthermore, we have shown that habituation of sniffing, locomotion, and rearing during the course of familiarization with an environment is profoundly retarded in congenic D_1 knockouts [62].

D_1 knockouts have also been examined in a number of behavioral paradigms designed to assess learning and reward. In the Morris water maze, hybrid D_1 knockouts exhibit a longer latency to locate a submerged escape platform [63, 66, 68], despite having a swim speed comparable to wild types and intact locomotor coordination [61, 63, 66]. Another study has found that hybrid D_1 knockouts require significantly more trials than wild types to learn an operant conditioning paradigm and demonstrate reduced responding for sucrose under various reinforcement schedules. Furthermore, D_1 knockouts were found to have a longer extinction time and impaired reversal learning following acquisition of the task [73]. More recently, impaired learning was observed in D_1 knockouts when the temporal relationship between reward-oriented behavior and reward retrieval was increased. Specifically, D_1 knockouts performed more poorly than wild types in the "triple T" maze in which a predetermined sequence of correct left–right turns is required to obtain a distal food reward [74]. Similar to the earlier report described above [73], the magnitude of the learning deficit in D_1 knockouts was greater when the required turn sequence was altered following acquisition of the task [74]. However, it may be that impaired learning in D_1 knockouts is context dependent, as they have been reported to perform normally in spontaneous alternation, passive avoidance, fear conditioning, and two odor discrimination paradigms [63, 66]. Evidence for blunted reward-related behavior in the absence of D_1 receptors has been provided by the recent report that D_1 knockouts travel less and obtain fewer brain stimulation rewards than controls in spatial learning tasks [67]. Indeed, more intense intracranial self-stimulation of the nucleus accumbens was required in D_1 knockouts to achieve wild type levels of responding in this study.

Additional studies have been performed in D_1 knockouts at other levels of behavioral investigation. With regard to dopaminergic regulation of spontaneous orofacial movements, congenic D_1 knockouts have been shown to display a marked reduction in horizontal jaw movements in the absence of any material alteration in vertical jaw movements, together with lower levels of tongue protrusions and incisor chattering [75]; an additional finding, that D1-like agonist-stimulated orofacial topographies are absent in D_1 knockouts, provides convincing evidence that D_1 receptors are indeed responsible for the observed phenotypic effects. Inconsistent findings have emerged in relation to the putative role for D_1 receptors in the behavioral manifestation of anxiety. In particular, one study found that hybrid D_1 knockouts spent less time in the more aversive open arms of the elevated plus maze than wild types and executed a reduced number of transitions between the open and closed arms [76], while other studies observed no effect of genotype in this test [68] or the light–dark paradigm [77].

13.3.2 D_1 Knockout: Drug-Induced Behavior

Pharmacological studies using selective D1-like and D2-like ligands in D_1 knockouts have helped to determine the relative involvement of D_1 vs. D_5 receptors in D1-like-dependent behaviors and to identify functional D_1:D2-like interactions. Initial phenotypic characterization of hybrid D_1 knockouts revealed that the locomotor-stimulant effects of the selective D1-like agonist, SKF 81297, are abolished in the absence of D_1 receptors. Furthermore, catalepsy induced by the selective D1-like antagonist, SCH 23390, in the ring test was absent in D_1 knockouts [10]. More recently, we elaborated these findings by demonstrating that constituent behaviors in the ethogram of congenic D_1 knockouts are unaltered by a D1-like agonist or antagonist [62]. Despite inconsistent findings in relation to the level of spontaneous grooming behavior in D_1 knockouts [71, 78], characteristic induction of grooming by SKF 83959 is reduced in these mutants relative to wild types [62, 79]. Furthermore, grooming induced by cocaine and various neuropeptides is attenuated in D_1 knockouts [80, 81]. Additionally, the report that haloperidol-induced catalepsy is dose dependently exaggerated in D_1 knockouts [82] indicates that inhibitory D_1:D2-like receptor interactions are involved in mediating the cataleptic response to D2-like receptor antagonism.

A significant body of evidence has accumulated supporting a role for D_1 receptors in the psychostimulant effects of cocaine. Specifically, locomotor activation in response to acute and repeated cocaine administration is absent in D_1 knockouts [65, 69, 78, 81, 83]. Furthermore, the prominent rearing and grooming response to repeated cocaine administration is markedly reduced in hybrid D_1 knockouts [81]. Although two studies have reported no difference between hybrid or incipient congenic D_1 knockouts and wild types in the cocaine conditioned place preference paradigm [69, 83], a recent study has demonstrated that hybrid D_1 knockouts exhibit impaired acquisition of cocaine self-administration [84]. The latter study also found that (a) wild types, but not D_1 knockouts, successfully recommence cocaine

self-administration following saline-induced extinction of responding and (b) selective agonists for D1-like or D2-like receptors function as positive reinforcers in wild types, but not D_1 knockouts.

Additional studies have investigated a role for D_1 receptors in the mechanisms of action of other commonly abused psychoactive substances. Indeed, independent observers have found that hybrid D_1 knockouts are unresponsive to the locomotor-stimulant effects of morphine and ketamine [85, 86]. Furthermore, two groups have reported a reduction in voluntary ethanol consumption and preference over water in hybrid D_1 knockouts relative to wild types, despite similar overall levels of fluid consumption across the genotypes [76, 87]. A specific role for D_1 receptors in these effects is supported by the observation that pretreatment with a selective D1-like antagonist produced a comparable level of alcohol consumption in wild type and D_1 knockout mice. Additionally, the finding that a selective D2-like antagonist reduced ethanol consumption in both genotypes indicates that cooperative D_1:D2-like interactions are involved in the reinforcing properties of alcohol in mice [87].

Results obtained from studies investigating D_1 receptor involvement in the behavioral response to amphetamine are less consistent. Specifically, some investigators have observed reduced locomotor stimulation and sensitization in response to both acute and repeated treatment with amphetamine, respectively, in hybrid D_1 knockouts [81, 88]. In contrast, another group has found that locomotor sensitization to repeated amphetamine administration in hybrid D_1 knockouts is indistinguishable from wild types across a wide range of doses [89]. More recently, a marked increase in locomotor sensitization to repeated amphetamine has been observed in hybrid D_1 knockouts when assessed in a novel but not familiar environment [90]. Pretreatment with a selective D1-like antagonist has been found to block the development of locomotor sensitization in wild types but not D_1 knockouts [89]. Collectively, these findings have led to speculation that external associative factors and endogenous compensatory mechanisms (not involving D_5 receptors) may be responsible for the development of amphetamine-induced behavioral sensitization in D_1 knockouts [89, 90].

Novel findings in D_1 knockouts have revealed an important role for D_1 receptors in modulating seizure threshold. We have previously shown that the ability of selective D1-like agonists to induce seizures is critically dependent on their intracellular signal transduction coupling effects [39]. Specifically, the D1-like agonist, SKF 83822, which selectively stimulates adenylate cyclase, induces prominent behavioral seizures in mice, whereas SKF 83959, which selectively stimulates phospholipase C, does not (see Section 13.2.1). Interestingly, we have recently found that SKF 83822-induced behavioral and EEG seizures are gene dose dependently abolished in congenic D_1 knockouts; in addition, EEG seizures in response to SKF 83822 are markedly reduced in knockout mice lacking the intracellular signal integration molecule, DARPP-32; furthermore, administration of SKF 83822 in wild types produced a fivefold increase in DARPP-32 phosphorylation at Thr34 in striatal slices and twofold increases in ERK1/2 and GluR1 AMPA receptor phosphorylation in both the striatum and the hippocampus [91].

13.3.3 Interpretation of D_1 Knockout Phenotype

A summary of the behavioral changes reported to date from studies in D_1 knockouts can be seen in Table 13.1. Despite uncertainty concerning the involvement of D_1 receptors in unconditioned psychomotor activity from early studies in hybrid D_1 knockouts [10, 61, 63], it is now clear that spontaneous locomotor and rearing behavior are increased in D_1 knockouts relative to wild types [62, 69, 92]. A hyperactive phenotype in mice lacking D_1 receptors is surprising given that selective D1-like receptor antagonism induces profound locomotor suppression and catalepsy in intact rodents [4]. Such incongruity between findings from pharmacological and gene knockout studies is not easily reconciled, although radioligand-binding data showing that D_5- and D2-like receptor expression is essentially unaltered in D_1 knockouts [10, 61, 65, 93] suggests that genetic compensation is not responsible. One possibility is that normally expressed D_5 receptors are supersensitive in mice lacking D_1 receptors, as manifested in spontaneous hyperactivity; indeed, modest hypoactivity is seen in congenic D_5 knockouts [94] (see Section 13.3.4). Paradoxically, locomotor stimulation in wild types following challenge with a selective D1-like agonist is absent in D_1 knockouts [10, 62, 91]. A ceiling effect on locomotion in D_1 knockouts (whereby phasic drug challenge cannot further stimulate spontaneous levels of activity) is unlikely, given that periods of behavioral inactivity are seen in D_1 knockouts under basal conditions [83]. Thus, it appears that D_1 receptors are critically involved in facilitating locomotion under phasic conditions.

Classical pharmacological studies in genetically intact rodents have shown that grooming behavior, and particularly ethologically complete grooming syntax, is controlled by activity at D1-like receptors. Therefore, findings that spontaneous grooming is only modestly reduced or even elevated in D_1 knockouts under different environmental conditions were surprising [62, 71, 72, 78]. However, similar to unconditioned locomotor activity, results from pharmacological studies in D_1 knockouts have been more consistent. Specifically, induction of grooming by SKF 83959 [62, 79], cocaine [81], and various neuropeptides [78] is attenuated in D_1 knockouts. Furthermore, Cromwell and colleagues have shown that sequencing of discrete patterns of grooming behavior is disrupted in mice lacking D_1 receptors [95]. Taken together, these findings indicate that D_1 receptor activation is indeed necessary for the expression of grooming behavior in mice. However, it should be mentioned that considering dopamine agonist-induced grooming is reduced, but not abolished, in D_1 knockouts, it is likely that other receptors are also involved. Indeed, we have recently found modest modulatory roles for D_4 and D_5 receptors in grooming behavior [94, 96] (see Sections 13.3.4 and 13.4.9).

Converging evidence indicates that D_1 receptors have an important functional role in shaping psychostimulant-induced reward-related behavior; that this is most evident for cocaine, but less so for amphetamine, raises the possibility that novel antagonists selective for D_1 receptors may be efficacious in the treatment of cocaine abuse. Precise reasons underlying the differential involvement of D_1 receptors in mediating the behavioral effects of two psychostimulants that are

Table 13.1 Behavioral changes identified in dopamine receptor knockout mice

	D1-like receptors				D2-like receptors							
Behavior	D_1 knockout		D_5 knockout		D_2 knockout		D_{2L} knockout		D_3 knockout		D_4 knockout	
Locomotion												
Spontaneous	↑	[10, 62, 65, 69–72]	↓	[94]	↓	[8, 108, 110, 111, 113, 114]	↓	[11, 12, 141]	↑	[7, 68, 72, 144–146, 148, 149]	↓	[9]
	↓	[63, 67]	↑	[15]								
Drug-induced												
D1-like agonist	↓↓	[10, 62]	↓	[64]			↓	[11]				
D2-like agonist			↑	[94]								
Cocaine	↓↓	[65, 69, 78, 81, 83]	↓	[104]			↓	[130]	↑	[145, 156]	↑	[9, 171]
									↓	[155]		
Amphetamine	↓	[81, 88]					↓	[141]	↑	[157]	↑	[172]
	↑	[90]										
Methamphetamine					↓	[126]					↑	[9]
Apomorphine							↓	[11, 141]				
Morphine	↓↓	[85]							↑	[149]		
Ketamine	↓↓	[86]										
MDMA					↓	[127]			↓	[127]		
Caffeine					↓	[123, 124]						
Rearing												
Spontaneous	↑	[10, 62, 65, 69, 71, 72]	↑	[15, 94]	↓	[8, 111]	↓	[12]	↑	[7, 68, 72, 144]	↓	[9]
	↓	[61, 66]	↓	[64]	↑	[114]						
Drug induced	↓	[81]	↑	[94]								
Grooming												
Spontaneous	↓	[62, 72, 78]	↓	[94]								
	↑	[71]										

Table 13.1 (continued)

	D1-like receptors				D2-like receptors							
Behavior	D_1 knockout		D_5 knockout		D_2 knockout		D_{2L} knockout		D_3 knockout		D_4 knockout	
Drug-induced												
D1-like agonist	↓	[62, 79]	↓	[94]							↓	[96]
Cocaine	↓	[81]										
Neuropeptide induced	↓	[80]										
Coordination			↑	[15]	↓	[8, 111, 114]	↓	[12, 137]			↑	[9]
Catalepsy												
Spontaneous					↑	[8, 111]						
D1-like antagonist	↓↓	[10]										
D2-like antagonist	↑	[82]			↓	[122, 137]	↓	[11, 12, 142]				
Learning/memory	↓	[63, 66–68, 73, 74]			↓	[114, 118–120]	↓	[137, 138]	↓	[121]		
Reward												
Natural stimuli	↓	[67]			↓	[117]	↓	[139]				
Psychoactive drugs	↓	[76, 84, 87]			↓	[116, 117, 130, 132–134]	↓	[138]	↑	[145, 149]	↑	[171]
Seizures												
D1-like agonist	↓↓	[91]	↓	[91]							↓	[96]
Non-dopamine agonist					↑	[135, 136]						
Novelty-seeking											↓	[166]
Anxiety									↓	[68, 144, 152]		
Antidepressant activity			↑	[64]					↑	[153]		

Table 13.1 (continued)

	D1-like receptors		D2-like receptors			
Behavior	D_1 knockout	D_5 knockout	D_2 knockout	D_{2L} knockout	D_3 knockout	D_4 knockout
Orofacial movements						
Horizontal jaw	↓ [75]	↓ [103]				
Vertical jaw		↑ [103]	↑ [115]			
Tongue protrusions	↓ [75]		↓ [115]			
Incisor chattering	↓ [75]		↓ [115]		↓ [115]	
Vibrissae		↓ [103]				

References are in square brackets
↑ or ↓: Behavior significantly increased or decreased, respectively, in knockout relative to wild types
↑↓: Evidence supporting the specified behavioral change in knockouts is insufficient or equivocal
↓↓: Behavioral change in wild type is abolished in knockout

each indirect agonists at dopamine receptors are currently unknown and warrant further investigation.

It is well recognized that dopaminergic mechanisms in general and D1-like receptors in particular are involved in mediating orofacial movements [18, 92]. Detailed understanding of the endogenous mechanisms that control orofacial movements is important given their criticality in consumptive behavior, self-care, offensive and defensive behaviors, vocalization and, in higher mammals, verbal and non-verbal communication [4]. Additionally, in the clinical setting, orofacial dyskinesia is a common and distressing side effect of certain antipsychotic drugs that requires resolution. The study of spontaneous and D1-like agonist-induced orofacial movements in D_1 knockouts has identified functional roles for D_1 receptors in positively modulating horizontal jaw movements, tongue protrusions, and incisor chattering. Furthermore, the finding that gross head movements in response to a selective D2-like agonist are excessive in D_1 knockouts suggests that the inhibitory D_1:D2-like receptor interactions known to modulate certain dopamine-dependent behaviors also regulate discrete topographies of orofacial movement [75].

There is now strong evidence that the ability of certain D1-like agonists to lower seizure threshold is critically dependent on their stimulation of adenylate cyclase-coupled, rather than phospholipase C-coupled, signal transduction cascades [39] that involve DARPP-32 and ERK1/2 [91]. Indeed, it has recently been reported that ERK signaling at D_1 receptors occurs via an adenylate cyclase/protein kinase A/DARPP-32 pathway, as the ability of amphetamine to induce ERK activation is absent in DARPP-32 mutants [97]. Future studies examining the seizure profile in D_1 knockouts (a) following local administration of SKF 83822 in discrete brain regions and (b) in response to convulsant agents acting on glutamatergic, GABAergic, and cholinergic systems will clarify further the precise involvement of D_1 receptors in seizure processes.

Genetic manipulations other than conventional gene knockout by homologous recombination in embryonic stem cells have been performed in mice to alter the expression of D_1 receptors in an attempt to elucidate their functional roles in vivo. The first report of a transgenic mouse over-expressing D_1 receptors appeared in 1999. Two heterozygote D_1 transgenic lines were noted to have increased D_1 receptor-binding sites in extra-striatal regions. Spontaneous behavior and locomotor stimulation by amphetamine or cocaine was indistinguishable between D_1 transgenics and controls. Surprisingly, locomotor, rearing, and climbing responses to the selective D1-like agonist SKF 81297 in wild types were reversed, not potentiated, in mice over-expressing D_1 receptors [98]. A similar spontaneous hypokinetic phenotype in D_2 knockouts led these investigators to speculate that stimulation of over-expressed D_1 receptors may suppress D_2 receptor function in this model; however, a subsequent study in D_1 transgenic/D_2 knockout mice failed to support this hypothesis [99]. These findings are not easily reconciled with data described above from pharmacological studies in intact rodents or mice with targeted gene deletion of D_1 receptors. However, the random integration of transgenes into the host genome using conventional transgenic techniques, leading to regional and quantitative differences in protein expression, may be responsible for

the observed phenotype in D_1 transgenic mice. Future development of genetically engineered mice with enhanced promoter activity of the native D_1 receptor gene would help to resolve this issue and may provide novel insights into D_1 receptor function.

In a separate study, Drago and colleagues used an elegant approach to specifically ablate D_1 receptor-expressing cells in mice [100]. Although most heterozygote mutants harboring activated diphtheria toxin in the D_1 receptor gene died in the neonatal period, those that survived were characterized by dystonic posturing, myoclonic jerks, and marked bradykinesia relative to wild types, consistent with an important role for neurons expressing D_1 receptors in the control of movement. More recently these investigators have used a similar approach to create a separate mutant in which progressive ablation of D_1 receptor-expressing cells begins in the postnatal period [101]; unlike mice with congenital loss of D_1 receptor-expressing cells [100, 102], these mutants survive to adulthood and have normal gait and coordination. Postnatal loss of D_1 receptor-expressing cells was associated with hind limb dystonia, hyperactivity in both novel and familiar environments, impaired oral behavior, and spontaneous seizures [101]. While these models are invaluable preclinical research tools in the study of neurological disorders characterized by loss of discrete neuronal cell populations, such as Huntington's disease, direct phenotypic comparison with D_1 knockouts is complicated by the concomitant loss of other cellular constituents in cell ablation mutants.

13.3.4 D_5 Knockout: Spontaneous Behavior

A preliminary account of D_5 knockouts maintained on a hybrid genetic background (129/SvJ1 $\times$ C57BL/6) first appeared in a review article of dopamine receptor function. Hybrid D_5 knockouts evidenced significantly higher levels of horizontal and vertical activity in an open-field environment and outperformed their wild type counterparts in the rotarod test for locomotor coordination, leading to the suggestion that D_5 receptors normally act to depress or inhibit spontaneous motor behavior [15]. A subsequent more comprehensive assessment of hybrid D_5 knockouts evidenced incongruent phenotypic effects [64]. In contrast to the earlier report outlined above, spontaneous locomotor activity was unaltered in hybrid D_5 knockouts, while rearing activity was reduced in one batch of knockouts, but not another. Additionally, no differences were observed between D_5 knockouts and wild types in a test of locomotor coordination.

Phenotypic assessments of hybrid D_5 knockouts have also been carried out at other levels of behavioral investigation. The finding that hybrid D_5 knockouts perform normally in the light–dark box and elevated plus maze argues against a functional role for D_5 receptors in anxiety-related behavior [15, 64]. Furthermore, in the Morris water maze, D_5 knockouts were found to exhibit normal learning and spatial memory capabilities. Acoustic startle responses were found to be reduced in one batch of D_5 knockouts but not another; however, prepulse inhibition did not differ between the genotypes in either batch suggesting that the involvement of D_5

receptors in gating responses to environmental stimuli, if any, is minimal and/or complex. A gender-specific genotypic effect in D_5 knockouts has been identified in the Porsolt forced swim test for antidepressant activity, with male D_5 knockouts exhibiting lower levels of immobility compared to male wild types in this behavioral paradigm [64].

To clarify the functional involvement of D_5 receptors in mediating spontaneous motor behaviors, we recently examined ethologically the phenotype of congenic D_5 knockout mice over the course of exploration and habituation in a novel environment. During the initial period of exploration, D_5 knockouts evidenced a modest reduction in locomotion and a modest increase in sifting (exploratory movements of the forepaws through the cage bedding), with no change in rearing or other behaviors relative to wild types. Over the course of subsequent habituation, topographical shifts in behavior revealed additional phenotypic differences in D_5 knockouts, specifically an overall reduction in the level of grooming and delayed habituation of rearing [94]. Furthermore, in a recent study to evaluate the involvement of D_5 receptors in mediating spontaneous orofacial movement topography, congenic D_5 knockouts were found to exhibit an increase in vertical jaw movements, which habituated more slowly than in wild types, together with a decrease in both horizontal jaw movements and movements of the vibrissae [103].

13.3.5 D_5 Knockout: Drug-Induced Behavior

As part of the original behavioral characterization of hybrid D_5 knockouts described in Section 13.3.4 above, Holmes and colleagues examined behavioral responsiveness to the selective D1-like agonist, SKF 81297, and the selective D1-like antagonist, SCH 23390. SKF 81297-induced horizontal activity was dose dependently reduced in hybrid D_5 knockouts over a 15-min period of assessment, while vertical activity did not differ between the genotypes. During the dark phase of rodent diurnal cycle, SCH 23390 was found to reduce horizontal and vertical activity indistinguishably between hybrid D_5 knockouts and wild types [64]. Collectively, these findings led the investigators to speculate that D_5 receptors might be involved in facilitating phasic D1-like receptor-mediated locomotor stimulation but not tonic baseline activity.

In 2003, Elliot and co-workers used hybrid D_5 knockouts to investigate the functional involvement of D_5 receptors in the locomotor and discriminative stimulus effects of cocaine. In an automated open-field environment, cocaine dose dependently stimulated horizontal activity over a 1-h period of assessment. Horizontal activity was gene dose dependently reduced in homozygous D_5 knockouts relative to wild types, consistent with a facilitative role for D_5 receptors in mediating the locomotor-stimulant effects of cocaine. In contrast, the ability to discriminate cocaine from saline did not differ between D_5 knockouts and wild types; the selective D1-like antagonist SCH 39166 was found to produce saline-appropriate cocaine responding in both genotypes [104]. Taken together, these

findings are consistent with a primary role for D_1, but not D_5, receptors in cocaine discrimination.

Recently, we assessed congenic D_5 knockouts in response to a range of selective D1-like agonists with varying efficacies to stimulate adenylate cyclase and/or phospholipase C, and the selective D2-like antagonist RU 24213 [94]. Administration of SKF 83959 characteristically induced a dose-dependent topographical shift in behavior from self-directed grooming at lower doses to prominent exploratory locomotion, sifting, and rearing at higher doses [62, 79, 96]. Whereas overall levels of grooming did not differ between the genotypes, episodes of ethologically complete syntactic, intense grooming were significantly reduced in D_5 knockouts. Challenge with SKF 83959 produced a reciprocal increase in rearing behavior in D_5 knockouts relative to wild types. Unexpectedly, characteristic induction of stereotyped "ponderous" locomotion by RU 24213 was dose dependently increased in D_5 knockout mice, indicating that inhibitory D_5:D2-like receptor interactions participate in discrete dopamine-mediated behaviors [94].

Interestingly, recent evidence has emerged to support a role for D_5 receptors in modulating seizure threshold. Specifically, cortical EEGs were recorded in congenic D_5 knockouts in response to a dose of the selective D1-like agonist SKF 83822 previously shown to readily induce behavioral seizures in mice [39]. In both heterozygous and homozygous D_5 knockouts, the latency to first seizure was significantly increased relative to wild types. Furthermore, the total number of EEG seizures and, particularly, the number of high amplitude, high-frequency polyspike EEG events were reduced in D_5 knockouts [91].

13.3.6 Interpretation of D_5 Knockout Phenotype

A summary of the behavioral changes reported to date from studies in D_5 knockouts can be seen in Table 13.1. Inconsistent behavioral phenotypes observed in D_5 knockouts maintained on a hybrid (129/SvJ1 $\times$ C57BL/6) genetic background between and within laboratories [15, 64] have complicated elucidating the functional roles of the D_5 receptor. Reduction in rearing in one batch of hybrid D_5 knockouts but not another within the same study [64] suggests permutational differences in 129/SvJ1 and C57BL/6 alleles between mice rather than deletion of the D_5 receptor may have produced the observed effect. Indeed, reduced rearing activity is characteristic of 129/SvJ mice when compared directly with the C57BL/6 inbred strain [105]. Alternatively, it might be that D_5 receptors are minimally involved in mediating discrete behaviors and large numbers of animals are required to resolve these subtle effects. Regardless, it is difficult to determine with confidence the relevance of isolated behavioral changes, such as increased antidepressant activity and decreased acoustic startle responses, identified to date from studies using hybrid D_5 knockouts alone. Replicating these studies in congenic D_5 knockouts would provide a more conclusive understanding of the precise behavioral roles of the D_5 receptor and possibly identify novel functions.

Spontaneous locomotion and locomotor stimulation to a selective D1-like agonist or cocaine are reduced in both hybrid and congenic D_5 knockouts [64, 94, 104], providing convincing evidence for a facilitative role of D_5 receptors in ambulation. In contrast, both spontaneous and D1-like agonist-induced rearing are increased in congenic D_5 knockouts. The latter finding combined with the reported increase in spontaneous sifting in congenic D_5 knockouts over an initial period of exploration in a novel environment suggest a modest inhibitory role for D_5 receptors in mediating complex patterned exploratory behavior [92, 94]. Collectively, these findings in D_5 knockouts complement localization studies demonstrating D_5 receptor expression in discrete areas of the basal ganglia [106], a brain region well established in processing signals relating to motor coordination and exploratory behavior.

Interestingly, D_1 knockouts maintained on both hybrid and congenic backgrounds exhibit a contrasting spontaneous behavioral phenotype to that observed in congenic D_5 knockouts (see Section 13.3.1); they demonstrate increased locomotion and decreased sifting upon initial exploration of a novel environment [62, 71]. Therefore, despite similarities in their respective pharmacological and biochemical profiles, reciprocal functional organization might exist between D_5 and D_1 receptors to regulate discrete topographies of behavior. In support of this hypothesis, opposing roles of D_5 and D_1 receptors in modulating locomotor behavior have been identified through the use of selective antisense oligonucleotide strategies in 6-OHDA-lesioned rats [107]. However, evidence from the study of orofacial movements in individual D_1 and D_5 knockout lines suggests that cooperative functional organization also exists within the D1-like receptor family. Specifically, both D_1 and D_5 receptors inhibit spontaneous vertical jaw movements and facilitate spontaneous horizontal jaw movements in mice, albeit with functional prepotency of the D_1 receptor [75, 92, 103]. The recent finding that characteristic induction of stereotyped "ponderous" locomotion following challenge with a selective D2-like agonist occurs to excess in congenic D_5 knockouts [94] indicates that D_5 receptors also interact functionally with D2-like receptors to modulate behavior. How such inhibitory D_5:D2-like receptor interactions are organized within the brain at a molecular, cellular, or network level and the relative involvement of D_2, D_3, and D_4 receptors remains to be established.

In congenic D_5 knockouts, spontaneous and drug-stimulated grooming behavior is reduced [94]. A critical role for D1-like receptors in regulating grooming is well recognized. Given promotion by D1-like agonists, attenuation by D1-like antagonists, and attenuation in congenic D_1 knockouts (see Section 13.3.2), it has traditionally been assumed that D_1 receptors play a primary, if not, exclusive role in regulating grooming on the basis of their dense localization relative to D_5 counterparts in brain regions such as the dorsolateral striatum that are known to mediate grooming [3, 4]. However, recent findings in congenic D_5 knockout mice indicate this conclusion to have been premature; both D_1 and D_5 receptors appear to be involved in the expression of grooming [94]. Furthermore, reduction in syntactic intense grooming, but not overall grooming events, in response to the selective D1-like agonist SKF 83959 implies a functional role for D_5 receptors in generating

sequential grooming movements, as distinct from grooming initiation. A comparable, more profound behavioral phenotype has also been observed in both hybrid and congenic D_1 knockout mice [62, 95]. Interestingly, lesion mapping studies in rats have identified the dorsolateral neostriatum as crucial for the induction of sequential grooming behavior without disrupting the ability to emit grooming actions [30]. This, together with the report that D_5 and D_1 receptor immunolocalization is found throughout the neostriatum in both rats and non-human primates [106], suggests that intact D_1 and D_5 receptor signaling within this region is necessary for the induction of sequential grooming behavior.

The recent finding in congenic D_5 knockouts that D_5 receptors participate in the induction of seizures by selective D1-like agonists [91] elaborates an earlier pharmacological study utilizing D1-like antagonists which could not determine the relative anticonvulsant properties of the individual D1-like receptors [39]. A functional role for D_5 receptors in the mechanisms of seizure generation by selective D1-like agonists has previously received little attention due to the well-established preponderance of D_1 vs. D_5 receptor expression in both cortical and striatal brain regions. Indeed, as the overall number of EEG seizures is reduced similarly in both heterozygous and homozygous D_5 knockouts, it would appear that, unlike for D_1 receptors (see Section 13.3.2), decreasing the level of D_5 receptor expression beyond 50% does not enhance the seizure protective effect. Regardless, these EEG findings in congenic D_5 knockouts have revealed a modest functional role for the D_5 receptor in mediating the convulsant effects of SKF 83822 that could not be determined using behavioral assessment alone [94].

13.4 D2-Like Receptor Family

Shortly after the generation of the first D_1 knockouts, mice lacking functional D_2 receptors were reported [8]. The introduction of a premature stop codon in exon 2 of the D_2 receptor gene prevented the expression of either short or long splice variants of the D_2 receptor protein. Other "complete" D_2 knockouts have since been created [108–110], followed by mutants with selective knockout of the long isoform (D_{2L}) of the D_2 receptor protein [11, 12]. Successful deletion of the D_3 receptor gene in mice was reported initially in 1996 [7], while D_4 knockouts emerged the following year [9].

13.4.1 D_2 Knockout: Spontaneous Behavior

In 1995, the first "complete" D_2 knockout constructed on a hybrid (129/SvJae × C57BL/6) genetic background was found to exhibit multiple physical and movement-related abnormalities. Specifically, during a 5-min period of assessment in an open field, D_2 knockouts were characterized by hypoactivity and a marked reduction in exploratory rearing behavior; posture, gait, and motor coordination were also abnormal in these mutants. Furthermore, D_2 knockouts demonstrated less

mobility on the ring test used for the assessment of catalepsy in mice [8]. The following year, as part of a study evaluating the functional involvement of D_2 receptors in regulating pituitary hormone secretion, hybrid (129/Sv × BDF1), D_2 knockouts created using a different gene targeting vector were similarly found to be hypoactive relative to wild types and to exhibit abnormal gait [110]. Consistent with these earlier reports, a third hybrid (129/Sv × C57BL/6) D_2 knockout line evidenced reduced body weight, hunched posture, and bradykinesia; surprisingly, the magnitude of these deficits was noted to improve significantly with increasing age [108]. In an experiment to investigate the effect of heterogeneity in the genetic background on mutant phenotypes, a fourth D_2 knockout line [109] was examined on the original hybrid (129/SvEv × C57BL/6) genetic background and also following multiple backcrosses to each of the parental strains. Profound deficits in motor coordination were observed in hybrid D_2 knockouts and the parental 129/SvEv strain, but not incipient congenic C57BL/6 D_2 knockouts, leading these investigators to conclude that strain-related differences, not deletion of the D_2 receptor, were responsible for the ataxia [111].

Realization that genetic background is a potential confounder in the interpretation of mutant phenotypes prompted additional studies in D_2 knockouts at increasing levels of congenicity. Indeed, in contrast to previous findings in hybrid D_2 knockouts [8, 110], postural abnormalities, spontaneous catalepsy, or tremor were not observed in D_2 knockouts comprising mainly C57BL/6 alleles [111, 112]. A more consistent effect of D_2 receptor deletion has been shown in relation to other movement-related behaviors. Specifically, we have demonstrated that, in agreement with their hybrid counterparts, incipient congenic D_2 knockouts exhibit a modest reduction in spontaneous locomotion and qualitative shifts in discrete topographies of rearing relative to wild types over an initial period of exploration in a novel environment [113]. Furthermore, reduced locomotion and increased topographies of rearing have also been described in congenic D_2 knockouts [114]; an additional finding that poor performance in congenic D_2 knockouts on the rotarod is unrelated to parental strain indicates that absence of D_2 receptors is indeed responsible for the observed deficit in motor coordination [114] and not epistasis, as previously suggested [111]. In relation to dopaminergic regulation of spontaneous orofacial movements, we have recently shown that incipient congenic D_2 knockouts are characterized by increased vertical jaw movements and unaltered horizontal jaw movements, with reductions in tongue protrusions, incisor chattering, and head movements relative to wild types [115], consistent with both facilitative and inhibitory roles for D_2 receptors in discrete topographies of orofacial movement.

D_2 knockouts have also been used to investigate the involvement of D_2 receptors in learning and reward-related behavior. In relation to the latter, an early study found no difference between hybrid D_2 knockouts and wild types in a conditioned place preference paradigm where food was available as a reward [116]. Conversely, in a more recent study using congenic D_2 knockouts where mice were trained to self-stimulate the lateral hypothalamus, stronger electrical currents were necessary to achieve a stable level of operant responding in mutants [117]; deficits have also been identified in D_2 knockouts in the acquisition of a task where intracranial

stimulation was available as a reward [118], indicating that reinforcement processes are impaired in the absence of D_2 receptors. A more consistent picture has emerged with regard to D_2 receptor involvement in associative learning. Specifically, one study has shown that congenic D_2 knockouts require more trials than wild types to achieve stable operant responding in a conditioned place preference paradigm [114]. More recently, impaired associative learning has been reported in congenic D_2 knockouts in a two-odor discrimination paradigm; in particular, whereas female, but not male, D_2 knockouts exhibited poor ability to discriminate between two odors to obtain a food reward [119], both genders required significantly more trials than wild types to demonstrate reversal learning of the task [120]. However, the possibility that reinforcement mechanisms are disturbed in D_2 knockouts renders it difficult to distinguish between the effects of D_2 receptor knockout on reinforcement vs. acquisition of cue value via associative learning.

In a delayed alternation paradigm, incipient congenic D_2 knockouts were characterized by impairments in spatial working memory, as determined by their inability to enter the correct arm on a T maze, when progressive time delays were introduced before commencing a previously learned task [121].

13.4.2 D_2 Knockout: Drug-Induced Behavior

A number of studies have examined D_2 knockouts following challenge with selective D1-like or D2-like agonists to further characterize the mechanisms through which functional dopamine receptor interactions modulate behavior and to identify additional behavioral phenotypes that might have been masked under basal conditions. In dopamine-depleted mice, combined administration of a selective D1-like and D2-like agonist but not the selective D1-like agonist alone was found to reverse locomotor deficits in wild types but not hybrid D_2 knockouts [111], indicating that cooperative D1-like:D_2 receptor interactions are necessary for the production of spontaneous ambulatory behavior. Our own finding that characteristic induction of stereotyped sniffing and ponderous locomotion in response to high doses of the selective D2-like agonist RU 24213 was markedly reduced in incipient congenic D_2 knockouts [113] provides additional evidence that D_2 receptors are involved in mediating discrete dopamine-dependent behaviors. Indeed, haloperidol-induced catalepsy is gene dose dependently abolished in both hybrid and incipient congenic D_2 knockouts [111, 122], indicating that among D2-like receptors, selective D_2 receptor blockade is responsible for the emergence of extrapyramidal side effects that frequently complicate the use of first-generation antipsychotic drugs. It also appears that D_2 receptors participate in inhibitory dopamine receptor processes to modulate particular behaviors. Specifically, Boulay and colleagues have shown that the D_2/D_3 agonists 7-OH-DPAT and PD 128907 reduce locomotor activity in wild types but not incipient congenic D_2 knockouts in a gene dose-dependent manner [112]. Furthermore, we have found that incipient congenic D_2 knockouts exhibit heightened orofacial movements, termed vacuous chewing, when compared with wild types following challenge with the selective D1-like agonist A 68930 [113].

Such findings are most readily explained by the absence in D_2 knockouts of D_2 receptors that exert inhibitory effects on these functions. Pharmacological studies in D_2 knockouts have also identified functional interactions between dopamine and other neuromodulators. Specifically, the locomotor-stimulant effects of the adenosine A_{2A} receptor antagonist caffeine are attenuated in D_2 knockouts [123, 124]. Furthermore, a selective adenosine A_{2A} receptor agonist has been shown to reduce spontaneous and amphetamine-stimulated locomotion in D_2 knockouts and wild types [123]. Taken together, these findings are consistent with antagonistic and independent roles for dopamine D_2 and adenosine A_{2A} receptors in controlling discrete motor functions.

Generation of D_2 knockout mice has enabled researchers to investigate a putative role for D_2 receptors in the locomotor-modulating effects of various drugs of abuse. With regard to opiates, separate findings in hybrid [116] and congenic [125] D_2 knockouts do not support a role for D_2 receptors in morphine-induced hyperactivity. Conversely, the opioid antagonist naloxone has been shown to suppress spontaneous locomotor activity in wild types but not congenic D_2 knockouts. Interestingly, naloxone was found to similarly reduce basal locomotion in β-endorphin knockouts but not in enkephalin knockouts or β-endorphin/enkephalin double knockouts. Collectively, these findings indicate that D_2 receptors are involved in modulating spontaneous locomotor activity stimulated by the endogenous opioid enkephalin [125]. Unlike morphine, the locomotor-stimulant effects of methamphetamine and MDMA are reduced in congenic D_2 knockouts [126, 127]. Furthermore, ethanol-induced ataxia is reduced in D_2 knockouts of incipient congenicity on either a C57BL/6 or a 129/Sv background [128, 129]. To date, no consensus has been reached concerning D_2 receptor involvement in cocaine-induced hyperactivity, with one study demonstrating reduced locomotor activity in hybrid D_2 knockouts [130] and another showing no difference between congenic D_2 knockouts and controls [131].

Other studies have evaluated drug-seeking behavior in D_2 knockout mice. Two groups have shown that morphine-induced conditioned place preference is absent in both hybrid and congenic D_2 knockouts under different reinforcement schedules [116, 132]. Furthermore, although morphine-induced conditioned place preference was unaltered in drug-naïve incipient congenic D_2 knockouts, opiate withdrawn and dependent D_2 knockouts did not show place preference for morphine [133]. More recently, morphine has been shown to potentiate the rewarding properties of self-administered hypothalamic brain stimulation in wild types but not congenic D_2 knockout mice. Interestingly, a similar effect of D_2 receptor deletion was not observed for amphetamine using identical experimental techniques [117]. In relation to cocaine, high, but not low, doses have been found to increase levels of self-administration [134] and induce conditioned place preference [130] in hybrid D_2 knockouts. Furthermore, the abilities of selective D2-like antagonists and agonists, respectively, to increase cocaine self-administration and serve as cocaine substitutes in wild types are absent in hybrid D_2 knockouts [134]. Collectively, these findings suggest that although D_2 receptors are not essential for cocaine self-administration, they are involved in positively modulating the rewarding effects of this drug.

Finally, additional studies in D_2 knockouts have evaluated the role of D_2 receptors in seizure processes. Interestingly, although D_2 knockouts do not evidence spontaneous behavioral seizures, Bozzi and colleagues have demonstrated that hybrid D_2 knockouts develop seizures at doses of kainic acid and pilocarpine that do not affect seizure threshold in wild types. Furthermore, seizure-related neuronal cell death in response to these agents was reduced in D_2 knockouts [135, 136]. These findings, when considered with recent results from seizure studies in D_1 knockouts [91] (see Section 13.3.2), indicate that D_1 and D_2 receptors have important functional roles in lowering and elevating seizure threshold, respectively.

13.4.3 D_{2L} Knockout

In 2000, two reports emerged describing the spontaneous behavioral phenotype in mice with selective knockout of the long isoform (D_{2L}) of the D_2 receptor [11, 12]. In the first study, no difference was found in locomotor activity between D_{2L} knockouts and wild types over a period of exploration in a novel environment [11]. Conversely, in the second study, locomotion and rearing were reduced in both hybrid and incipient congenic D_{2L} knockouts over 5 min of assessment in an open field. Furthermore, latency to fall from the rotarod was significantly reduced in incipient congenic D_{2L} knockouts on the first trial but not during subsequent trials [12]. Additional evidence for movement-related abnormalities in D_{2L} knockouts has been provided by a study comparing incipient congenic mutants and wild types at various developmental stages [137]. Interestingly, deficits in locomotion and coordination in young adult D_{2L} knockouts mirrored those in aged, but not young, wild types, consistent with a Parkinsonian-like phenotype in D_{2L} mutants in a manner similar to that previously suggested in "complete" knockout mice [8].

D_{2L} knockouts have also been used to determine the relative contribution of individual D_2 receptor isoforms to other dopamine-dependent behaviors. In relation to associative learning, a significant impairment has been observed in incipient congenic D_{2L} knockouts in the acquisition of a conditioned avoidance task [137, 138], suggesting that D_{2L} receptors participate in the behavioral response to aversive stimuli. Additional evidence has emerged supporting the involvement of D_{2L} receptors in reward-related behavior. Specifically, in the pleasurable wheel-running paradigm, incipient congenic D_{2L} knockouts were found to perform fewer wheel turns than wild types, in the absence of any detectable difference between the genotypes on the rotarod, suggesting that blunted reward and not impaired motor coordination was responsible for the observed effect [139].

Other investigators have investigated D_{2L} receptor involvement in the regulation of discrete emotion-driven behaviors. There were no difference between D_{2L} knockouts and wild types in terms of general activity or open sector entries on the zero maze, indicating that D_{2L} receptors are not involved in the behavioral manifestation of anxiety [137]. In support of this finding, a separate study found no difference in defecation between the genotypes following exposure to a stressful environment [138]. In fact, hybrid D_{2L} knockouts have been characterized by

a lower bite frequency and shorter attack duration than wild types in the resident intruder test, consistent with attenuated stress-induced offensive aggression in these mutants [140].

Many of the pharmacological studies conducted in "complete" D_2 knockouts have also been performed in D_{2L} knockouts. A low dose of the selective D2-like agonist quinpirole suppressed locomotion in hybrid D_{2L} knockouts but not wild types [11]. Conversely, in an alternative hybrid D_{2L} knockout line, a similarly low dose of quinpirole was not associated with an observable genotypic difference in locomotion [12]; however, methodological differences in the time between injection of the drug and commencing behavioral assessments may explain the discrepant findings between these two studies. Locomotor stimulation by the non-selective dopamine receptor agonist apomorphine and the selective D1-like agonist, SKF 81297 were also markedly reduced in D_{2L} knockouts [11]. More recently, it has been reported that climbing behavior induced by amphetamine, apomorphine, and combined D1-like and D2-like agonists is significantly attenuated in incipient congenic D_{2L} knockouts. In addition, a higher level of stereotyped non-offensive biting was observed in D_{2L} knockouts than wild types in response to the combined administration of quinpirole and SKF 81297, but not when these agonists were administered individually [141] leading these authors to speculate that cooperative D1-like/D_{2L} and D1-like/D_{2S} receptor interactions are involved in mediating locomotor and stereotyped behavior, respectively. A number of studies have shown that catalepsy induced by the antipsychotics haloperidol and raclopride is essentially abolished in both hybrid and incipient congenic D_{2L} knockouts [11, 12, 142]. Furthermore, locomotor suppression following challenge with these antagonists in wild types is attenuated in D_{2L} knockouts [12, 142].

In relation to D_{2L} receptor involvement in mediating behavioral responsiveness to drugs of abuse, one study has shown that the locomotor-stimulant effect of morphine is unaltered in incipient congenic D_{2L} knockouts. In contrast, conditioned place preference for morphine and conditioned place avoidance induced by naloxone-precipitated morphine withdrawal was disrupted in D_{2L} knockouts [138]. Taken together, these results suggest that while D_{2L} receptors do not appear to participate in morphine-induced hyperactivity, they are important in the rewarding properties of morphine and aversive properties of opiate withdrawal. Interestingly, additional findings that cocaine-induced locomotor stimulation is moderately reduced [130] while cocaine conditioned place preference is unaltered in D_{2L} knockouts [130, 138] indicate that D_{2L} receptors may have different functional roles for individual drugs of abuse.

13.4.4 Interpretation of D_2 and D_{2L} Knockout Phenotypes

A summary of the behavioral changes reported to date from studies in D_2 and D_{2L} knockouts can be seen in Table 13.1. The availability of independent knockout lines lacking both short and long isoforms of the D_2 receptor or selective

deletion of the latter allows systematic comparison of results obtained from studies in these mutants to identify isoform-specific D_2 receptor functions. Before discussing the results from studies in D_{2L} knockouts and their contribution to our understanding of D_2 receptor function at the level of behavior, it should be mentioned that, as expected, northern blot analysis in both of the original D_{2L} knockout lines revealed no difference in D_2 receptor mRNA between knockouts and wild types [11, 12]. Therefore, the short isoform of the D_2 receptor is over-expressed in D_{2L} knockouts and may be functionally relevant. In an elegant approach to resolve this issue, Centonze and colleagues mated "complete" D_2 knockouts and D_{2L} knockouts to achieve more physiological levels of D_{2S} receptor expression [143]; however, this strategy has not been adopted in behavioral studies to date.

Initial phenotypic characterization of D_2 knockouts on a hybrid genetic background exhibited some of the clinical features found in Parkinson's disease, leading the originators to speculate that the D_2 knockout mouse constitutes a reliable model of the human neurodegenerative condition [8]. However, subsequent studies have failed to identify abnormal posture, gait, or spontaneous tremor in incipient or fully congenic D_2 knockouts [111, 112]. Despite these inconsistencies, converging evidence indicates that spontaneous locomotion and coordination are significantly reduced in D_2 knockouts [8, 108, 110, 111, 113, 114]. More detailed assessment of locomotion in D_2 knockouts has revealed that initiation of movement, time spent in motion, and distance traveled are all decreased in these mutants [111]. Thus it would appear that akinesia rather than bradykinesia is responsible for spontaneous hypoactivity in D_2 knockouts. Interestingly, similar locomotor and coordination deficits have been observed in D_{2L} knockouts [11, 12, 137], indicating that the long isoform of the D_2 receptor may be responsible for movement-related phenotypes in D_2 knockouts.

A comparative study between congenic D_2 knockouts and the inbred 129/Sv and C57BL/6 parental strains has identified additional D_2 receptor-dependent behaviors that are unrelated to genetic background effects [114]. First, rearing directed toward the walls of the testing apparatus is significantly increased in D_2 knockouts. Excessive rearing confined to the perimeter of the cage together with reduced locomotion could indicate increased anxiety in D_2 knockouts. However, D_2 or D_{2L} knockouts do not exhibit increased defecation during exploration of an unfamiliar environment [138, 144] and D_{2L} knockouts are indistinguishable from wild types on the zero maze [137], consistent with normal levels of anxiety in these mutants. Furthermore, our finding that D_2 knockouts are characterized by significant shifts in multiple topographies of rearing during the course of habituation to a novel environment [113] indicates that D_2 receptor involvement in rearing is more complex and cannot be loosely classified as either facilitative of inhibitory. Second, D_2 knockouts are supersensitive to the tremor-inducing effect of the indole alkaloid, harmaline [114]. It is possible that increased tremor may be due to known learning deficits in D_2 knockouts [114, 118–120] in a manner where movement-induced tremor acts as an aversive stimulus in wild type mice. Alternatively, increased tremor may

represent phasic exacerbation of Parkinsonian-like abnormalities in D_2 knockouts that are compensated for under tonic conditions.

Induction of catalepsy in rodents is a characteristic feature of antipsychotics whose pharmacological effects are mediated via selective antagonism at D2-like receptors. It is generally accepted that antipsychotics with low potency to induce catalepsy are associated with fewer extrapyramidal side effects that frequently complicate the treatment of psychosis. Interestingly, haloperidol-induced catalepsy is markedly reduced in D_2 knockouts [111, 122], suggesting that, within the D2-like family, D_2 receptors are predominantly involved in the cataleptic response to typical antipsychotics. Similar observations in D_{2L} knockouts [11, 12, 142] indicate that the long isoform of the D_2 receptor may be responsible for the induction of cataleptic behavior. It therefore remains possible that novel antagonists with preferential affinity for D_3 receptors may retain therapeutic efficacy in treating psychosis in the absence of motoric side effects.

The extent to which D_2 receptors modulate locomotor activity induced by psychostimulants is less clear. While D_2 receptors appear to facilitate hyperactivity induced by caffeine, methamphetamine, and MDMA [123, 124, 126, 127], they do not appear to modulate locomotor stimulation induced by morphine [116, 125] or cocaine [130, 131]. Conversely, there is convincing evidence that D_2 receptors are important, but not essential, in the reinforcing properties of both morphine [116, 117, 132, 133] and cocaine [130, 134]. Interestingly, D_2 receptors do not appear to participate in the rewarding effects of amphetamine [117] and D_{2L} receptors appear to be differentially involved in the mechanisms of action of various drugs of abuse [130, 138], highlighting the complexity of neural processes underpinning substance abuse-related behavior. It should be noted that learning and coordination deficits in D_2 and D_{2L} knockouts [8, 12, 111, 114, 118–120, 137, 138] may impair performance in operant conditioning paradigms that are frequently employed in substance abuse studies.

Pharmacological studies using selective dopamine agonists in D_2 and D_{2L} knockouts have broadened our understanding of the mechanisms through which D_2 receptors functionally interact with their D1-like counterparts to modulate locomotor behavior. Findings that locomotor stimulation by indirect and non-selective dopamine agonists is attenuated in both D_2 and D_{2L} knockouts [11, 111, 141] indicate that cooperative D1-like/D_{2L} receptor interactions are necessary for the production of normal ambulatory behavior. How such interactions are organized within the brain is presently unclear; one possibility involves D_1/D_2 heterodimers formed by direct protein–protein interactions (reviewed in Chapter 10). Our finding that vacuous chewing following challenge with a selective D1-like agonist occurs to significant excess in D_2 knockouts [113] suggests that inhibitory D1-like/D_2 receptor interactions also exist. Such inhibitory functional interactions may be conceptualized by the absence of pre-synaptic D_{2S} receptors in D_2 knockouts causing increased dopamine synthesis and enhanced activation of D1-like receptors. Similar mechanisms might also explain excessive D1-like receptor-dependent induction of grooming following challenge with cocaine in D_2 knockouts [130].

The study of spontaneous orofacial movements in dopamine receptor knockouts has provided additional insight into the mechanisms by which signaling through individual dopamine receptor subtypes can influence behavioral phenotype. Specifically, D_2 knockouts are characterized by increased vertical jaw movements and unaltered horizontal jaw movements [115] while D_1 knockouts evidence reduced horizontal jaw movements and unaltered vertical jaw movements [75] (see Section 13.3.1), consistent with reciprocal roles for D_1 and D_2 receptors in regulating composite oral behavior [4, 20].

Finally, classical seizure studies using selective D2-like agonists and antagonists in rodents have suggested that D2-like receptors elevate seizure threshold under normal conditions [45]. Studies with convulsant agents in D_2 knockouts have elaborated these earlier findings by demonstrating that, among the D2-like family, D_2 receptors are primarily involved in these effects and further suggest that D_2 receptors prevent seizure-induced neuronal cell death [135, 136]. To date, seizure studies have not been performed in D_{2L} knockouts so the relative importance of short and long isoforms of the D_2 receptor in these actions is currently unknown. Regardless, these findings raise the possibility that development of novel agonists selective for D_2 receptors may have therapeutic utility as anticonvulsant agents.

13.4.5 D_3 Knockout: Spontaneous Behavior

Generation and behavioral characterization of the first D_3 receptor knockout mouse was reported in 1996 [7]. Homozygous hybrid (129/SvJ1 × C57BL/6) D_3 knockouts displayed significant increases in both horizontal and rearing activity relative to wild types throughout 15 min of exploration in an open field, while locomotor activity was increased to a similar level in heterozygous D_3 knockouts in a time-dependent manner. The following year, phenotypic evaluation of a second D_3 knockout mouse line created using a different gene targeting construct was reported [145]. Although overall levels of spontaneous horizontal activity in these hybrid (129/Sv × C57BL/6) D_3 knockouts did not differ from wild types over 30 min in a novel environment, post hoc analysis revealed that D_3 knockouts were hyperactive during the first 5 min of the test. Subsequent assessments of locomotor activity following habituation to the testing environment were not associated with any observable genotypic effects. A similar lack of significant genotypic effect for horizontal activity and total distance traveled over 30 min in an unfamiliar environment has been reported in a third independently generated D_3 knockout line [108]. In the most recently constructed D_3 knockout mouse, non-significant increases in total distance traveled and vertical activity relative to wild types have been observed under similar experimental conditions [146]. However, analysis of the data over constituent time bins, which revealed significant time × genotype interactions for locomotion in the first two D_3 knockout lines [7, 145], was not documented in the latter two studies.

A number of behavioral studies incorporating phenotypic assessments of locomotion and/or rearing have since been conducted on the original D_3 knockout

lines. In separate studies comparing single and double dopamine receptors knockouts, hybrid D_3 knockouts created by Accili and colleagues evidenced significant increases in horizontal and vertical activity relative to wild types over 15 min of exploration in an unfamiliar environment [68, 144]. Backcrossing this particular hybrid D_3 knockout line to the C57BL/6 inbred strain for one generation produced discrepant behavioral results across two laboratories. In one study, activity levels in D_3 knockouts were indistinguishable from wild types over the course of exploration of and habituation to a novel environment when tested during either the light or the dark phase of the rodent diurnal cycle [147]. Conversely, in another study, although no genotypic difference was observed for spontaneous locomotor activity in a novel environment during the light phase, D_3 knockouts were consistently hyperactive relative to wild types over a 3-h period during the dark phase [148]. Results from more recent experiments in D_3 knockouts of increasing congenicity demonstrate similar incongruity. Specifically, among three studies evaluating spontaneous locomotion in a novel and/or familiar environment in incipient or fully congenic D_3 knockouts [69, 149, 150], only one [149] observed a significantly greater level of locomotion in D_3 knockout mice.

In our own laboratory, when assessed ethologically over a 1-h period of exploration in a novel environment, hybrid D_3 knockouts were characterized by significant increases in spontaneous sniffing, locomotion, and discrete topographies of rearing, together with a decrease in grooming relative to their wild type counterparts. Over the course of habituation to the environment, initially high levels of sniffing and rearing endured in D_3 knockouts but not in wild types [72]. In contrast, when evaluated using identical phenotypic assessment techniques by the same observer, congenic D_3 knockouts were characterized by no alteration in any topography of spontaneous behavior present in the mouse repertoire during exploration in a novel environment; continued assessment over several hours revealed only delayed habituation of rearing that was limited to females [151].

A number of studies have utilized D_3 knockouts to investigate the possible involvement of D_3 receptors in the behavioral manifestation of anxiety. Xu and colleagues noted that hybrid D_3 knockouts and wild types made a similar number of entries into the more aversive open arms of an elevated plus maze, leading these investigators to speculate that increased locomotion previously reported in these mutants is not secondary to a heightened state of anxiety [145]. In contrast, two subsequent studies using the original hybrid line created by Accili and colleagues found that D_3 knockouts made significantly more entries and spent more time in the open arms of an elevated plus maze [68, 152], consistent with an anxiolytic effect of D_3 receptor deletion in these mice. In support of this finding, D_3 knockouts were more inclined than wild types to enter the central section of an unfamiliar open field [152]. Furthermore, in a separate study characterizing the behavioral phenotype of hybrid D_3 knockouts over the course of exploration in a Lat maze, defecation during the test, which is another objective measure of anxiety, was reduced in heterozygous D_3 knockouts [144].

D_3 knockouts have also been investigated in other spontaneous behavioral paradigms. In relation to orofacial movement topography, we have demonstrated

that, relative to wild types, congenic D_3 knockouts evidence transient increases and reductions in head movements and horizontal jaw movements, respectively; vertical jaw movements, tongue protrusions, and movements of the vibrissae are unaltered in these mutants. Furthermore, habituation of incisor chattering was significantly reduced in D_3 knockouts in a gender-specific manner [115]. On the T maze, which is a delayed alternation paradigm used to evaluate spatial working memory, modest impairments were noted in incipient congenic D_3 knockouts when successive increments in retention time before commencing the next trial were introduced [121]. Finally, although a recent study found no genotypic differences in spontaneous immobility during the forced swim test for antidepressant behavior, an increase in immobility was seen following vehicle injection in wild types but not incipient congenic D_3 knockout mice [153]. Interestingly, other studies involving D_3 knockouts, including isolated findings in our own laboratory, have reported similar genotypic differences in response to vehicle injection [147, 151, 154], raising the possibility that D_3 receptors might be involved in the behavioral response to stress in mice.

13.4.6 D_3 Knockout: Drug-Induced Behavior

As part of the original characterization of their hybrid D_3 knockout, Xu and colleagues found that D_3 knockouts exhibit locomotor supersensitivity in response to the combined administration of selective D1-like and D2-like agonists but not when these agents are administered separately [145]. In support of this finding, we have recently demonstrated that wild type locomotor responses to either the selective D1-like agonist SKF 83959 or the selective D2-like agonist RU 24213 are unaltered in congenic D_3 knockouts over the course of exploration and subsequent habituation to a novel environment [151]. Interestingly, the enhanced locomotion in response to the combined administration of D1-like and D2-like agonists is preserved in dopamine-depleted D_3 knockouts [145]. Taken together, these results suggest that D_3 receptors modulate ambulatory behavior by inhibiting the cooperative effects of post-synaptic D1-like and D2-like receptors.

Locomotor hyperresponsivity in hybrid D_3 knockouts has also been observed following acute challenge with low, but not high, doses of the indirect dopamine agonist cocaine [145]. In contrast, a subsequent experiment demonstrated that D_3 knockouts are characterized by a blunted locomotor response to both acute and repeated cocaine administration; however, the progressive emergence of stereotyped head-bobbing behavior associated with serial cocaine dosing in this study was markedly elevated in D_3 knockouts relative to wild types [155]. Similar studies investigating the behavioral effects of cocaine have also been performed in incipient congenic D_3 knockouts and produced inconsistent findings. Specifically, whereas repeated cocaine administration was associated with a higher level of locomotion in D_3 knockouts relative to wild type controls in one study [156], another group reported that locomotor stimulation and stereotyped behavior in response to acute cocaine administration did not differ between the genotypes [148]. Furthermore, in a recent study evaluating the role individual dopamine receptors and interactions

thereof in the behavioral effects of cocaine, locomotor stimulation, and conditioned place preference were unaltered in D_3 knockouts [69].

A number of studies in D_3 knockout mice have investigated the role of D_3 receptors in mediating behavioral responses to other psychostimulant drugs of abuse. Xu and colleagues have documented that a lower dose of amphetamine is required to produce conditioned place preference in hybrid D_3 knockouts than wild types [145]. In addition, low, but not high, dose amphetamine has been shown to stimulate locomotion to a greater extent in incipient congenic D_3 knockouts than in wild types over 3 h under partially habituated conditions [157]. A similar place preference and locomotor profile in response to morphine has been reported in incipient congenic D_3 knockouts. With regard to the former, an antagonist selective for μ-opioid receptors prevented the morphine-induced place preference effect, suggesting that functional D_3:μ-opioid receptor interactions mediate the reinforcing effects of this drug [149]. These findings of exaggerated locomotor responses to cocaine, amphetamine, and morphine in D_3 knockouts are consistent with an inhibitory role for D_3 receptors in psychostimulant-induced hyperactivity. Conversely, Risborough and colleagues have recently observed a modest gender-specific reduction in MDMA-induced locomotor activation in congenic D_3 knockouts, suggesting that D_3 receptors may normally facilitate the stimulant effects of this drug [127].

In addition to investigating the functional involvement of D_3 receptors in the locomotor and reinforcing properties of psychostimulant drugs of abuse, pharmacological studies in D_3 knockouts have also been performed on the basis of previous behavioral findings using preferential D_3 receptor ligands in intact animals. In an early study, locomotor activity in a novel environment following challenge with a range of D_3-preferring agonists (7-OH-DPAT, quinelorane, and PD 128907) or a selective D_3 antagonist (PNU 99194A) was indistinguishable between incipient congenic D_3 knockouts and wild types [147]. In contrast, a subsequent study found that novelty-induced locomotor activity following challenge with either 7-OH-DPAT or PD 128907, at lower doses than those used previously, was significantly reduced in wild type but not incipient congenic D_3 knockouts, supporting previous pharmacological and genetic evidence that D_3 receptors inhibit locomotion under normal conditions [158]. Of note, higher doses of these agents, below those used in the earlier study [147], inhibited locomotion similarly between the genotypes, indicating that both D_2 and D_3 receptors are activated at these higher doses. Interestingly, there is evidence that the mechanisms through which D_3 receptor activation inhibits novelty-induced locomotion do not appear to operate in a familiar environment [158]. Other studies have investigated the ability of selective D_3 ligands to modulate the locomotor-stimulant effects of non-dopaminergic compounds in D_3 knockout mice. Specifically, Leriche and colleagues documented that inhibition of locomotor stimulation by the selective NMDA receptor antagonist MK-801 in wild types by a partial D_3 agonist or a D_3 antagonist is absent in incipient D_3 congenic knockouts [159]. More recently, the novel selective D_3 receptor antagonist, NGB 2904, has been shown to exaggerate amphetamine-stimulated locomotion in wild types but not incipient congenic D_3 knockouts. Furthermore, high doses of

NGB 2904 stimulated spontaneous locomotion in wild types but not D_3 knockouts [159, 160]. Thus, it appears that some newer agents are indeed highly selective for the D_3 receptor and may be used in intact animals and knockout mice to further elucidate the behavioral roles of the D_3 receptor.

Emerging evidence that dopaminergic dysregulation is present in certain mood disorders has prompted studies investigating the role of D_3 receptors therein. However, in the forced swim test, increased immobility in wild types following administration of the antiparkinsonian and putatively antidepressant D2-like agonist pramipexole was unaltered in either hybrid or congenic D_3 knockouts [150], suggesting that D_3 receptors are not involved in any antidepressant effect of this drug. A similar result has been observed with imipramine in hybrid D_3 knockouts [161]. In contrast, a recent study has demonstrated that incipient congenic D_3 knockouts are more sensitive to a range of antidepressant drugs in the forced swim test [153], leading these authors to speculate that elevated dopamine levels in mesolimbic pathways [146, 162] may be responsible for the observed effect in D_3 knockout mice.

13.4.7 Interpretation of D_3 Knockout Phenotype

A summary of the behavioral changes reported to date from studies in D_3 knockouts can be seen in Table 13.1. Numerous studies have evaluated spontaneous locomotor activity in the four available D_3 knockout lines in an effort to resolve inconsistent findings obtained from initial experiments in these mutants. These studies have described either modest hyperactivity [68, 72, 144, 148, 149] or no material locomotor phenotype [69, 147, 150, 151] in D_3 knockout mice. Methodological differences related to the novelty of or familiarity with the test environment appear to have contributed to discrepant findings across laboratories. Specifically, the transient hyperactive phenotype in D_3 knockouts occurs very early during the course of exploration and is not apparent following habituation to the environment [7, 145, 149, 158]. As hybrid or incipient congenic strains were used in all instances where a significant locomotor phenotype has been identified in D_3 knockouts, it is possible that heterogeneity in the genetic background of these mutants may have produced the observed phenotypic effects. However, considering that (a) an increase in locomotion has been observed in D_3 knockouts across various laboratories using differently constructed D_3 knockout lines, (b) in the majority of instances the hyperactivity in D_3 knockouts was found to dissipate over time such that post hoc analysis over constituent time bins was required to detect these differences, (c) in other studies unaltered locomotion, but not hypoactivity, has been reported in D_3 knockouts, and (d) D_3 knockouts have been repeatedly found to exhibit an exaggerated locomotor response to phasic dopaminergic stimulation, it is plausible that D_3 receptors have a subtle functional role in negatively modulating locomotion under normal conditions. Indeed, D_3 receptor-mediated inhibition of locomotor activity is supported by recent pharmacological studies in intact rodents showing that low doses of novel selective D_3 agonists and antagonists attenuate and increase locomotion, respectively [158, 160].

Interestingly, additional pharmacological approaches in D_3 knockouts indicate that the inhibitory action of D_3 receptors on ambulatory behavior requires the cooperative activation of D1-like and D2-like receptors. Specifically, whereas locomotor stimulation in response to administration of either a selective D1-like or D2-like agonist is unaltered in D_3 knockouts relative to wild types [151], combined administration of these agonists induces exaggerated locomotor activity in D_3 knockouts [145]. In agreement with this hypothesis, the indirect dopamine agonists cocaine and amphetamine have also been shown to induce exaggerated locomotor responses in D_3 knockouts [145, 156, 157]. While one study has demonstrated a reduction in cocaine-stimulated locomotion in D_3 knockouts [155], the high dose of cocaine used by these investigators was not associated with a heightened locomotor responses in D_3 knockouts in a previous study demonstrating genotypic effects at a lower dose of the drug [145]. High-dose cocaine was associated with the emergence of stereotyped head-bobbing behavior that occurred to significant excess in D_3 knockouts. Given that increasing doses of dopamine agonists induce a behavioral phenotype characterized by progressive locomotor stimulation and leading to the emergence of competitive motor stereotypy (see Section 13.2), the findings of Carta and colleagues [155] are also consistent with an inhibitory role for D_3 receptors in psychostimulant-induced locomotion. Results obtained from studies evaluating the behavior of D_3 knockouts in a drug-conditioned place preference paradigm indicate that D_3 receptors are also functionally involved in the rewarding effects of psychostimulants [145, 149]. These findings complement previous immunocytochemical studies demonstrating high levels of D_3 receptor expression in the nucleus accumbens, a brain area well established in mediating reinforcement processes.

Although the original characterization of the hybrid D_3 knockout created by Xu and colleagues found no significant genotypic effects on the elevated plus maze [145], subsequent findings in D_3 knockouts in this paradigm [68] and also the open field [152] and Lat maze [144] suggest a functional role for D_3 receptors in facilitating behavioral responses to anxiety-provoking stimuli. When evaluated in tandem with the previous finding of novelty-induced hyperactivity in D_3 knockouts, a phenotype consistent with reduced anxiety in these mutants raises some additional points for consideration. Specifically, in the absence of any material increase in topographies of exploratory behavior such as sifting in D_3 knockouts [72, 151], it is possible that increased locomotion relative to wild types during initial exploration in a novel environment is secondary to lower levels of anxiety in these mutants. Alternatively, it could be argued that increased locomotor drive and not decreased anxiety is responsible for the higher number of entries into the open arms of the elevated plus maze and the centre of an open field in D_3 knockouts [68, 152]; however, increased locomotor drive cannot explain the lower level of defecation by D_3 knockouts under anxiety-provoking conditions [144]. Furthermore, indirect evidence that D_3 knockouts exhibit less behavioral reactivity to stress and invasive procedures than wild types [147, 151, 154] provides additional support for a facilitative role of D_3 receptors in mediating behavioral responses to anxiety.

The availability of D_3 knockouts has enabled testing of a growing hypothesis that D_3 receptors have a functional role in psychotic disorders including schizophrenia [163]. NMDA receptor antagonists such as phencyclidine and ketamine can induce psychosis in healthy individuals and precipitate psychotic episodes in patients with schizophrenia. In rodents, these agents produce robust behavioral changes including marked hyperactivity that constitutes a well-established animal model of psychosis. Interestingly, Leriche and colleagues have reported that hyperactivity induced by NMDA is attenuated in D_3 knockouts. Furthermore, the ability of the antipsychotics haloperidol and clozapine to inhibit MK 801-induced hyperactivity in wild types was essentially unaltered in D_3 knockouts, leading these investigators to speculate that agents demonstrating selective D_3 receptor blockade may have antipsychotic activity [159].

Additional evidence linking D_3 receptors and schizophrenia has emerged from the assessment of spatial memory in D_3 knockout mice. Working memory relates to the temporary storage of external information and is known to be impaired in patients with schizophrenia. The prefrontal cortex is responsible for consolidating working memory through a mechanism that requires activation of D_1 receptors. A study in D_3 knockout mice has shown that deletion of D_3 receptors is associated with blunted responsivity to stimulation of D_1 receptors in this region [121]. Furthermore, this study demonstrated impaired working memory in D_3 knockouts using a spatial memory paradigm, suggesting that abnormal D_3 receptor function may be involved in the cognitive deficits of schizophrenia. Conversely, the finding that individual topographies of orofacial movement are essentially unaltered in D_3 knockouts [115] argues against a role for D_3 receptors in the manifestation of orofacial dyskinesias that often develop consequent to prolonged antipsychotic treatment in schizophrenia.

An enduring controversy in the interpretation of results generated from studies in D_3 knockouts relates to the relative importance of pre-synaptic vs. post-synaptic sites in mediating the observed phenotypic effects. Reports that (a) discrete behavioral effects in D_3 knockouts are preserved following depletion of endogenous dopamine [145] and (b) extracellular dopamine levels and the dopamine metabolites DOPAC and HVA are unaltered in D_3 knockouts [149] have led to the postulate that post-synaptic D_3 receptors are involved. Furthermore, the fact that electrophysiological changes in single neurons following the combined administration of D1-like and D2-like agonists are unaltered in D_3 knockouts [145] suggests that post-synaptic D_3 receptors involved are located on neuronal cells distinct from those expressing D1-like and other D2-like receptor subtypes. Indeed, D_3 receptor expression on inhibitory GABAergic interneurons would result in disinhibition of dopaminergic function and explain many of the results obtained to date from studies in D_3 knockout mice. On the other hand, microdialysis studies have found a significant increase in the level of dopamine in the striatum [146, 162], consistent with an autoreceptor role for D_3 receptors in dopamine release/synthesis. Elevated extracellular dopamine in mesolimbic pathways has been hypothesized to be a possible mechanism for the antidepressant behavioral phenotype observed in mice lacking functional D_3 receptors [153]. In reality, as immunocytochemical

studies have confirmed the presence of pre-synaptic and post-synaptic D_3 receptors [164], it is likely that both populations are functionally involved in modulating D_3 receptor-dependent behaviors.

13.4.8 D_4 Knockout: Spontaneous Behavior

Initial phenotypic characterization of D_4 knockouts maintained on a hybrid genetic background (129/OlaHsd × C57BL/6) identified multiple spontaneous behavioral abnormalities. In a novel environment, D_4 knockouts covered less horizontal distance, exhibited fewer rearing episodes and moved more slowly than wild type mice. Following habituation to the testing environment, D_4 knockouts also initiated fewer movements and spent less time in motion than controls [9], indicating that D_4 receptors are involved in facilitating a variety of normal locomotor behaviors. Furthermore, in a test of motor coordination, D_4 knockouts outperformed their wild type counterparts, experiencing 50% fewer falls on the rotarod and remaining on the apparatus 2.5 times longer; this led to the postulate that D_4 knockouts are more adept at complex, coordinated motor tasks, possibly due to altered dopamine metabolism in the dorsal striatum of these animals [9].

To examine the putative functional involvement of D_4 receptors in the personality trait of novelty-seeking suggested from genetic linkage/association studies [165], Dulawa and colleagues evaluated hybrid D_4 knockouts in three approach-avoidance paradigms [166]. In contrast to the original report by Rubinstein and colleagues [9], no differences were observed between D_4 knockouts and wild types in terms of gross spontaneous locomotor activity in the open-field environment under either novel or familiar conditions. D_4 knockouts did show reduced behavioral responses to novelty, as indicated by fewer entries into the center of the field; however, the total time spent in the center of the arena did not differ significantly between the genotypes. In the emergence test, a free exploration paradigm in which animals can explore the open field or retreat into a cylinder, D_4 knockouts displayed a longer latency to emerge from the cylinder, made fewer entries into the cylinder, and spent more time inside the cylinder. Furthermore, in the novel object test, another free exploration paradigm which allows animals to explore a novel object in a non-threatening and familiar environment, D_4 knockouts were found to spend proportionately less time in the center after introduction of the stimulus [166].

Reduced exploratory activity in hybrid D_4 knockouts in the conflict-producing environments reported above might be due to avoidance behavior associated with heightened anxiety and not a reduced response to novelty per se. Accordingly, a subsequent study used hybrid D_4 knockouts to investigate the functional involvement of D_4 receptors in unconditioned anxiety-related behaviors. In the elevated plus maze, D_4 knockouts made fewer open-arm entries and spent less time in the open arms [167]. The total number of entries into either the open or closed arms was similar between the genotypes, indicating that the reduced number of open-arm entries exhibited by D_4 knockouts was not due to a locomotor or motivational deficit. In the light–dark test, D_4 knockouts displayed an increase in their initial latency to

enter the light compartment and spent less time on the illuminated side compared to wild types. In both paradigms, avoidance behavior in D_4 knockouts was completely prevented by pretreatment with anxiolytic drugs [167].

Several genetic linkage/association studies have identified discrete polymorphisms in the dopamine D_4 receptor gene as conferring increased risk for developing ADHD [168, 169]. In 2004, Avale and colleagues presented evidence that neonatal 6-OHDA lesioning in wild type mice represents a valid animal model of this clinical disorder. Specifically, lesioned mice exhibit hyperactivity that wanes after puberty, paradoxical hypolocomotor responses to amphetamine and methylphenidate, poor behavioral inhibition in approach/avoidance conflict paradigms, and deficits in continuously performed motor coordination tasks. Interestingly, congenic D_4 knockout mice with superimposed neonatal 6-OHDA lesioning failed to develop the hyperactive phenotype. Furthermore, wild type mice lesioned with 6-OHDA exhibited behavioral disinhibition when tested in an open field and the elevated plus maze, whereas D_4 knockouts demonstrated normal avoidance of the unprotected areas [170]. Collectively, the results from this study are consistent with a facilitative role for D_4 receptors in manifesting discrete behavioral features in an animal model of ADHD.

The most recent behavioral studies involving D_4 knockouts have been conducted in our laboratory [96, 103]. At the level of spontaneous ethological behavior, congenic D_4 knockouts were characterized by only a marginal reduction in exploratory sniffing together with delayed habituation of sifting. While such phenotypic differences might be interpreted as reduced novelty seeking or heightened anxiety in D_4 knockouts, other related behaviors such as rearing and locomotion were unaltered. Thus, the small magnitude and topographical specificity of these phenotypic effects indicate that any functional role for D_4 receptors in mediating spontaneous locomotor or exploratory/anxiety-related behavior is subtle [96]. In relation to dopaminergic regulation of orofacial movement topography, congenic D_4 knockouts were indistinguishable from wild type mice when assessed quantitatively for vertical and horizontal jaw movements, tongue protrusions, and incisor chattering [103], arguing against any material role for D_4 receptors in these processes.

13.4.9 D_4 Knockout: Drug-Induced Behavior

Original pharmacological characterization of hybrid (129/OlaHsd × C57BL/6) D_4 knockouts evaluated the locomotor-stimulant effects of clozapine, ethanol, cocaine, and methamphetamine in these animals [9]. Low-dose clozapine was found to attenuate apomorphine-induced locomotion in wild type but not in hybrid D_4 knockouts, whereas high-dose clozapine blocked apomorphine-induced behavior in both genotypes, perhaps via antagonism at other D2-like receptor sites. In contrast to the depressant effect of D_4 receptor deletion on spontaneous locomotor topographies reported by these investigators (see Section 13.4.8), hybrid D_4 knockouts were found to be hyperreactive to the locomotor-stimulant effects of ethanol, cocaine, and methamphetamine [9].

To further characterize the involvement of D_4 receptors in mediating the effects of psychostimulant drugs of abuse, subsequent studies evaluated additional behavioral parameters in D_4 knockout mice in response to cocaine [171] and amphetamine [172]. In agreement with the earlier report of Rubinstein and colleagues, locomotor responses to cocaine were greater in hybrid D_4 knockouts compared to wild type controls [171]. As an extension of this work, Katz and co-workers examined the involvement of D_4 receptors in mediating the discriminative stimulus effects of cocaine. While lower doses of cocaine were able to initiate cocaine responding in D_4 knockouts relative to wild types, the observation that the D_2/D_3 antagonist raclopride antagonized cocaine responding to a similar extent in both genotypes suggests that D_4 receptors are minimally and indirectly involved in mediating the discriminative effects of cocaine, possibly via inhibitory functional interactions with other D2-like receptors [171]. In response to amphetamine, locomotor activity was unaltered in congenic D_4 knockouts relative to wild types over a 1-h period following acute administration of amphetamine; however, post hoc analysis of activity over constituent time bins during the assessment revealed a time $\times$ genotype interaction at higher doses of the drug, characterized by a transient increase in locomotor activity in D_4 knockout mice. Furthermore, following a chronic administration schedule, congenic D_4 knockouts displayed an enhanced dose-dependent sensitized response to amphetamine compared to wild types, consistent with a functional role for D_4 receptors in psychostimulant-mediated neural plasticity [172].

The above pharmacological studies in D_4 knockout mice employed non-selective or indirect dopamine agonists, having indiscriminate activity across the five cloned dopamine receptor subtypes, to probe the functional roles of the D_4 receptor. Recently, we sought to elaborate these findings by evaluating the behavioral phenotype of congenic D_4 knockouts in response to selective D2-like and D1-like agonists using an ethologically based behavioral assessment technique [96]. The characteristic shift from normal fluid ambulation to stereotyped ponderous locomotion in response to the selective D2-like agonist RU 24213 was reduced in D_4 knockouts, consistent with a role for D_4 receptors in facilitating the expression of stereotyped locomotor topographies. Interestingly, induction of intense grooming syntax by the selective D1-like antagonist SKF 83959 was reduced in D_4 knockout mice. Furthermore, the ability of the structurally related but pharmacologically distinct selective D1-like agonist SKF 83822 to lower seizure threshold [39, 91, 94] was attenuated in D_4 knockouts in accordance with a minor facilitative role for D_4 receptors in mediating dopamine-dependent seizures [96].

13.4.10 Interpretation of D_4 Knockout Phenotype

A summary of the behavioral changes reported to date from studies in D_4 knockouts can be seen in Table 13.1. It is clear from the preceding sections that considerable inconsistency exists between findings relating to spontaneous locomotion in hybrid (129/OlaHsd $\times$ C57BL/6) D_4 knockouts. One study observed significant reductions in gross spontaneous locomotor and rearing activity in both novel

and familiar environments in D_4 knockouts [9], whereas a subsequent investigation reported no significant locomotor differences between D_4 knockouts and wild types under similar experimental conditions [166]. Furthermore, in a study evaluating the locomotor-stimulant effects of cocaine in hybrid D_4 knockouts, locomotor activity in saline-treated groups did not differ significantly between the genotypes [171]. Collectively, these results indicate that factors unrelated to loss of the D_4 receptor may have produced these phenotypic effects. In relation to genetic background, when C57BL/6 and 129/OlaHsd inbred mouse strains are compared directly, 129/OlaHsd mice are hypoactive in the open-field arena [173]. This might explain the lack of any material spontaneous locomotor phenotype in congenic D_4 knockouts for which the contribution of 129/OlaHsd alleles is expected to be below 0.1% [96].

Assessment of hybrid D_4 knockouts in targeted behavioral paradigms has identified facilitatory and inhibitory roles for D_4 receptors in the related traits of novelty-seeking [166] and anxiety [167], respectively, suggesting that D_4 receptors may be involved in the expression of certain emotionally driven behaviors. In addition, an elegant experiment utilizing a combined gene knockout and pharmacological lesioning approach has provided evidence that D_4 receptors are necessary to recapitulate many of the core behavioral features in an animal model of ADHD [170], a relationship supported by clinical genetic studies in humans. However, it is pertinent to mention that any putative relationship between genetically determined D_4 receptor-mediated phenotypes in humans and mice must be interpreted with caution given that the 48 bp repeat polymorphism in exon III of the human D_4 receptor gene associated with ADHD (and novelty-seeking behavior) is absent from the murine homologue [174].

Absence of D_4 receptors has consistently been shown to augment the locomotor-stimulant effects of various drugs of abuse including the indirect dopamine agonists cocaine and amphetamine [9, 171, 172]. In contrast, the characteristic stereotyped locomotor response to the selective D2-like agonist, RU 24213, is reduced in D_4 knockouts. Taken together, these findings are important at two levels. Firstly, D_4 receptors appear to be integral to the development of motor stereotypy, a hypothesis further supported by anatomical reports localizing D_4 receptors to discrete areas of the basal ganglia purported to be involved in the expression of behavioral stereotypies. Secondly, these findings provide indirect evidence that functional D_4:D1-like receptor interactions are operating to produce locomotor supersensitivity in D_4 knockouts. Direct evidence for functional D_4:D1-like receptor interactions in vivo has emerged from behavioral studies examining selective D1-like agonists in congenic D_4 knockout mice [96].

Until recently, no evidence has been reported to suggest the functional involvement of individual D2-like receptor subtypes in mediating grooming behavior. Specifically, induction of syntactic, intense grooming in response to the selective D1-like agonist SKF 83959 is conserved in congenic D_3 receptor knockouts [151]; SKF 83959 has yet to be tested in D_2 knockout mice. The finding that such SKF 83959-induced grooming is reduced in congenic D_4 knockouts indicates, for the first

time, that subtle D_4:D1-like receptor interactions participate in mediating grooming behavior. Furthermore, the observation that induction of seizures by the selective D1-like agonist SKF 83822 is reduced in D_4 knockouts suggests that cooperative functional D_4:D1-like receptor interactions also participate in the regulation of neuronal excitability. Behavioral indices of such functional D_4:D1-like interactions are reinforced by the recent observation that D_1 receptor expression is up-regulated in D_4 knockouts [175]. However, how these interactions are organized within the brain at a molecular, cellular, or network level and the relative involvement of D_1 and D_5 receptors remains to be established.

13.5 Double Knockouts Involving Dopamine Receptors

13.5.1 D_1/D_2 Double Knockout

The relative abundance of D_1 and D_2 receptors vis-à-vis other dopamine receptor subtypes in the brain prompted the generation of D_1/D_2 double knockouts to investigate their involvement in functional D1-like:D2-like interactions [176]. Although the progeny from heterozygous D_1/D_2 double knockout matings follows the expected Mendelian ratio, homozygous D_1/D_2 double knockouts die in the early postnatal period in the absence of any identifiable macroscopic or microscopic brain abnormalities; this is most likely due to altered feeding behavior and a dysfunctional gastrointestinal system. In contrast, heterozygous D_1/homozygous D_2 double knockouts evidence reduced body weight but survive to adulthood, while homozygous D_1/heterozygous D_2 double knockouts develop ulcers in the small intestine and only survive if fed a semi-liquid diet. Collectively, these findings indicate that cooperative D_1:D_2 receptor interactions are involved in feeding behavior and gastrointestinal function, with functional primacy of D_1 receptors in these effects. Interestingly, spontaneous locomotor behavior and coordination in homozygous D_1 or homozygous D_2 single knockouts are unaltered by concomitant deletion of one functional D_2 or D_1 receptor allele, respectively [176]. However, given that meaningful locomotor assessment cannot be performed in homozygous D_1/D_2 double knockouts for the reasons outlined above, it would be premature to exclude a role for D_1:D_2 receptor interactions in these behaviors based on this finding. Future development of tissue specific and/or conditional homozygous D_1/D_2 double knockouts or studies with selective D1-like or D2-like drugs in this model may help to clarify the issue.

13.5.2 D_1/D_3 Double Knockout

In situ hybridization studies have shown the D_1 and D_3 receptor mRNAs are co-localized in single neurons, suggesting that these receptors functionally interact at

a cellular level [177]. In 2000, individual D_1 and D_3 knockouts and D_1/D_3 double knockouts maintained on a hybrid genetic background were compared in a number of behavioral paradigms [68]. No evidence was found for receptor interaction in tests of spatial memory or motor coordination. Conversely, a lower level of locomotion and rearing in D_1 knockouts relative to wild types during exploration in an open field was exacerbated in D_1/D_3 double knockouts, leading these authors to conclude that D_1 and D_3 receptors interact to modulate discrete topographies of exploratory behavior. However, it should be mentioned that abundant evidence indicates that D_1 knockouts are characterized by spontaneous hyperactivity (see Section 13.3.3), and not hypoactivity as reported in this study. Indeed, a subsequent study by these investigators using incipient congenic lines reported excessive locomotion in both D_1 and D_1/D_3 knockouts under novel and familiar conditions [69]. Furthermore, we have shown that increased locomotion, sniffing, and rearing in D_1 knockouts during exploration in a novel environment are unaltered in hybrid D_1/D_3 double knockouts [72]. Interestingly, although isolated features of D_3 knockout phenotype are apparent in mice lacking both D_1 and D_3 receptors, the overall spontaneous behavioral phenotype in D_1/D_3 double knockouts mirrors considerably that of D_1 knockout mice [68, 72]; this suggests that D_1 receptors facilitate discrete behaviors in D_3 knockout mice. In support of this hypothesis, increased anxiety in D_3 knockouts is abolished in the absence of D_1 receptors [68]. More recent findings in D_1/D_3 double knockouts indicate that while D_1 receptors are important in the locomotor-stimulant effects of cocaine (see Section 13.3.3), functional interaction with D_3 receptors may be involved in the reinforcing properties of this drug [69].

13.5.3 D_2/D_3 Double Knockout

Within the D2-like family, the high degree of structural homology between D_2 and D_3 receptors led to the early hypothesis that these receptors may have complementary functions in regions of shared expression [108]. Indeed, both D_2 and D_3 (but not D_4) receptors are known to function as pre-synaptic autoreceptors to regulate dopamine synthesis/release. An initial comparative study between hybrid D_2 and D_3 single knockouts and derived D_2/D_3 double knockouts found that impaired ambulation in mice lacking D_2 receptors is more pronounced in the absence of D_3 receptors. Furthermore, D_3 receptor expression was increased in D_2 knockouts in this study, leading these authors to postulate that cooperative D_2:D_3 receptor interactions normally facilitate locomotion in mice and D_3 receptors may partially compensate for D_2 receptor functions in D_2 knockout mice [108]. However, these findings are incongruent with other evidence from single mutants that D_3 receptors normally inhibit motor behavior (see Section 13.4.7) and previous data demonstrating unaltered D_3 (or D_4) RNA expression in an independently created D_2 knockout line [8]. A subsequent study has shown that in a Lat maze, locomotion and rearing frequency and duration in D_2 knockouts are essentially unaltered in D_2/D_3 double knockouts [144]. In addition, a recent study has found that D_2 receptor-mediated

motor stereotypies are not influenced by D_3 receptors [178]. Thus, inconsistent findings from the small number of studies conducted to date involving D_2/D_3 double knockouts do not support the existence of prominent functional D_2:D_3 receptor interactions in the behaviors examined; this does not exclude (a) the possibility that methodological factors including genetic background effects may have obscured subtle interactions and (b) a role for D_2/D_3 receptor interactions in modulating other dopamine-dependent behaviors.

13.6 Challenges

There are few domains of behavior for which regulation by one or more dopamine receptors has been excluded and those domains where such regulation is known to be either fundamental or an important modulatory factor are multiple and diverse. However, it is now many years since the continuing identification of new, molecular biologically defined dopamine receptor subtypes exceeded the capacity of medicinal chemists to identify selective agonists and antagonists for each of those subtypes. This psychopharmacological vacuum reflects at least two factors: difficulties in identifying chemical structures that can distinguish between receptor proteins whose amino acid sequences evidence only subtle differences and some concern in the pharmaceutical industry as to whether such selectivity is of therapeutic potential in "volume" disorders.

The adage that "nature abhors a vacuum" appears just as true in neuroscience as elsewhere; hence this psychopharmacological vacuum has been filled by an alternative approach to parcellation of function between dopamine receptor subtypes, namely mutant mice with targeted gene manipulation. This approach is a "two-edged sword": the potential of these techniques is not yet fully realized, particularly in terms of conditional mutants that afford the investigator temporal and/or regional control over expression of the receptor mutation, and the ability to target specific cell types; yet these techniques engender their own concerns, particularly in terms of genetic background effects, compensation processes, and sex-specific phenotypes; also, concerns endure over non-replicability between laboratories that may reflect differing molecular genetic strategies for targeting the same receptor and/or differing test paradigms for targeting the same domain of behavior [4]. Nevertheless, the yield to date from mutant studies has been substantial and is likely to increase. This yield would likely be facilitated by combining studies in mutants with an enhanced psychopharmacological "toolbox," to the extent that further selective agents are forthcoming, and systematic studies that allow more valid comparisons between laboratories. We have learned much over the past 50 years, but there is still more to understand.

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References

1. Holmes A, Lachowicz JE, Sibley DR. Phenotypic analysis of dopamine receptor knockout mice; recent insights into the functional specificity of dopamine receptor subtypes. Neuropharmacology 2004;47:1117–34.
2. Tan S, Hermann B, Borrelli E. Dopaminergic mouse mutants: investigating the roles of the different dopamine receptor subtypes and the dopamine transporter. Int Rev Neurobiol 2003;54:145–97.
3. Waddington JL, Clifford JJ, McNamara FN, Tomiyama K, Koshikawa N, Croke DT. The psychopharmacology-molecular biology interface: exploring the behavioural roles of dopamine receptor subtypes using targeted gene deletion ('knockout'). Prog Neuropsychopharmacol Biol Psychiatry 2001;25:925–64.
4. Waddington JL, O'Tuathaigh C, O'Sullivan G, Tomiyama K, Koshikawa N, Croke DT. Phenotypic studies on dopamine receptor subtype and associated signal transduction mutants: insights and challenges from 10 years at the psychopharmacology-molecular biology interface. Psychopharmacology 2005;181:611–38.
5. Zhou QY, Palmiter RD. Dopamine-deficient mice are severely hypoactive, adipsic, and aphagic. Cell 1995;83:1197–209.
6. Giros B, Jaber M, Jones SR, Wightman RM, Caron MG. Hyperlocomotion and indifference to cocaine and amphetamine in mice lacking the dopamine transporter. Nature 1996;379:606–12.
7. Accili D, Fishburn CS, Drago J, et al. A targeted mutation of the D3 dopamine receptor gene is associated with hyperactivity in mice. Proc Natl Acad Sci USA 1996;93:1945–9.
8. Baik JH, Picetti R, Saiardi A, et al. Parkinsonian-like locomotor impairment in mice lacking dopamine D2 receptors. Nature 1995;377:424–8.
9. Rubinstein M, Phillips TJ, Bunzow JR, et al. Mice lacking dopamine D4 receptors are supersensitive to ethanol, cocaine, and methamphetamine. Cell 1997;90:991–1001.
10. Xu M, Moratalla R, Gold LH, et al. Dopamine D1 receptor mutant mice are deficient in striatal expression of dynorphin and in dopamine-mediated behavioral responses. Cell 1994;79:729–42.
11. Usiello A, Baik JH, Rouge-Pont F, et al. Distinct functions of the two isoforms of dopamine D2 receptors. Nature 2000;408:199–203.
12. Wang Y, Xu R, Sasaoka T, Tonegawa S, Kung MP, Sankoorikal EB. Dopamine D2 long receptor-deficient mice display alterations in striatum-dependent functions. J Neurosci 2000;20:8305–14.
13. Drago J, Padungchaichot P, Accili D, Fuchs S. Dopamine receptors and dopamine transporter in brain function and addictive behaviors: insights from targeted mouse mutants. Dev Neurosci 1998;20:188–203.
14. Glickstein SB, Schmauss C. Dopamine receptor functions: lessons from knockout mice. Pharmacol Ther 2001;91:63–83.
15. Sibley DR. New insights into dopaminergic receptor function using antisense and genetically altered animals. Annu Rev Pharmacol Toxicol 1999;39:313–41.
16. Viggiano D, Ruocco LA, Sadile AG. Dopamine phenotype and behaviour in animal models: in relation to attention deficit hyperactivity disorder. Neurosci Biobehav Rev 2003;27:623–37.
17. Zhang J, Xu M. Toward a molecular understanding of psychostimulant actions using genetically engineered dopamine receptor knockout mice as model systems. J Addict Dis 2001;20:7–18.
18. Niznik HB, Sugamori KS, Clifford JJ, Waddington JL. D1-like dopamine receptors: molecular biology and pharmacology. In: Di Chiara G, ed. Handbook of experimental pharmacology: Dopamine in the CNS 1. Berlin: Springer; 2002:121–58.

19. Waddington JL, Daly SA, Downes RP, Deveney AM, McCauley PG, O'Boyle KM. Behavioural pharmacology of 'D1-like' dopamine receptors: further subtyping, new pharmacological probes and interactions with 'D2-like' receptors. Prog Neuropsychopharmacol Biol Psychiatry 1995;19:811–31.
20. Waddington JL, Daly SA, McCaulay PG, O'Boyle KM. Levels of functional interaction between D1-like and D2-like dopamine receptor systems. In: Niznik HB, ed. Dopamine receptors and transporters: Pharmacology, structure and function. New York: Marcel Dekker; 1994:511–37.
21. Adachi K, Hasegawa M, Fujita S, et al. Prefrontal, accumbal [shell] and ventral striatal mechanisms in jaw movements and non-cyclase-coupled dopamine D1-like receptors. Eur J Pharmacol 2003;473:47–54.
22. Adachi K, Ikeda H, Hasegawa M, Nakamura S, Waddington JL, Koshikawa N. SK&F 83959 and non-cyclase-coupled dopamine D1-like receptors in jaw movements via dopamine D1-like/D2-like receptor synergism. Eur J Pharmacol 1999;367:143–9.
23. Fujita S, Lee J, Kiguchi M, et al. Topographical resolution of jaw movements mediated by cyclase- vs. non-cyclase-coupled dopamine D(1)-like receptors: studies with SK&F 83822. Eur J Pharmacol 2006;538:94–100.
24. Hasegawa M, Adachi K, Nakamura S, Sato M, Waddington JL, Koshikawa N. Ventral striatal vs. accumbal (shell) mechanisms and non-cyclase-coupled dopamine D(1)-like receptors in jaw movements. Eur J Pharmacol 2001;423:171–8.
25. Makihara Y, Okuda Y, Kawada C, et al. Differential involvement of cyclase- versus non-cyclase-coupled D1-like dopamine receptors in orofacial movement topography in mice: studies with SKF 83822. Neurosci Lett 2007;415:6–10.
26. Makihara Y, Yamamoto H, Inoue M, Tomiyama K, Koshikawa N, Waddington JL. Topographical effects of D1-like dopamine receptor agonists on orofacial movements in mice and their differential regulation via oppositional versus synergistic D1-like: D2-like interactions: cautionary observations on SK&F 82958 as an anomalous agent. J Psychopharmacol 2004;18:484–95.
27. Tomiyama K, McNamara FN, Clifford JJ, Kinsella A, Koshikawa N, Waddington JL. Topographical assessment and pharmacological characterization of orofacial movements in mice: dopamine D(1)-like vs. D(2)-like receptor regulation. Eur J Pharmacol 2001;418:47–54.
28. Setler PE, Sarau HM, Zirkle CL, Saunders HL. The central effects of a novel dopamine agonist. Eur J Pharmacol 1978;50:419–30.
29. Molloy AG, Waddington JL. Dopaminergic behaviour stereospecific promoted by the D1 agonist R-SK & F 38393 and selectively blocked by the D1 antagonist SCH 23390. Psychopharmacology 1984;82:409–10.
30. Cromwell HC, Berridge KC. Implementation of action sequences by a neostriatal site: a lesion mapping study of grooming syntax. J Neurosci 1996;16:3444–58.
31. Kalueff AV, Aldridge JW, LaPorte JL, Murphy DL, Tuohimaa P. Analyzing grooming microstructure in neurobehavioral experiments. Nat Protoc 2007;2:2538–44.
32. Waddington JL, O'Boyle KM. Drugs acting on brain dopamine receptors: a conceptual re-evaluation five years after the first selective D1 antagonist. Pharmacol Ther 1989;43:1–52.
33. Andersen PH, Jansen JA. Dopamine receptor agonists: selectivity and dopamine D1 receptor efficacy. Eur J Pharmacol 1990;188:335–47.
34. O'Boyle KM, Gaitanopoulos DE, Brenner M, Waddington JL. Agonist and antagonist properties of benzazepine and thienopyridine derivatives at the D1 dopamine receptor. Neuropharmacology 1989;28:401–5.
35. Jenner P. The rationale for the use of dopamine agonists in Parkinson's disease. Neurology 1995;45:S6–12.
36. Graham DL, Hoppenot R, Hendryx A, Self DW. Differential ability of D1 and D2 dopamine receptor agonists to induce and modulate expression and reinstatement of cocaine place preference in rats. Psychopharmacology 2007;191:719–30.

37. Braun AR, Laruelle M, Mouradian MM. Interactions between D1 and D2 dopamine receptor family agonists and antagonists: the effects of chronic exposure on behavior and receptor binding in rats and their clinical implications. J Neural Transm 1997;104: 341–62.
38. Beninger RJ, Miller R. Dopamine D1-like receptors and reward-related incentive learning. Neurosci Biobehav Rev 1998;22:335–45.
39. O'Sullivan GJ, Roth BL, Kinsella A, Waddington JL. SK&F 83822 distinguishes adenylyl cyclase from phospholipase C-coupled dopamine D1-like receptors: behavioural topography. Eur J Pharmacol 2004;486:273–80.
40. Rashid AJ, So CH, Kong MM, et al. D1-D2 dopamine receptor heterooligomers with unique pharmacology are coupled to rapid activation of Gq/11 in the striatum. Proc Natl Acad Sci USA 2007;104:654–9.
41. Arnt J, Hyttel J. Differential involvement of dopamine D-1 and D-2 receptors in the circling behaviour induced by apomorphine, SK & F 38393, pergolide and LY 171555 in 6-hydroxydopamine-lesioned rats. Psychopharmacology 1985;85: 346–52.
42. Starr BS, Starr MS. Differential effects of dopamine D1 and D2 agonists and antagonists on velocity of movement, rearing and grooming in the mouse. Implications for the roles of D1 and D2 receptors. Neuropharmacology 1986;25:455–63.
43. Jenner P. Dopamine agonists, receptor selectivity and dyskinesia induction in Parkinson's disease. Curr Opin Neurol 2003;16(Suppl 1):S3–7.
44. Yamamoto M, Usuda S, Tachikawa S, Maeno H. Pharmacological studies on a new benzamide derivative, YM-09151-2, with potential neuroleptic properties. Neuropharmacology 1982;21:945–51.
45. Starr MS. The role of dopamine in epilepsy. Synapse 1996;22:159–94.
46. Ahlenius S, Salmi P. Behavioral and biochemical effects of the dopamine D3 receptor-selective ligand, 7-OH-DPAT, in the normal and the reserpine-treated rat. Eur J Pharmacol 1994;260:177–81.
47. Pugsley TA, Davis MD, Akunne HC, et al. Neurochemical and functional characterization of the preferentially selective dopamine D3 agonist PD 128907. J Pharmacol Exp Ther 1995;275:1355–66.
48. Kagaya T, Yonaga M, Furuya Y, Hashimoto T, Kuroki J, Nishizawa Y. Dopamine D3 agonists disrupt social behavior in rats. Brain Res 1996;721:229–32.
49. Rogoz Z, Skuza G, Klodzinska A. Anxiolytic-like effects of preferential dopamine D3 receptor agonists in an animal model. Pol J Pharmacol 2003;55:449–54.
50. Chagas-Martinich L, Carey RJ, Carrera MP. 7-OH-DPAT effects on latent inhibition: low dose facilitation but high dose blockade: implications for dopamine receptor involvement in attentional processes. Pharmacol Biochem Behav 2007;86:441–8.
51. Micheli F, Heidbreder C. Selective dopamine D3 receptor antagonists: a review 2001–2005. Recent Patents CNS Drug Discov 2006;1:271–88.
52. Joyce JN, Millan MJ. Dopamine D3 receptor antagonists as therapeutic agents. Drug Discov Today 2005;10:917–25.
53. Oak JN, Oldenhof J, Van Tol HH. The dopamine D(4) receptor: one decade of research. Eur J Pharmacol 2000;405:303–27.
54. Clifford JJ, Waddington JL. Topographically based search for an 'Ethogram' among a series of novel D(4) dopamine receptor agonists and antagonists. Neuropsychopharmacology 2000;22:538–44.
55. Zhang K, Grady CJ, Tsapakis EM, Andersen SL, Tarazi FI, Baldessarini RJ. Regulation of working memory by dopamine D4 receptor in rats. Neuropsychopharmacology 2004;29:1648–55.
56. Arnsten AF, Murphy B, Merchant K. The selective dopamine D4 receptor antagonist, PNU-101387G, prevents stress-induced cognitive deficits in monkeys. Neuropsychopharmacology 2000;23:405–10.

57. Zhang K, Davids E, Tarazi FI, Baldessarini RJ. Effects of dopamine D4 receptor-selective antagonists on motor hyperactivity in rats with neonatal 6-hydroxydopamine lesions. Psychopharmacology 2002;161:100–6.
58. Shah AA, Sjovold T, Treit D. Selective antagonism of medial prefrontal cortex D4 receptors decreases fear-related behaviour in rats. Eur J Neurosci 2004;19:3393–7.
59. Bernaerts P, Tirelli E. Facilitatory effect of the dopamine D4 receptor agonist PD168,077 on memory consolidation of an inhibitory avoidance learned response in C57BL/6 J mice. Behav Brain Res 2003;142:41–52.
60. Powell SB, Paulus MP, Hartman DS, Godel T, Geyer MA. RO-10-5824 is a selective dopamine D4 receptor agonist that increases novel object exploration in C57 mice. Neuropharmacology 2003;44:473–81.
61. Drago J, Gerfen CR, Lachowicz JE, et al. Altered striatal function in a mutant mouse lacking D1A dopamine receptors. Proc Natl Acad Sci USA 1994;91:12564–8.
62. McNamara FN, Clifford JJ, Tighe O, et al. Congenic D1A dopamine receptor mutants: ethologically based resolution of behavioural topography indicates genetic background as a determinant of knockout phenotype. Neuropsychopharmacology 2003;28:86–99.
63. Smith DR, Striplin CD, Geller AM, et al. Behavioural assessment of mice lacking D1A dopamine receptors. Neuroscience 1998;86:135–46.
64. Holmes A, Hollon TR, Gleason TC, et al. Behavioral characterization of dopamine D5 receptor null mutant mice. Behav Neurosci 2001;115:1129–44.
65. Xu M, Hu XT, Cooper DC, et al. Elimination of cocaine-induced hyperactivity and dopamine-mediated neurophysiological effects in dopamine D1 receptor mutant mice. Cell 1994;79:945–55.
66. El-Ghundi M, Fletcher PJ, Drago J, Sibley DR, O'Dowd BF, George SR. Spatial learning deficit in dopamine D(1) receptor knockout mice. Eur J Pharmacol 1999;383:95–106.
67. Tran AH, Tamura R, Uwano T, Kobayashi T, Katsuki M, Ono T. Dopamine D1 receptors involved in locomotor activity and accumbens neural responses to prediction of reward associated with place. Proc Natl Acad Sci USA 2005;102:2117–22.
68. Karasinska JM, George SR, El-Ghundi M, Fletcher PJ, O'Dowd BF. Modification of dopamine D(1) receptor knockout phenotype in mice lacking both dopamine D(1) and D(3) receptors. Eur J Pharmacol 2000;399:171–81.
69. Karasinska JM, George SR, Cheng R, O'Dowd BF. Deletion of dopamine D1 and D3 receptors differentially affects spontaneous behaviour and cocaine-induced locomotor activity, reward and CREB phosphorylation. Eur J Neurosci 2005;22:1741–50.
70. Centonze D, Grande C, Saulle E, et al. Distinct roles of D1 and D5 dopamine receptors in motor activity and striatal synaptic plasticity. J Neurosci 2003;23:8506–12.
71. Clifford JJ, Tighe O, Croke DT, Sibley DR, Drago J, Waddington JL. Topographical evaluation of the phenotype of spontaneous behaviour in mice with targeted gene deletion of the D1A dopamine receptor: paradoxical elevation of grooming syntax. Neuropharmacology 1998;37:1595–602.
72. Wong JY, Clifford JJ, Massalas JS, Kinsella A, Waddington JL, Drago J. Essential conservation of D1 mutant phenotype at the level of individual topographies of behaviour in mice lacking both D1 and D3 dopamine receptors. Psychopharmacology 2003;167:167–73.
73. El-Ghundi M, O'Dowd BF, Erclik M, George SR. Attenuation of sucrose reinforcement in dopamine D1 receptor deficient mice. Eur J Neurosci 2003;17:851–62.
74. Nitz DA, Kargo WJ, Fleischer J. Dopamine signaling and the distal reward problem. Neuroreport 2007;18:1833–6.
75. Tomiyama K, McNamara FN, Clifford JJ, et al. Phenotypic resolution of spontaneous and D1-like agonist-induced orofacial movement topographies in congenic dopamine D1A receptor 'knockout' mice. Neuropharmacology 2002;42:644–52.
76. Short JL, Ledent C, Drago J, Lawrence AJ. Receptor crosstalk: characterization of mice deficient in dopamine D1 and adenosine A2A receptors. Neuropsychopharmacology 2006;31:525–34.

77. El-Ghundi M, O'Dowd BF, George SR. Prolonged fear responses in mice lacking dopamine D1 receptor. Brain Res 2001;892:86–93.
78. Drago J, Gerfen CR, Westphal H, Steiner H. D1 dopamine receptor-deficient mouse: cocaine-induced regulation of immediate-early gene and substance P expression in the striatum. Neuroscience 1996;74:813–23.
79. Clifford JJ, Tighe O, Croke DT, et al. Conservation of behavioural topography to dopamine D1-like receptor agonists in mutant mice lacking the D1A receptor implicates a D1-like receptor not coupled to adenylyl cyclase. Neuroscience 1999;93:1483–9.
80. Drago F, Contarino A, Busa L. The expression of neuropeptide-induced excessive grooming behavior in dopamine D1 and D2 receptor-deficient mice. Eur J Pharmacol 1999;365: 125–31.
81. Xu M, Guo Y, Vorhees CV, Zhang J. Behavioral responses to cocaine and amphetamine administration in mice lacking the dopamine D1 receptor. Brain Res 2000;852:198–207.
82. Hiroi N, Martin AB, Grande C, Alberti I, Rivera A, Moratalla R. Molecular dissection of dopamine receptor signaling. J Chem Neuroanat 2002;23:237–42.
83. Miner LL, Drago J, Chamberlain PM, Donovan D, Uhl GR. Retained cocaine conditioned place preference in D1 receptor deficient mice. Neuroreport 1995;6:2314–6.
84. Caine SB, Thomsen M, Gabriel KI, et al. Lack of self-administration of cocaine in dopamine D1 receptor knock-out mice. J Neurosci 2007;27:13140–50.
85. Becker A, Grecksch G, Kraus J, et al. Loss of locomotor sensitisation in response to morphine in D1 receptor deficient mice. Naunyn Schmiedebergs Arch Pharmacol 2001;363:562–8.
86. Miyamoto S, Mailman RB, Lieberman JA, Duncan GE. Blunted brain metabolic response to ketamine in mice lacking D(1A) dopamine receptors. Brain Res 2001;894:167–80.
87. El-Ghundi M, George SR, Drago J, et al. Disruption of dopamine D1 receptor gene expression attenuates alcohol-seeking behavior. Eur J Pharmacol 1998;353:149–58.
88. Crawford CA, Drago J, Watson JB, Levine MS. Effects of repeated amphetamine treatment on the locomotor activity of the dopamine D1A-deficient mouse. Neuroreport 1997;8: 2523–7.
89. Karper PE, De la Rosa H, Newman ER, et al. Role of D1-like receptors in amphetamine-induced behavioral sensitization: a study using D1A receptor knockout mice. Psychopharmacology 2002;159:407–14.
90. McDougall SA, Reichel CM, Cyr MC, Karper PE, Nazarian A, Crawford CA. Importance of D(1) receptors for associative components of amphetamine-induced behavioral sensitization and conditioned activity: a study using D(1) receptor knockout mice. Psychopharmacology 2005;183:20–30.
91. O'Sullivan GJ, Dunleavy M, Hakansson K, et al. Dopamine D1 vs D5 receptor-dependent induction of seizures in relation to DARPP-32, ERK1/2 and GluR1-AMPA signalling. Neuropharmacology 2008;54:1051–61.
92. O'Sullivan GJ, Clifford JJ, Tomiyama K, et al. D1-like dopamine receptor-mediated function in congenic mutants with D1 vs. D5 receptor 'knockout'. J Recept Signal Transduct Res 2004;24:107–16.
93. Montague DM, Striplin CD, Overcash JS, Drago J, Lawler CP, Mailman RB. Quantification of D1B(D5) receptors in dopamine D1A receptor-deficient mice. Synapse 2001;39:319–22.
94. O'Sullivan GJ, Kinsella A, Sibley DR, Tighe O, Croke DT, Waddington JL. Ethological resolution of behavioural topography and D1-like versus D2-like agonist responses in congenic D5 dopamine receptor mutants: identification of D5:D2-like interactions. Synapse 2005;55:201–11.
95. Cromwell HC, Berridge KC, Drago J, Levine MS. Action sequencing is impaired in D1A-deficient mutant mice. Eur J Neurosci 1998;10:2426–32.
96. O'Sullivan GJ, Kinsella A, Grandy DK, Tighe O, Croke DT, Waddington JL. Ethological resolution of behavioral topography and D2-like vs. D1-like agonist responses in congenic D4 dopamine receptor 'knockouts': identification of D4:D1-like interactions. Synapse 2006;59:107–18.

97. Valjent E, Pascoli V, Svenningsson P, et al. Regulation of a protein phosphatase cascade allows convergent dopamine and glutamate signals to activate ERK in the striatum. Proc Natl Acad Sci USA 2005;102:491–6.
98. Dracheva S, Xu M, Kelley KA, et al. Paradoxical locomotor behavior of dopamine D1 receptor transgenic mice. Exp Neurol 1999;157:169–79.
99. Dracheva S, Haroutunian V. Locomotor behavior of dopamine D1 receptor transgenic/D2 receptor deficient hybrid mice. Brain Res 2001;905:142–51.
100. Drago J, Padungchaichot P, Wong JY, et al. Targeted expression of a toxin gene to D1 dopamine receptor neurons by cre-mediated site-specific recombination. J Neurosci 1998;18:9845–57.
101. Gantois I, Fang K, Jiang L, et al. Ablation of D1 dopamine receptor-expressing cells generates mice with seizures, dystonia, hyperactivity, and impaired oral behavior. Proc Natl Acad Sci USA 2007;104:4182–7.
102. Wong JY, Padungchaichot P, Massalas JS, Drago J. Late direct and transneuronal effects in mice with targeted expression of a toxin gene to D1 dopamine receptor neurons. Neuroscience 2000;95:1035–41.
103. Tomiyama K, Makihara Y, Yamamoto H, et al. Disruption of orofacial movement topographies in congenic mutants with dopamine D5 but not D4 receptor or DARPP-32 transduction 'knockout'. Eur Neuropsychopharmacol 2006;16:437–45.
104. Elliot EE, Sibley DR, Katz JL. Locomotor and discriminative-stimulus effects of cocaine in dopamine D5 receptor knockout mice. Psychopharmacology 2003;169:161–8.
105. Homanics GE, Quinlan JJ, Firestone LL. Pharmacologic and behavioral responses of inbred C57BL/6 J and strain 129/SvJ mouse lines. Pharmacol Biochem Behav 1999;63:21–6.
106. Ciliax BJ, Nash N, Heilman C, et al. Dopamine D(5) receptor immunolocalization in rat and monkey brain. Synapse 2000;37:125–45.
107. Dziewczapolski G, Menalled LB, Garcia MC, Mora MA, Gershanik OS, Rubinstein M. Opposite roles of D1 and D5 dopamine receptors in locomotion revealed by selective antisense oligonucleotides. Neuroreport 1998;9:1–5.
108. Jung MY, Skryabin BV, Arai M, et al. Potentiation of the D2 mutant motor phenotype in mice lacking dopamine D2 and D3 receptors. Neuroscience 1999;91:911–24.
109. Kelly MA, Rubinstein M, Asa SL, et al. Pituitary lactotroph hyperplasia and chronic hyperprolactinemia in dopamine D2 receptor-deficient mice. Neuron 1997;19: 103–13.
110. Yamaguchi H, Aiba A, Nakamura K, et al. Dopamine D2 receptor plays a critical role in cell proliferation and proopiomelanocortin expression in the pituitary. Genes Cells 1996;1:253–68.
111. Kelly MA, Rubinstein M, Phillips TJ, et al. Locomotor activity in D2 dopamine receptor-deficient mice is determined by gene dosage, genetic background, and developmental adaptations. J Neurosci 1998;18:3470–9.
112. Boulay D, Depoortere R, Perrault G, Borrelli E, Sanger DJ. Dopamine D2 receptor knockout mice are insensitive to the hypolocomotor and hypothermic effects of dopamine D2/D3 receptor agonists. Neuropharmacology 1999;38:1389–96.
113. Clifford JJ, Kinsella A, Tighe O, et al. Comparative, topographically-based evaluation of behavioural phenotype and specification of D(1)-like:D(2) interactions in a line of incipient congenic mice with D(2) dopamine receptor 'knockout'. Neuropsychopharmacology 2001;25:527–36.
114. Fowler SC, Zarcone TJ, Vorontsova E, Chen R. Motor and associative deficits in D2 dopamine receptor knockout mice. Int J Dev Neurosci 2002;20:309–21.
115. Tomiyama K, McNamara FN, Clifford JJ, et al. Comparative phenotypic resolution of spontaneous, D2-like and D1-like agonist-induced orofacial movement topographies in congenic mutants with dopamine D2 vs. D3 receptor 'knockout'. Synapse 2004;51: 71–81.
116. Maldonado R, Saiardi A, Valverde O, Samad TA, Roques BP, Borrelli E. Absence of opiate rewarding effects in mice lacking dopamine D2 receptors. Nature 1997;388:586–9.

117. Elmer GI, Pieper JO, Levy J, et al. Brain stimulation and morphine reward deficits in dopamine D2 receptor-deficient mice. Psychopharmacology 2005;182:33–44.
118. Tran AH, Tamura R, Uwano T, et al. Altered accumbens neural response to prediction of reward associated with place in dopamine D2 receptor knockout mice. Proc Natl Acad Sci USA 2002;99:8986–91.
119. Kruzich PJ, Grandy DK. Dopamine D2 receptors mediate two-odor discrimination and reversal learning in C57BL/6 mice. BMC Neurosci 2004;5:12.
120. Kruzich PJ, Mitchell SH, Younkin A, Grandy DK. Dopamine D2 receptors mediate reversal learning in male C57BL/6 J mice. Cogn Affect Behav Neurosci 2006;6:86–90.
121. Glickstein SB, Hof PR, Schmauss C. Micelacking dopamine D2 and D3 receptors have spatial working memory deficits. J Neurosci 2002;22:5619–29.
122. Boulay D, Depoortere R, Oblin A, Sanger DJ, Schoemaker H, Perrault G. Haloperidol-induced catalepsy is absent in dopamine D(2), but maintained in dopamine D(3) receptor knock-out mice. Eur J Pharmacol 2000;391:63–73.
123. Chen JF, Moratalla R, Impagnatiello F, et al. The role of the D(2) dopamine receptor (D(2)R) in A(2A) adenosine receptor (A(2A)R)-mediated behavioral and cellular responses as revealed by A(2A) and D(2) receptor knockout mice. Proc Natl Acad Sci USA 2001;98:1970–5.
124. Zahniser NR, Simosky JK, Mayfield RD, et al. Functional uncoupling of adenosine A(2A) receptors and reduced response to caffeine in mice lacking dopamine D2 receptors. J Neurosci 2000;20:5949–57.
125. Hayward MD, Low MJ. Naloxone's suppression of spontaneous and food-conditioned locomotor activity is diminished in mice lacking either the dopamine D(2) receptor or enkephalin. Brain Res Mol Brain Res 2005;140:91–8.
126. Glickstein SB, Schmauss C. Effect of methamphetamine on cognition and repetitive motor behavior of mice deficient for dopamine D2 and D3 receptors. Ann NY Acad Sci 2004;1025:110–8.
127. Risbrough VB, Masten VL, Caldwell S, Paulus MP, Low MJ, Geyer MA. Differential contributions of dopamine D1, D2, and D3 receptors to MDMA-induced effects on locomotor behavior patterns in mice. Neuropsychopharmacology 2006;31:2349–58.
128. Palmer AA, Low MJ, Grandy DK, Phillips TJ. Effects of a Drd2 deletion mutation on ethanol-induced locomotor stimulation and sensitization suggest a role for epistasis. Behav Genet 2003;33:311–24.
129. Phillips TJ, Brown KJ, Burkhart-Kasch S, et al. Alcohol preference and sensitivity are markedly reduced in mice lacking dopamine D2 receptors. Nat Neurosci 1998;1:610–5.
130. Welter M, Vallone D, Samad TA, Meziane H, Usiello A, Borrelli E. Absence of dopamine D2 receptors unmasks an inhibitory control over the brain circuitries activated by cocaine. Proc Natl Acad Sci USA 2007;104:6840–5.
131. Chausmer AL, Elmer GI, Rubinstein M, Low MJ, Grandy DK, Katz JL. Cocaine-induced locomotor activity and cocaine discrimination in dopamine D2 receptor mutant mice. Psychopharmacology 2002;163:54–61.
132. Elmer GI, Pieper JO, Rubinstein M, Low MJ, Grandy DK, Wise RA. Failure of intravenous morphine to serve as an effective instrumental reinforcer in dopamine D2 receptor knock-out mice. J Neurosci 2002;22:RC224.
133. Dockstader CL, Rubinstein M, Grandy DK, Low MJ, van der Kooy D. The D2 receptor is critical in mediating opiate motivation only in opiate-dependent and withdrawn mice. Eur J Neurosci 2001;13:995–1001.
134. Caine SB, Negus SS, Mello NK, et al. Role of dopamine D2-like receptors in cocaine self-administration: studies with D2 receptor mutant mice and novel D2 receptor antagonists. J Neurosci 2002;22:2977–88.
135. Bozzi Y, Borrelli E. Dopamine D2 receptor signaling controls neuronal cell death induced by muscarinic and glutamatergic drugs. Mol Cell Neurosci 2002;19:263–71.

136. Bozzi Y, Vallone D, Borrelli E. Neuroprotective role of dopamine against hippocampal cell death. J Neurosci 2000;20:8643–9.
137. Fetsko LA, Xu R, Wang Y. Effects of age and dopamine D2L receptor-deficiency on motor and learning functions. Neurobiol Aging 2005;26:521–30.
138. Smith JW, Fetsko LA, Xu R, Wang Y.Dopamine D2L receptor knockout mice display deficits in positive and negative reinforcing properties of morphine and in avoidance learning. Neuroscience 2002;113:755–65.
139. Vargas-Perez H, Borrelli E, Diaz JL. Wheel running use in dopamine D2L receptor knockout mice. Neurosci Lett 2004;366:172–5.
140. Vukhac KL, Sankoorikal EB, Wang Y. Dopamine D2L receptor- and age-related reduction in offensive aggression. Neuroreport 2001;12:1035–8.
141. Fetsko LA, Xu R, Wang Y. Alterations in D1/D2 synergism may account for enhanced stereotypy and reduced climbing in mice lacking dopamine D2L receptor. Brain Res 2003;967:191–200.
142. Xu R, Hranilovic D, Fetsko LA, Bucan M, Wang Y. Dopamine D2S and D2L receptors may differentially contribute to the actions of antipsychotic and psychotic agents in mice. Mol Psychiatry 2002;7:1075–82.
143. Centonze D, Usiello A, Costa C, et al. Chronic haloperidol promotes corticostriatal long-term potentiation by targeting dopamine D2L receptors. J Neurosci 2004;24:8214–22.
144. Vallone D, Pignatelli M, Grammatikopoulos G, et al. Activity, non-selective attention and emotionality in dopamine D2/D3 receptor knock-out mice. Behav Brain Res 2002;130: 141–8.
145. Xu M, Koeltzow TE, Santiago GT, et al. Dopamine D3 receptor mutant mice exhibit increased behavioral sensitivity to concurrent stimulation of D1 and D2 receptors. Neuron 1997;19:837–48.
146. Joseph JD, Wang YM, Miles PR, et al. Dopamine autoreceptor regulation of release and uptake in mouse brain slices in the absence of D(3) receptors. Neuroscience 2002;112: 39–49.
147. Boulay D, Depoortere R, Rostene W, Perrault G, Sanger DJ. Dopamine D3 receptor agonists produce similar decreases in body temperature and locomotor activity in D3 knock-out and wild-type mice. Neuropharmacology 1999;38:555–65.
148. Betancur C, Lepee-Lorgeoux I, Cazillis M, Accili D, Fuchs S, Rostene W. Neurotensin gene expression and behavioral responses following administration of psychostimulants and antipsychotic drugs in dopamine D(3) receptor deficient mice. Neuropsychopharmacology 2001;24:170–82.
149. Narita M, Mizuo K, Mizoguchi H, Sakata M, Tseng LF, Suzuki T. Molecular evidence for the functional role of dopamine D3 receptor in the morphine-induced rewarding effect and hyperlocomotion. J Neurosci 2003;23:1006–12.
150. Siuciak JA, Fujiwara RA. The activity of pramipexole in the mouse forced swim test is mediated by D2 rather than D3 receptors. Psychopharmacology 2004;175:163–9.
151. McNamara FN, Clifford JJ, Tighe O, et al. Phenotypic, ethologically based resolution of spontaneous and D(2)-like vs D(1)-like agonist-induced behavioural topography in mice with congenic D(3) dopamine receptor 'knockout'. Synapse 2002;46:19–31.
152. Steiner H, Fuchs S, Accili D. D3 dopamine receptor-deficient mouse: evidence for reduced anxiety. Physiol Behav 1997;63:137–41.
153. Leggio GM, Micale V, Drago F. Increased sensitivity to antidepressants of D3 dopamine receptor-deficient mice in the forced swim test (FST). Eur Neuropsychopharmacol 2008;18:271–7.
154. Le Foll B, Diaz J, Sokoloff P. Neuroadaptations to hyperdopaminergia in dopamine D3 receptor-deficient mice. Life Sci 2005;76:1281–96.
155. Carta AR, Gerfen CR, Steiner H. Cocaine effects on gene regulation in the striatum and behavior: increased sensitivity in D3 dopamine receptor-deficient mice. Neuroreport 2000;11:2395–9.

156. Le Foll B, Frances H, Diaz J, Schwartz JC, Sokoloff P. Role of the dopamine D3 receptor in reactivity to cocaine-associated cues in mice. Eur J Neurosci 2002;15:2016–26.
157. McNamara RK, Logue A, Stanford K, Xu M, Zhang J, Richtand NM. Dose-response analysis of locomotor activity and stereotypy in dopamine D3 receptor mutant mice following acute amphetamine. Synapse 2006;60:399–405.
158. Pritchard LM, Logue AD, Hayes S, et al. 7-OH-DPAT and PD 128907 selectively activate the D3 dopamine receptor in a novel environment. Neuropsychopharmacology 2003;28:100–7.
159. Leriche L, Schwartz JC, Sokoloff P. The dopamine D3 receptor mediates locomotor hyperactivity induced by NMDA receptor blockade. Neuropharmacology 2003;45:174–81.
160. Pritchard LM, Newman AH, McNamara RK, et al. The dopamine D3 receptor antagonist NGB 2904 increases spontaneous and amphetamine-stimulated locomotion. Pharmacol Biochem Behav 2007;86:718–26.
161. Le Foll B, Sokoloff P, Stark H, Goldberg SR. Dopamine D3 receptor ligands block nicotine-induced conditioned place preferences through a mechanism that does not involve discriminative-stimulus or antidepressant-like effects. Neuropsychopharmacology 2005;30:720–30.
162. Koeltzow TE, Xu M, Cooper DC, et al. Alterations in dopamine release but not dopamine autoreceptor function in dopamine D3 receptor mutant mice. J Neurosci 1998;18:2231–8.
163. Schwartz JC, Diaz J, Pilon C, Sokoloff P. Possible implications of the dopamine D(3) receptor in schizophrenia and in antipsychotic drug actions. Brain Res Brain Res Rev 2000;31:277–87.
164. Diaz J, Pilon C, Le Foll B, et al. Dopamine D3 receptors expressed by all mesencephalic dopamine neurons. J Neurosci 2000;20:8677–84.
165. Munafo MR, Yalcin B, Willis-Owen SA, Flint J. Association of the dopamine D4 receptor (DRD4) gene and approach-related personality traits: meta-analysis and new data. Biol Psychiatry 2008;63:197–206.
166. Dulawa SC, Grandy DK, Low MJ, Paulus MP, Geyer MA. Dopamine D4 receptor-knock-out mice exhibit reduced exploration of novel stimuli. J Neurosci 1999;19:9550–6.
167. Falzone TL, Gelman DM, Young JI, Grandy DK, Low MJ, Rubinstein M. Absence of dopamine D4 receptors results in enhanced reactivity to unconditioned, but not conditioned, fear. Eur J Neurosci 2002;15:158–64.
168. Faraone SV, Doyle AE, Mick E, Biederman J. Meta-analysis of the association between the 7-repeat allele of the dopamine D(4) receptor gene and attention deficit hyperactivity disorder. Am J Psychiatry 2001;158:1052–7.
169. Grady DL, Chi HC, Ding YC, et al. High prevalence of rare dopamine receptor D4 alleles in children diagnosed with attention-deficit hyperactivity disorder. Mol Psychiatry 2003;8:536–45.
170. Avale ME, Falzone TL, Gelman DM, Low MJ, Grandy DK, Rubinstein M. The dopamine D4 receptor is essential for hyperactivity and impaired behavioral inhibition in a mouse model of attention deficit/hyperactivity disorder. Mol Psychiatry 2004;9:718–26.
171. Katz JL, Chausmer AL, Elmer GI, Rubinstein M, Low MJ, Grandy DK. Cocaine-induced locomotor activity and cocaine discrimination in dopamine D4 receptor mutant mice. Psychopharmacology 2003;170:108–14.
172. Kruzich PJ, Suchland KL, Grandy DK. Dopamine D4 receptor-deficient mice, congenic on the C57BL/6 J background, are hypersensitive to amphetamine. Synapse 2004;53:131–9.
173. Montkowski A, Poettig M, Mederer A, Holsboer F. Behavioural performance in three substrains of mouse strain 129. Brain Res 1997;762:12–8.
174. Fishburn CS, Carmon S, Fuchs S. Molecular cloning and characterisation of the gene encoding the murine D4 dopamine receptor. FEBS Lett 1995;361:215–9.
175. Gan L, Falzone TL, Zhang K, Rubinstein M, Baldessarini RJ, Tarazi FI. Enhanced expression of dopamine D(1) and glutamate NMDA receptors in dopamine D(4) receptor knockout mice. J Mol Neurosci 2004;22:167–78.

176. Kobayashi M, Iaccarino C, Saiardi A, et al. Simultaneous absence of dopamine D1 and D2 receptor-mediated signaling is lethal in mice. Proc Natl Acad Sci USA 2004;101:11465–70.
177. Ridray S, Griffon N, Mignon V, et al. Coexpression of dopamine D1 and D3 receptors in islands of Calleja and shell of nucleus accumbens of the rat: opposite and synergistic functional interactions. Eur J Neurosci 1998;10:1676–86.
178. Glickstein SB, Schmauss C. Focused motor stereotypies do not require enhanced activation of neurons in striosomes. J Comp Neurol 2004;469:227–38.

Chapter 14
Dopamine Modulation of the Prefrontal Cortex and Cognitive Function

Jeremy K. Seamans and Trevor W. Robbins

Abstract This chapter will review the basic neurobiology of the dopamine (DA) system in the prefrontal cortex (PFC) and its functional role in cognition. It will consider the properties of DA release and of DA receptors in the PFC and how they may contribute to the overall function of the mesocortical DA system. DA release in the PFC occurs in response to a variety of events that can be appetitive or aversive in nature and this release may prepare the PFC networks to deal with environmental or cognitive challenges. The amount of DA released may selectively affect the different subtypes of receptors on PFC neurons, which in turn have different modulatory actions on PFC networks. It has been proposed that the PFC DA system and especially D1 receptors are tuned according to an inverted-U dose–response function such that too much or too little DA or D1 receptor activation is detrimental to cognitive performance. At optimal levels, DA acting via D1 receptors may increase the signal-to-noise ratio to improve the efficiency of active PFC networks, while levels higher or lower levels may reduce the overall signal to noise but allow PFC networks to deal with information in a more flexible manner. The key to understanding the PFC DA system may lie in understanding how a balance is achieved to promote optimal modulation across a variety of situations.

Keywords Prefrontal cortex · Working memory · Response flexibility · Inverted-U · Computational models

14.1 Introduction

This chapter focuses on the properties of the dopamine (DA) system in the prefrontal cortex (PFC) and how these properties may contribute to higher cognitive function.

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Here we will discuss the basic anatomy, chemistry, physiology, and behavioral pharmacology of the PFC DA system. The chapter ends with an attempt to present an integrative overview of the possible function of the mesofrontal DA system.

14.2 Basic Anatomy of DA Release

The dopaminergic innervation of the forebrain is constituted by ~30,000–40,000 highly collateralized neurons residing in the ventral mesencephalon [1–3]. These mesencephalic cell groups are designated as A8, A9, and A10, according to the nomenclature of Dahlström and Fuxe [4], and generally correspond to the DA cells of the substantia nigra (SN, A9), ventral tegmental area (VTA, A10), and the retrorubral area (A8) [3, 5, 6].

DA neurons show two predominant patterns of firing activity termed tonic and phasic [7, 8]. Tonic activity consists of a pace-maker like firing pattern of ~1–6 Hz that DA neurons usually exhibit in the absence of salient stimuli [9–11]. Tonic firing patterns maintain basal extracellular levels of DA in afferent regions and can be affected by visceral stimuli that can moderately increase or decrease efferent DA levels to provide a "tone" on DA receptors [7].

Basal DA levels in PFC recorded using in vivo microdialysis are typically on the order of 0.1–0.4 nM [12, 13]. However, when interpreting these values it is important to note that (a) the values are often not corrected for in vitro recovery which is on the order of 10–13% [12], (b) the microdialysis probe itself may cause an ~10-fold drop in its vicinity as revealed by simultaneous voltammetric recordings [14], and (c) these measurements reflect extrasynaptic DA levels. Taking into account ~10% recovery and the 10-fold drop caused by the probe, the measured extrasynaptic concentrations of ~0.1–0.4 nM roughly correspond to extrasynaptic DA levels of 10–40 nM in the PFC. PFC DA levels were close to this range as measured using the more accurate no-net-flux procedure [15]. If 10–40 nM DA exists in the extrasynaptic space then based on the results of diffusion modeling, one would expect perisynaptic DA concentrations 10-fold higher on the order of 100–400 nM [16, 17] with intrasynaptic levels being an order of magnitude higher than that. Activation of the mesocortical DA system would then increase DA levels above this baseline level.

Salient environmental stimuli, particularly rewarding, aversive, and novel stimuli, all have been shown to modulate the phasic firing pattern of DA neurons [11, 18, 19]. Physiologically patterned burst stimulation of DA fibers causes DA levels in PFC to increase by ~40–200 nM while aversive stimuli increased PFC DA levels by 100–200 nM, as measured in the prelimbic region of the PFC using carbon-fiber electrodes [20–24]. Therefore, based on the known biochemical properties of the mesofrontal system, basal perisynaptic levels of DA in the PFC are on the order of hundreds of nanomolars with extrasynaptic concentrations in the range of tens of nanomolars while phasic physiological activation increases DA by 40–200 nM over these basal levels.

14.3 Behavioral Activation of the Mesocortical DA System

14.3.1 Aversive Events

The most studied aspect of PFC DA release is how it changes when the animal is presented with stimuli of negative emotional valence. Early studies showed that metabolic activation of the DA system in the PFC increased in association with electric footshock, conditioned fear, or swim stress [25–27]. Later studies using microdialysis reported that DA levels in the PFC were increased by a wide array of negative events such as tail pinch [28, 29], footshock or conditioned footshock [30, 32, 33], handling stress [13, 32, 34], pharmacological stressors [29, 35, 36], social defeat stress [37], and electrical stimulation of the midbrain tectum [38]. The changes in the DA signal associated with these stimuli were typically on the order of 100–200% above baseline. Again assuming a basal extrasynaptic level of ~10–40 nM DA, this would mean that the increase detected in these microdialysis studies was on the order of 20–120 nM. The tail pinch induced increases in extrasynaptic PFC DA levels measured in the prelimbic cortex with voltammetry were of similar magnitude and on the order of 100–200 nM [21, 23, 24].

One of the most striking aspects of the response of the PFC DA system to stress is its remarkably protracted nature. PFC DA levels can remain elevated for tens of minutes following even light handling as measured with microdialysis [13, 34]. Remarkably, aversive electrical stimulation of the inferior colliculus for 15 s when delivered at stimulation intensities that evoked escape responses in awake animals, increased PFC DA for >3 h [38]. Even with voltammetry, which is able to measure evoked changes in DA levels on millisecond time scales, similar long-lasting changes have been reported. For instance, tail-pinch stress in the form of a wooden clothespin placed at the base of the tail for 15 min caused an increase in DA measured with nafion-coated carbon-fiber electrodes in the PFC that lasted from 25 min to over an hour after removal of the clothespin [21, 23, 24]. Even if the tail pinch was given for only 2 min, it caused an increase in PFC DA that again lasted tens of minutes [39]. Therefore, the protracted nature of the PFC DA response to stress appears to be a genuine property of the system and does not mask some more transient phenomenon.

The protracted nature of release may provide insights into the function of the mesofrontal DA signal. One possible explanation for these prolonged increases in PFC DA is that they may reflect or even produce emotional arousal [34, 40]. In accordance with this idea, anxiolytic agents that reduce emotional arousal also reverse stress-induced increases in DA levels and DA turnover [27, 35, 36, 41]. Accordingly, the PFC DA response also varies across rat strains that have intrinsically different levels of arousal. For instance, Roman low-avoidance rats are characterized as more fearful than Roman high-avoidance rats that show less freezing behaviors, better coping, and lower HPA activity [42]. Giorgi et al. [29] showed that tail-pinch or pharmacological stressors increased PFC DA levels of Roman high-avoidance but not low-avoidance rats, suggesting that the rise in PFC DA levels

may be related to a more effective coping strategy rather than the stress response per se. Furthermore, long-term administration of antidepressants had no marked effect on the stress-induced physiological responses (increases in neuroactive steroids or corticosterone), yet prevented the stress-induced raise in PFC DA [43], again implying that the DA response may not be related to emotional arousal per se but rather the marshalling of resources to deal with a stressor [29]. Thus, one reason why the elevations in PFC DA are so long lasting may be because the processing of the significance of environmental stressors often far outlasts their presence.

14.3.2 Appetitive Events

Food rewards also increase PFC DA release but the increases are typically less than those observed for aversive stimuli [28, 34, 41, 44, 45]. Although appetitive stimuli, like aversive stimuli, can increase PFC DA for tens of minutes, more transient changes have also been reported. Richardson and Gratton [47] observed changes in the voltammetric signal just in a short 45 s consumption period following a lever press for milk reward. When the delivery of the milk was delayed by 20–30 s, the PFC DA signal increased by a few nanomolars until the point where the milk was delivered. The DA levels also increased if the reward was unexpectedly withheld or decreased or if the response requirement was increased by changing the fixed ratio schedule. This way, the transient DA signal appeared to be related to the animal's uncertainty about how much reward it would receive. This implies that the PFC DA system may be engaged as reward contingencies change in anticipation of cognitive effort. Hence the PFC DA system may become relevant in situations requiring cognitive effort perhaps in a parallel manner to the way the mesolimbic DA system is activated in situations requiring motivation and physical effort [48].

14.3.3 Cognitive Processing

The PFC DA system is also activated during various forms of learning. Robust and sustained DA release is associated with classical appetitive or aversive conditioning to a context [30, 32] or to an auditory cue [31, 33]. In primates, DA levels were elevated on the delayed-response working memory task, but not a nondelayed task [49]. Likewise, in rats, PFC DA levels increased during and in anticipation of the performance of a delayed alternation working memory task [50]. On the delayed win-shift working memory task on a radial maze, Phillips et al. [51] showed that DA levels increased by >100% during training and test phases of the task where the trial-unique information for a given trial was acquired and used but decreased to baseline levels during the delay period. DA levels often began to rise toward the end of the delay, again suggesting that the DA signal reflected anticipation of the forthcoming cognitive challenge. When the delay period was unexpectedly increased, DA levels did not increase and task performance worsened. These data suggest that

the PFC DA system may be recruited to solve the task at hand and an inability to appropriately raise DA levels could lead to poor task performance.

DA levels were also increased in another form of executive function involving the PFC, namely response flexibility. van der Meulen et al. [52] observed a large and extended increase in DA efflux in rats performing initial reversals on a spatial 2-lever discrimination task. Stefani and Moghaddam [53] measured PFC DA levels using microdialysis during a rodent set-shifting task on a plus maze. In addition to rats performing the actual task there was a separate yoked group of rats that received intermittent nonpredictable rewards and a "reward retrieval" group that was rewarded on every trial. Increases in PFC DA were observed for the first two groups but not the passively rewarded group. The increases in DA levels were on the order of 100–200% and again these increases greatly outlasted the period of the actual task. A correlation was also observed between magnitude of PFC DA and the rapidity of the shift to the new response rule. Notably however, the yoked rats exhibited the highest DA levels, suggesting that the DA system may have been recruited in a vain attempt to figure out the arbitrary response–reward mappings. Likewise, the cognitive load imposed by the set-shift task may also have raised the DA levels in that group, while the only group without a cognitive load, the passively rewarded group, showed no change in PFC DA levels. Hence although there is an extensive neural network responsible for how attentional resources are allocated during behavior, the PFC and in particular the PFC DA system may provide the attentional or cognitive resources needed to understand the meaning of stimuli and strategies to deal with them.

The PFC DA system also becomes activated on tasks specifically designed to assess attention, such as the five choice serial reaction time task (5CSRTT) which is a good rodent analogue of the continuous performance attentional task in humans [54]. In this task, rats must monitor five light ports simultaneously so that if a light comes on, a nose poke must be made to obtain reward. PFC DA and DOPAC levels are increased in the medial PFC over baseline levels during the performance of this task, as measured by in vivo microdialysis [55]. The tendency shown by some rats to respond prematurely on the attentional task – a form of impulsivity – was not correlated with individual levels of DA or the DA metabolite DOPAC, in contrast to what was found for 5-HT. However, *post mortem* analysis revealed that those rats that were the most impulsive had a higher DA turnover in the PFC. Accordingly, on a temporal discounting of reward task that specifically assesses impulsivity, DOPAC (and not 5-HT or 5-HIAA) levels increased by 200–250% during the delay period following choice [56]. These data suggest that DA turnover can also significantly increase in association specifically with attention or in response to impulsive forms of choice.

14.3.4 Release Conclusions

DA release in the PFC is modulated in association with a variety of events and behaviors. Aversive stimuli are particularly potent in this regard and can elevate DA

levels by ~200%, which based on the calculations above, would produce extrasynaptic DA levels of >100 nM with presumed perisynaptic levels well over 1 μM. DA release is also often extremely long lasting even when measured with techniques that have millisecond precision such as voltammetry. Finally a common conclusion across studies was that the DA signal might become activated with cognitive demands, when the animal needs to attend to a stimulus, figure out its meaning as well as the rules for attaining rewards and avoiding potentially harmful stimuli.

14.4 DA Receptors in PFC

Once released from presynaptic axonal terminals, DA interacts with at least five receptor subtypes: D_1, D_2, D_3, D_4, D_5 receptors. Based on G protein coupling and the length of the 3rd cytoplasmic loop and the carboxy tail of the receptors, the current classification of all the cloned DA receptors subdivides them into the G_s-, G_q,- or G_{olf}-coupled D1 family and $G_{i/o}$ D2 family [57–60]. For simplicity, we will refer to D_1/D_5 receptors as D1 receptors and $D_2/D_3/D_4$ receptors as D2 receptors throughout unless otherwise noted.

D2 receptors exist in high ($D2^{high}$) or low ($D2^{low}$) affinity states [61] and these two affinity states have been observed in a variety of brain regions and species including rat and human PFC [62, 63]. The affinities for DA are on the order of 5–50 nM vs. 1–5 μM for $D2^{high}$ versus $D2^{low}$ states, respectively [63–65]. The exact percentages of receptors in each state depends on a variety of factors, but for rat striatal slices, Richfield et al. [63] reported 77% vs. 23% of D2 receptors in their high and low states, respectively, while for tissue homogenates others found numbers of 20–45% vs. 50–80% for $D2^{high}$ and $D2^{low}$ respectively [62, 64–66]. Likewise, D1 receptors appear to have high and low affinity sites on the order of 1–4 nM and 1–2 μM, respectively, although the affinity of D_5 receptors is 10-fold higher [63, 67, 68]. Therefore, the affinities of these receptors based on available methods, is such that either receptor could be activated by the levels of DA released during the behaviors described above. The relative activation of each receptor may be determined mainly by the prevailing affinity state and their locations relative to sites of release.

In rodent and monkey PFC, the abundance of both D_1 receptor mRNA and D1 receptor binding sites is significantly greater than the other DA receptor subtypes [69–72]. Anatomically, in the primate PFC, DA-immunoreactive terminals converge on pyramidal cells and parvalbumin-containing, DA-receptive fast-spiking interneurons [73–75]. In rodent PFC, both D1- and D2-like receptors are also found on pyramidal and nonpyramidal neurons [76–79]. D1 receptors are located primarily on the dendritic spines and shafts of pyramidal neurons and on the dendrites and axon terminals of putative GABAergic interneurons [80, 81]. D_1 receptors are usually displaced to the side of the postsynaptic density of large asymmetric synapses that showed profiles not indicative of DA synapses. Likewise, Bergson et al. [80] reported that D_1 and D_5 receptors are usually found on asymmetric synapses and not

at DA synapses. D_1 receptor labeling was found more often in spines than D_5 receptor labeling which was found in shafts or at some distance from a specialization. There is also a high density of D_5 receptors in the perisomatic plasma membrane that may form a perisomatic "microdomain" [82]. Likewise, most D_2 immunogold labeling in dendrites and spines is associated with asymmetric synapses, but is also found outside the postsynaptic density at extrasynaptic and perisynaptic sites [83]. However, the D_2 receptor is likely closer to the release sites than the D1 receptor [84].

Based on these biochemical and anatomical considerations, it appears that a DA terminal in the PFC does not often oppose DA receptors and that DA likely acts on its receptors mainly through volume transmission. The affinities and locations of the DA receptors in PFC probably dictate which receptor is activated under which conditions. We have argued [85–87] that D2 receptors, perhaps in their low affinity state, may be closer to release sites and would be activated by the μM levels of DA thought to be present in the perisynaptic region. In contrast, D1 receptors, which are located exclusively outside of the perisynaptic region (and perhaps other DA receptors in their high affinity state) would be activated by the hundreds of nM levels of DA present in the extrasynaptic space.

In support of this view, DA applied at concentrations of 10–500 nM increased synaptic responses in PFC neurons via D1 receptors while DA at concentrations >1 μM decreased the same synaptic currents via D2 receptors [88]. As a result, the amount of DA released and the time following a release event may differentially activate D1 versus D2 receptors potentially having different functional outcomes. Indeed, D1 receptors appear to contribute to different cognitive processes than D2 receptors in the PFC.

14.5 Contribution of PFC DA Receptors to Stress

As reviewed above the PFC DA system responds potently to stress. Stress (footshock or tailshock) has been shown to increase the density of D_2 dopamine receptors in the PFC and to change their relative affinity state [89]. Changes in D_4 receptors as a result of stress in turn have been shown to impair cognitive function [90]. The pre- and postsynaptic changes in the PFC DA system are relevant to the stress response itself. On an elevated plus maze, PFC DA depletion using 6 hydroxydopamine (6OHDA) caused a lower preference to stay on open arms, lower open arm entries and hypomobility [91]. D_4 knockout mice also exhibited reduced exploration of the open arms of the plus maze and longer latencies to explore the illuminated compartment of the light/dark shuttle box, again indicating an increase in anxiety [92].

One interpretation of these data may be that PFC DA is producing or modulating the physiological response to stress, thereby producing anxiety. However, a variety of DA drugs acting on D1 and D2 classes of DA receptors (SKF82958, SKF83566, raclopride, or quinpirole) did not affect the tail pinch evoked increase

in heart rate [93]. Rather, as argued above, the PFC DA system appears to help to evaluate the meaning of a stressor and how to deal with it. As noted above, this process would presumably occur in the period following the experience, which may explain why manipulations of the PFC DA system have a strong effect on the consolidation of fear memories. For example, 6OHDA lesions of the PFC do not affect the acquisition of conditioned fear but rather delay its extinction [94, 95]. Likewise, a D_4 receptor antagonist microinjected into PFC before a fear extinction paradigm reduced the memory of the extinction when tested a day later [96]. Finally, a D1 receptor antagonist when delivered immediately after training on an inhibitory avoidance-learning task impaired recall 24 h later [97]. These data therefore are consistent with the idea that the PFC DA receptors do not generate an anxiety response per se, but rather are involved in the processes allowing an organism to learn about and adapt to stress-related changes in the environment.

14.6 Contribution of PFC DA Receptors to Cognition

14.6.1 DA Modulation of Working Memory

Perhaps the most notable and investigated effects of PFC DA receptor manipulation are with regards to their role in working memory. Both D1 agonists or antagonists and DA depletions in the PFC are highly detrimental to working memory performance [98–104]. These studies suggested that the DA receptor activity is finely tuned in the PFC and perhaps may exhibit an inverted-U-shaped dose–response function [105, 106]. This idea arose from both pharmacological studies in the 1990s where it was reported that systemic administration of higher doses of D1 agonists impaired performance of the delayed-response working memory task, while very low doses could improve performance [107, 108]. The theory of an inverted-U-shaped dose–response function for D1 receptor (or more broadly DA) regulation of working memory has since been supported in a variety of studies.

The DA releaser amphetamine at a low dose (<1 mg/kg) produced a delay-dependent improvement in a discrete paired trial variable delay task or a delayed alternation task on a T-maze, while higher doses (>2 mg/kg) impaired performance [109, 110]. Rather than using amphetamine to artificially increase PFC DA levels, Romanides et al. [111] injected a μ-opioid agonist into the VTA to selectively activate the mesofrontal pathway and showed that this manipulation also produced impairments in a delayed alternation task in a T-maze that were blocked by a D1 antagonist injected into the PFC. Conversely, Mizoguchi et al. [112] chronically stressed (4 weeks) and then recovered (10 days) rats as a means to depress PFC DA transmission and found that this impaired the spatial working memory evaluated by a T-maze task. They also reported a concomitant increase in DA D1 receptor density in the PFC, which may have contributed to the working memory deficits.

Analogous data have been found on a delayed win-shift working memory task. Floresco and Phillips [113] showed that infusions of a D1 receptor agonist into the

PFC improved performance after a long delay but disrupted performance after a shorter delay. This result is notable because these authors also showed that DA levels measured using microdialysis on the same task were increased at short delays and decreased at long delays [51]. The interpretation of these data fit well into the inverted-U framework. Specifically, rats with either an intrinsically or an imposed decrease in performance may have a suboptimal activation of D1 receptors and therefore benefit more from D1 receptor activation. Rats performing optimally at baseline may have optimal D1 receptor activation and suffer from further exogenous D1 receptor activation.

Another situation where differential effects of DA receptor activation occur is with respect to individual levels of performance. Granon et al. [114] divided rats into two groups, with low and high baseline levels of accuracy on the five choice serial reaction time task and found that a partial D1 agonist (SKF38393) enhanced performance only in the lower baseline group. By contrast, only higher performing rats were susceptible to the detrimental effects of an intra-PFC D1 receptor antagonist (but not sulpiride, a D2 receptor antagonist). A plausible interpretation of these data is that the mesofrontal DA system is primarily engaged to optimize signal detection in this task. A follow-up study by Chudasama and Robbins [115] used a combined attention and working memory (CAM) task in which rats had to commit to working memory over a short delay the location of visual events they had successfully detected. A full DA D1 receptor agonist, SKF81297, produced dose-dependent effects on the attentional component, thereby replicating and expanding the findings of Granon et al. [114]. However, effects on the working memory component of the task were much less clear; some improvements were evident when the stimulus duration was reduced at certain doses and delays. At the highest dose, good memory at short delays was impaired whilst poor memory at longer delays was improved. Thus, D1 receptor stimulation sufficient to improve attentional accuracy could facilitate or disrupt working memory performance in a dose- and delay-dependent manner but the effect appears to depend on the individual's baseline level of performance and perhaps basal levels of DA.

Support for the inverted-U dose–function relationship has also been obtained from studies in humans with genetically determined differences in intrinsic DA levels. A functional polymorphism for COMT involves a methionine (Met) to valine (Val) substitution at codon 158 [116, 117]. The Met allele has ¼ the enzyme activity as the Val allele [116] and therefore Met individuals should have higher DA levels specifically in PFC. The inverted-U theory would predict that Val individuals who have intrinsically lower DA levels would be off the optimal point of the inverted-U curve and therefore do poorly on tests of working memory and executive function, but would benefit from manipulations that increase PFC DA levels. In contrast Met carriers should possess near optimal levels of DA receptor stimulation at baseline, and further elevations in DA levels would be detrimental [118]. Accordingly, human COMT Val/Val genotype individuals often exhibit poorer performance on tests of working memory and executive function that involve the PFC compared to Met/Met individuals [119]. These patterns can be changed by manipulating DA levels pharmacologically: Val/Val subjects showed either improved performance on tests of

executive function when given a COMT inhibitor (Tolcapone) or amphetamine, whereas the Met/Met individuals got less efficient, consistent with moving them off the optimal point of the inverted-U curve (3-back task in [120, 121]). Therefore, although the inverted-U theory, as initially proposed, was specific for D1 receptor activation, evidence across species now provides compelling support for the idea that inverted- U dose–function relationship may hold for PFC DA signaling in general.

14.6.2 How Is DA Improving Working Memory?

Early electrophysiological investigations showed that putative D1 receptor activation produced a (relative to baseline) stronger amplification of delay- and response-related single unit activity during the delayed-response task [100]. Yet D1 agonists were also shown to diminish non-target-related activity to a much larger degree than target-related activity [105, 122]. In this way, at the appropriate activation range D1 receptors might be said to increase the gain or increase the signal and decrease the noise, as suggested by early experimental and theoretical work [123–125].

In terms of the human PFC, an explanation of what signal to noise means and how it might be modulated by DA has been provided by Winterer and Weinberger [126–132]. Their definition of "noise" is the variability in the phase relationship of evoked electroencephalic activity (event-related potentials, ERP) with respect to a stimulus. It represents increased stimulus-related variability both across time and across the PFC [126]. Noise measures, according to the definition of Winterer et al. [126–131], are significantly affected by COMT genotype. Winterer and colleagues showed that on attentional tasks frontal noise in the EEG was lower in Met/Met individuals [133] while Val/Val individuals had greater frontal noise [129]. Prefrontal noise in these individuals was also negatively correlated with performance on the N-back working memory task [134]. Therefore, these EEG data indicate that, relative to homozygous Met individuals, homozygous Val carriers with presumed lower PFC DA levels show more "noise" based on various measures. Along with the differences in noise there is also a difference in the "signal" between groups as homozygous Met/Met individuals had a peak blood-oxygen-level-dependent (BOLD) response that was stronger than that found in Val/Val carriers, indicating that Met/Met genotype may also confer an increase in signal processing [131].

How might a modulation of signal to noise occur at a cellular level? DA exerts its impact on working memory and neural activity through a multitude of effects on presynaptic release, NMDA and $GABA_A$ currents, the persistent Na^+ current, various Ca^{2+} currents, the slowly inactivating K^+ current, and the H current [135–143]. To understand the functional implications of this intricate pattern of DA-induced cellular and synaptic changes for neural processing and PFC-dependent cognition, biophysically realistic computational models have proven useful [144–148]. Such models consist of sets of differential equations for each neuron that describe the evolution of the somatic and dendritic membrane potentials according to various

voltage-gated, Ca^{2+}-gated, and synaptically gated ionic currents. The appeal of these models is their close relation to biophysical quantities measured electrophysiologically, which allows the effects of DA measured in vitro to be implemented rather directly with few additional assumptions. These systems can reproduce the low and high activity states classically associated with spontaneous (baseline) activity and stimulus-specific delay activity in working memory tasks, respectively [149, 150]. Starting from a configuration that mimics certain functional characteristics of a working memory network, the models explored how the D1- or D2-receptor-mediated changes found in vitro could affect widespread cortical network dynamics. The results revealed that the combined effects of D1-induced conductance modulations led to a change in network dynamics that made it more difficult to switch between various high activity (active memory) states, i.e., to an increase in the "energy barrier" between different discrete states of network activity [144–148, 151]. These effects are partly rooted in the differential contribution of various D1-modulated currents to different activity regimes: The D1-induced increase in NMDA and modulation of other voltage-dependent currents, by boosting recurrent excitation within cell assemblies, increases the strength of the currently active memory state. The concomitant increase in $GABA_A$ currents leads to fiercer competition among different active ensembles of neurons, thereby limiting the number of items maintained by recurrent excitation. At the same time, this D1 mediated enhancement of $GABA_A$ currents and the reduction in glutamate release probability make it harder to evoke activity in cell assemblies in the first place [145]. At the in vivo electrophysiological level, these dynamic changes would predict an increased signal-to-noise ratio in the sense of an increased differentiation of firing rates associated with currently active memory states from those associated with nonactivated states or spontaneous activity. A related complementary idea, namely an increased signal-to-noise ratio via a DA-induced change in gain of the single neuron input/output function, had been proposed in more abstract terms within connectionist-like models more than 15 years ago by Servan-Schreiber et al. [125], and has received experimental support recently [151]. These processes provide a mechanistic basis for the putative increase in signal to noise thought to occur at the peak or the optimal region of the inverted-U curve.

14.7 DA Modulation of Working Memory or Working Attention?

Whilst there is considerable evidence to support a role for PFC DA in working memory, it is clear that the original concept of working memory from animal studies based on the spatial delayed response, delayed matching, or delayed saccade tasks, may require some modification to accommodate results that indicate a role for DA in attentional processing per se in the PFC. Thus, for example, depletion of PFC DA produced impairments in the development of an attentional set, as tested by a series of repeated intradimensional shifts in discrimination learning by marmoset monkeys [152], yet it both increased distractibility and improved the performance of

extra-dimensional shifting. This is consistent with the hypothesis that DA modulates the stability of rules for representation of responses. These data are also consistent with evidence that distraction during working memory tasks is particularly important for detecting deficits after prefrontal DA depletion [153] and with the effects of systemic or intra-PFC agents on attention and working memory in rats ([114, 115, 154, 155], see below). Therefore, as noted above, DA levels in PFC become elevated upon cognitive load and in this way may focus attentional resources to the task at hand, including focusing attention on critical aspects of working memory tasks, putatively by increasing signal-to-noise ratios.

14.8 DA Modulation of Response Flexibility

14.8.1 D2 Receptors and Response Flexibility

If the main purpose of DA is to increase signal to noise in PFC, then why have the optimal signal-to-noise ratio occur only within limited range at the midpoint of the inverted-U curve? One reason may be that the "off peak" regions of the inverted-U may create conditions that are optimal for other forms of cognition. As noted above, 6OHDA lesions can improve the performance of extra-dimensional shifting [152]. Furthermore, Met carriers are less flexible and Val carriers more flexible during reversals on a competing programs task [156]. Val carriers were also more flexible in processing emotional stimuli [157]. Behavioral and neurochemical lesioning studies have suggested a partly antagonistic nature of working memory and cognitive flexibility requirements [153, 158] and therefore response flexibility may be optimal at other portions of the curve if one assumes the inverted-U theory is based on DA concentrations and not just D1 receptor activation. More specifically, the high and low ends of the curve may be more optimal for D2 receptor activation, and D2 receptor activation may be more optimal for flexible modes of responding [85, 87]. There is some support for the idea that D2 receptors in PFC may be particularly involved in flexible modes of responding. In monkeys the D2 receptor antagonist raclopride impaired the performance of a reversal task of response flexibility [159]. In the rat, the role of PFC D2 receptors in set shifting was demonstrated clearly by Floresco et al. [155] using a 4 arm + maze task. Here the rats were trained to respond using an egocentric strategy of always going to the arm that was in the same spatial location relative to their frame of reference (i.e., always go right or left). The rule then changed, and in order to perform optimally rats now had to visit arms according to a visual cue, regardless of its egocentric location (or vice versa). Floresco et al. [155] found that a D2 antagonist but not a D1 antagonist disrupted performance and caused perseverative errors on the task. In humans, a D2 antagonist has been shown to deteriorate performance on tests of attentional set shifting and response flexibility [160]. Moreover, in a group of healthy volunteers, Mehta et al. [161] showed that the D2 antagonist sulpiride not only impaired task set-switching but actually protected against the deleterious effects of intervening task-irrelevant distractors on a spatial

working memory task, as would be expected with a blockade of D2 receptors and a shift of PFC networks into the peak of the curve optimal for D1 receptor activation. Conversely, activation of D2 receptors with the D2 agonist bromocriptine improved performance on the quintessential task of response flexibility, the Wisconsin Card-Sorting Task, in patients with traumatic brain injuries [162]. Collectively, these data provide support for the idea that PFC D1 receptor activation is optimal for working memory, while D2 receptor activation may be optimal for cognitive flexibility.

Other data provide apparent difficulties for this simple idea. For example, sulpiride a D2 receptor antagonist impaired, whilst a D2 agonist, bromocriptine, improved delayed spatial working memory performance in human studies [161, 163–165]. Furthermore, the D2 agonist bromocriptine impaired the ability to reverse a learned probabilistic discrimination, which can be considered a form of behavioral flexibility [161, 164]. These apparently contradictory findings highlight the complexity of the DA system and how multiple factors come into play in any study of DA function.

One critical factor that must be taken into account is the baseline performance of the subjects as discussed above. For instance, the improvement in aspects of spatial span memory by the D2 agonist bromocriptine [164] was notable for being baseline-dependent; those subjects with worse spans on placebo showed the largest improvements. This finding is consistent with what had been found earlier by Kimberg et al. [166] who showed that subjects with lower reading spans improved on bromocriptine on various tests of executive functioning. This also parallels the findings of Frank and O'Reilly [167] that another D2 receptor agonist, cabergoline, improved the flexible updating (i.e., switching) of working memory in healthy volunteers with low reading spans.

A similar baseline dependency has also recently been found for the effects of bromocriptine on attentional switching and working memory [168]. In this case, the effect depended on the baseline level of impulsivity, as measured by the Barratt Impulsivity Scale. Notably, the high-impulsive subjects also exhibited lower reading spans than low-impulsive individuals. These authors used a cued delayed matching to sample task in which healthy volunteers were required to retrieve either briefly-presented faces or scenes, depending on the color of the fixation cross. Distractors were also presented in the retention interval. The required category was switched in such a way as to induce switching or maintenance of the encoding "set," switching being slower and more error prone. In this study, bromocriptine reduced behavioral switch costs as expected for a drug that activates D2 receptors and facilitates behavioral flexibility. Yet this occurred only in high-impulsive subjects, while in low-impulsive subjects, if anything, performance was impaired.

These data highlight an important point; that many cognitive functions other than working memory depend on baseline DA levels, perhaps in regions other than the PFC. Accordingly, at least in some subject populations, the level of impulsivity is correlated with genetic determinants of intrinsic DA levels [169, 170] especially in the striatum [170, 171]. Likewise, the drug-induced improvement by bromocriptine in the work of Cools et al. [168] was also accompanied by a drug-induced modulation of activity in the putamen, whereas lateral frontal activity was unaltered during

switching. Thus, impulsivity and flexibility may be determined by the intrinsic levels of DA in brain regions other than the PFC and may be optimal at other portions of the putative inverted-U curve than for working memory.

Another recent study [172] has highlighted yet another important factor in determining the effects of DA acting at receptors in PFC and striatum: genetic polymorphisms that predict effects on reward and avoidance learning. A polymorphism in the DARPP-32 gene, associated with striatal dopamine function (putatively allied to D1 receptor function), predicted relatively better probabilistic reward learning. Conversely, the C957T polymorphism of the DRD2 gene, associated with D_2 receptor function, predicted the degree to which participants learned to avoid choices that had been probabilistically associated with negative outcomes. The Val/Met polymorphism of the COMT gene, associated with prefrontal cortical DA function, predicted participants' ability to rapidly adapt behavior on a trial-to-trial basis – in other words utilizing working memory. Finally, a computational model was used to account for these effects based on three independent genetic effects on three parameters of a reinforcement learning-based model.

These important studies highlight a number of key points; first that D2 receptors exert effects on cognition in humans at striatal as well as prefrontal cortical sites. Notably, in human psychopharmacological studies drugs are necessarily administered systemically and may therefore affect receptors in noncortical sites, especially the striatum, which may function in a reciprocal manner to the cortex in terms of its regulation by DA. Second, in human subjects, brain imaging methodology (PET or fMRI) will probably be necessary for unraveling such effects. Thirdly, knowledge of individual differences (possibly at the level of genetic polymorphisms) will also be vital for predicting effects of DA-ergic agents. This may well prove to be a key consideration when investigating effects of DA-ergic agents on cognition for another reason; with DA D2 agents there is the possibility of low-dose autoreceptor activation, which produces opposite effects on striatal DA-ergic activity to that normally produced by agonist activity. These effects particularly afflict human studies where low doses must be employed to avoid debilitating nausea or extrapyramidal side-effects. Virtually all of the studies reviewed above are susceptible to this issue. Indeed, one of them [167] actually assumed that their effects of cabergoline and haloperidol indeed result from autoreceptor actions. One way of resolving this issue will be to perform experiments using PET to measure displacement of the PET DA ligand by DA. Mehta et al. [173] used PET with the DA D2 receptor ligand raclopride to study the effects of sulpiride on spatial working memory. They replicated the earlier finding with this task of impaired accuracy but also showed that this impairment was significantly but *inversely* related to raclopride receptor occupancy – leading them to speculate that the detrimental effects of sulpiride were actually related to presynaptic effects and that extrastriatal D2 receptors may also play a role. Clearly, the future availability of methods for visualizing cortical D2 receptors which now are very difficult to detect, will enable a more satisfactory resolution of these issues.

Collectively, these data suggest that D2 receptors both within and outside of the PFC have a preferential role in the regulation of behavioral flexibility. The effects

may be determined by the genetically regulated intrinsic levels of DA system activity. These levels in turn determine where a particular individual sits on the putative inverted-U curve for DA. Furthermore there may be multiple inverted-U-type curves for DA in various brain regions that influence not only response flexibility but also other processes such as impulsivity.

14.8.2 How Is DA Modulating Response Flexibility?

In addition to working memory, computational models based on the known biophysical effects of DA have also provided insights into the question of how DA might regulate response flexibility. As noted above, the striatal DA system contributes to a number of "PFC-mediated" behaviors and the theoretical models of Frank and O'Reilly provide an account of how D2 receptors in the striatum might influence flexible modes of responding [167, 172, 174]. In their models, information within the PFC is discarded and updated based on the preferential activation of the striatal "Go" and "no-Go" systems. Within the striatum, unlike the PFC, neurons tend to contain either D1 or D2 receptors, and the idea is that activation of neurons with D2 receptors form the "no-GO" system and promote avoidance learning. Avoidance learning is of course a key aspect of flexible responding since a behavior must first be inhibited when it is no longer rewarded. The striatal D1 containing "Go" pathway neurons then are suggested to update network ensembles in PFC that maintain information in working memory. There is currently a growing literature supporting the predictions of this model of striatal DA function.

At the level of the PFC, D2 receptors may achieve a similar function but through different biophysical means. D2 agonists tend to act oppositely from D1 receptors on NMDA and $GABA_A$ currents, as well as on pyramidal cell excitability in PFC [85, 88, 136, 141]. The collective effect of simulating the D2-mediated reduction in NMDA and GABA currents was a reduction in the "barrier" separating activity states in the model networks, i.e., the valleys of the energy landscape become so flat and so near that noise may easily push the system from one representation into the other. This resulted in spontaneous pop-out of activity states caused by noisy fluctuations, highly unstable representations, and fast and spontaneous transitions between many different activity states [87, 141, 145, 148]. The D2-state, due to the decreased energy barrier among activity states, would allow easier access to PFC networks and faster switching among different PFC activity patterns such that the network may quickly cycle through multiple representations that could become active nearly simultaneously [87, 141, 145, 148]. Therefore, the D1-mediated changes in PFC neurons and networks may explain why their activation is optimal for working memory and attention, while the D2-mediated changes may explain why these receptors are more involved in other types of cognition, such as response flexibility and impulsivity.

While the PFC DA system is activated during the various cognitive processes discussed above, recall that the most striking activation occurs in response to various

stressors. Also recall that it appeared to be mainly D2-like or more specifically D_4 receptors in the PFC that are the critical modulators of fear or stress-related behaviors. It is of note that the low affinity state of the D_4 receptor is comparable to that of the D_2 receptor discussed above, while the high affinity state of the D_4 receptor is an order of magnitude lower than the high affinity state of the D_2 receptor [67, 68]. Therefore, the large stress-induced increases in PFC DA may be particularly well-suited to activate PFC D2-like and especially D_4 receptors. Remarkably the electrophysiological effects induced by D_4 receptors are similar to those produced by D_2 receptors, including a reduction in NMDA and $GABA_A$ currents [175, 176]. As described above, modulation of these currents was the critical determinant of the D2-mediated effects in the computational models and therefore, D_4 receptor stimulation should produce a similar dynamic. This implies that the strong release of DA in response to stress may activate D_4 receptors and establish a "D2-like" dynamic in PFC, which is associated with increased flexibility characteristic of effective problem solving under stress.

14.9 Summary and Conclusions

Anatomical, biochemical, neuropharmacological, and electrophysiological data on the PFC DA system were reviewed. The data suggested that the PFC DA system was activated under a variety of circumstances, usually for periods that outlasted the initiating event. Across studies, a consistent theme that emerged was that the PFC DA system becomes activated in response to a cognitive challenge when the organism has to understand the meaning of a particularly salient stimuli and how to deal with it. The levels of DA evoked by stressful stimuli were either measured or extrapolated to be on the order of 200–500 nM in the extrasynaptic space, with perisynaptic levels in the low μM range. These levels of DA released would be sufficient to activate D1-like and/or D2-like receptors, depending on their affinity state and anatomical location. However, in terms of functional measures, such as the modulation of NMDA and GABA currents, D1-like receptors tended to modulate currents in the range of hundreds of nM while D_2 and possibly D_4 receptors tended to modulate the same currents at much higher levels. D_2 receptors in their high affinity state may occupy the far left side of the curve, responding to very low levels of DA and paradoxically producing a similar dynamic to that observed at high DA levels. Once activated, D1-like and D2-like receptors initiated a variety of changes that modulated both intrinsic and synaptic currents on PFC neurons, including importantly NMDA and GABA. Computational models showed that the D1-like receptor-mediated changes tended to increase the signal to noise of cortical representations and protect these representations from distraction. In contrast, D_2/D_4 receptor-mediated changes tended to be in the opposite direction and collectively aided in avoidance learning and subsequently decreased the robustness of cortical representations by putting the PFC in a mode that could handle information in a more flexible manner.

One way in which these data could be integrated would be to suggest that DA is released in the PFC under conditions of cognitive load when cognitive resources must be dedicated to understand a stressful or rewarding stimulus and how to deal with it most effectively. D_2/D_4 receptors closer to the sites of release may be first activated by the higher levels of prevailing DA locally and initiate a flexible mode of processing in an attempt to find solutions. As the DA diffuses away, the exclusively extrasynaptically located D1-like receptors may become active and help to protect this processing from distractors that could interfere with the cognitive search. This implies that PFC networks are processing information perhaps simultaneously in different modes so as to most effectively deal with the events at hand. Whether this interpretation of the data or another interpretation is correct, it would appear that the PFC DA system does exist in some sort of a balance and it is clear that different receptors have different functions under different conditions. Therefore, one of the greatest challenges to the field will be to understand how PFC DA maintains a balance so as to become activated in a manner that is optimal for dealing with the task at hand.

References

1. Fallon JH, Loughlin SE. Monoamine innervation of the forebrain: collateralization. Brain Res Bull 1982;9(1–6):295–307.
2. Lindvall O, Bjorklund A, Skagerberg G. Selective histochemical demonstration of dopamine terminal systems in rat di- and telencephalon: new evidence for dopaminergic innervation of hypothalamic neurosecretory nuclei. Brain Res 1984;306(1–2):19–30.
3. Williams SM, Goldman-Rakic PS. Widespread origin of the primate mesofrontal dopamine system. Cereb Cortex 1998;8(4):321–45.
4. Dahlstrom A, Fuxe K. Localization of monoamines in the lower brain stem. Experientia 1964;20(7):398–9.
5. Berger B, Gaspar P, Verney C. Dopaminergic innervation of the cerebral cortex: unexpected differences between rodents and primates. Trends Neurosci 1991;14(1):21–7.
6. Porrino LJ, Goldman-Rakic PS. Brainstem innervation of prefrontal and anterior cingulate cortex in the rhesus monkey revealed by retrograde transport of HRP. J Comp Neurol 1982;205(1):63–76.
7. Grace AA. Phasic versus tonic dopamine release and the modulation of dopamine system responsivity: a hypothesis for the etiology of schizophrenia. Neuroscience 1991;41(1):1–24.
8. Grace AA. The tonic/phasic model of dopamine system regulation and its implications for understanding alcohol and psychostimulant craving. Addiction 2000;95(Suppl 2):S119–28.
9. Grace AA, Bunney BS. The control of firing pattern in nigral dopamine neurons: burst firing. J Neurosci 1984;4(11):2877–90.
10. Grace AA, Bunney BS. The control of firing pattern in nigral dopamine neurons: single spike firing. J Neurosci 1984;4(11):2866–76.
11. Schultz W. Dopamine neurons and their role in reward mechanisms. Curr Opin Neurobiol 1997;7(2):191–7.
12. Del Arco A, Mora F. Endogenous dopamine potentiates the effects of glutamate on extracellular GABA in the prefrontal cortex of the freely moving rat. Brain Res Bull 2000;53(3):339–45.
13. Del Arco A, Segovia G, Garrido P, et al. Prefrontal cortex and enviromental enrichment: studies on dopamine and acetylcholine release and working memory performance in rats. Behav Brain Res 2007;176(2):267–73.

14. Borland LM, Shi G, Yang H, Michael AC. Voltammetric study of extracellular dopamine near microdialysis probes acutely implanted in the striatum of the anesthetized rat. J Neurosci Methods 2005;146(2):149–58.
15. Chen NH, Lai YJ, Pan WH. Effects of different perfusion medium on the extracellular basal concentration of dopamine in striatum and medial prefrontal cortex: a zero-net flux microdialysis study. Neurosci Lett 1997;225(3):197–200.
16. Cragg SJ, Nicholson C, Kume-Kick J, Tao L, Rice ME. Dopamine-mediated volume transmission in midbrain is regulated by distinct extracellular geometry and uptake. J Neurophysiol 2001;85(4):1761–71.
17. Cragg SJ, Rice ME. DAncing past the DAT at a DA synapse. Trends Neurosci 2004;27(5):270–7.
18. Redgrave P, Prescott TJ, Gurney K. Is the short-latency dopamine response too short to signal reward error? Trends Neurosci 1999;22(4):146–51.
19. Ungless MA, Magill PJ, Bolam JP. Uniform inhibition of dopamine neurons in the ventral tegmental area by aversive stimuli. Science 2004;303(5666):2040–2.
20. Garris PA, Collins LB, Jones SR, Wightman RM. Evoked extracellular dopamine in vivo in the medial prefrontal cortex. J Neurochem 1993;61(2):637–47.
21. Doherty MD, Gratton A. Medial prefrontal cortical D1 receptor modulation of the meso-accumbens dopamine response to stress: an electrochemical study in freely-behaving rats. Brain Res 1996;715(1–2):86–97.
22. Lavin A, Nogueira L, Lapish CC, Wightman RM, Phillips PE, Seamans JK. Mesocortical dopamine neurons operate in distinct temporal domains using multimodal signaling. J Neurosci 2005;25(20):5013–23.
23. Zhang TY, Chretien P, Meaney MJ, Gratton A. Influence of naturally occurring variations in maternal care on prepulse inhibition of acoustic startle and the medial prefrontal cortical dopamine response to stress in adult rats. J Neurosci 2005;25(6):1493–502.
24. Brake WG, Sullivan RM, Gratton A. Perinatal distress leads to lateralized medial prefrontal cortical dopamine hypofunction in adult rats. J Neurosci 2000;20(14):5538–43.
25. Thierry AM, Tassin JP, Blanc G, Glowinski J. Selective activation of mesocortical DA system by stress. Nature 1976;263(5574):242–4.
26. Fadda F, Argiolas A, Melis MR, Tissari AH, Onali PL, Gessa GL. Stress-induced increase in 3,4-dihydroxyphenylacetic acid (DOPAC) levels in the cerebral cortex and in n. accumbens: reversal by diazepam. Life Sci 1978;23(22):2219–24.
27. Claustre Y, Rivy JP, Dennis T, Scatton B. Pharmacological studies on stress-induced increase in frontal cortical dopamine metabolism in the rat. J Pharmacol Exp Ther 1986;238(2): 693–700.
28. Taber MT, Fibiger HC. Activation of the mesocortical dopamine system by feeding: lack of a selective response to stress. Neuroscience 1997;77(2):295–8.
29. Giorgi O, Lecca D, Piras G, Driscoll P, Corda MG. Dissociation between mesocortical dopamine release and fear-related behaviours in two psychogenetically selected lines of rats that differ in coping strategies to aversive conditions. Eur J Neurosci 2003;17(12):2716–26.
30. Feenstra MG, Teske G, Botterblom MH, De Bruin JP. Dopamine and noradrenaline release in the prefrontal cortex of rats during classical aversive and appetitive conditioning to a contextual stimulus: interference by novelty effects. Neurosci Lett 1999;272(3):179–82.
31. Feenstra MG, Vogel M, Botterblom MH, Joosten RN, de Bruin JP. Dopamine and noradrenaline efflux in the rat prefrontal cortex after classical aversive conditioning to an auditory cue. Eur J Neurosci 2001;13(5):1051–4.
32. Yoshioka M, Matsumoto M, Togashi H, Saito H. Effect of conditioned fear stress on dopamine release in the rat prefrontal cortex. Neurosci Lett 1996;209(3):201–3.
33. Wilkinson LS, Humby T, Killcross AS, Torres EM, Everitt BJ, Robbins TW. Dissociations in dopamine release in medial prefrontal cortex and ventral striatum during the acquisition and extinction of classical aversive conditioning in the rat. Eur J Neurosci 1998;10(3):1019–26.

34. Feenstra MG, Botterblom MH. Rapid sampling of extracellular dopamine in the rat prefrontal cortex during food consumption, handling and exposure to novelty. Brain Res 1996;742(1–2):17–24.
35. Murphy BL, Arnsten AF, Goldman-Rakic PS, Roth RH. Increased dopamine turnover in the prefrontal cortex impairs spatial working memory performance in rats and monkeys. Proc Natl Acad Sci USA 1996;93(3):1325–9.
36. Murphy BL, Arnsten AF, Jentsch JD, Roth RH. Dopamine and spatial working memory in rats and monkeys: pharmacological reversal of stress-induced impairment. J Neurosci 1996;16(23):7768–75.
37. Tidey JW, Miczek KA. Social defeat stress selectively alters mesocorticolimbic dopamine release: an in vivo microdialysis study. Brain Res 1996;721(1–2):140–9.
38. Cuadra G, Zurita A, Macedo CE, Molina VA, Brandao ML. Electrical stimulation of the midbrain tectum enhances dopamine release in the frontal cortex. Brain Res Bull 2000;52(5):413–8.
39. Sullivan RM, Gratton A. Relationships between stress-induced increases in medial prefrontal cortical dopamine and plasma corticosterone levels in rats: role of cerebral laterality. Neuroscience 1998;83(1):81–91.
40. D'Angio M, Serrano A, Driscoll P, Scatton B. Stressful environmental stimuli increase extracellular DOPAC levels in the prefrontal cortex of hypoemotional (Roman high-avoidance) but not hyperemotional (Roman low-avoidance) rats. An in vivo voltammetric study. Brain Res 1988;451(1–2):237–47.
41. Beaufour CC, Le Bihan C, Hamon M, Thiebot M. Extracellular dopamine in the rat prefrontal cortex during reward-, punishment- and novelty-associated behaviour. Effects of diazepam. Pharmacol Biochem Behav 2001;69(1–2):133–42.
42. Driscoll P, Escorihuela RM, Fernandez-Teruel A, et al. Genetic selection and differential stress responses. The Roman lines/strains of rats. Ann N Y Acad Sci 1998;851:501–10.
43. Dazzi L, Serra M, Spiga F, Pisu MG, Jentsch JD, Biggio G. Prevention of the stress-induced increase in frontal cortical dopamine efflux of freely moving rats by long-term treatment with antidepressant drugs. Eur Neuropsychopharmacol 2001;11(5):343–9.
44. D'Angio M, Scatton B. Feeding or exposure to food odors increases extracellular DOPAC levels (as measured by in vivo voltammetry) in the prefrontal cortex of food-deprived rats. Neurosci Lett 1989;96(2):223–8.
45. Bassareo V, De Luca MA, Di Chiara G. Differential expression of motivational stimulus properties by dopamine in nucleus accumbens shell versus core and prefrontal cortex. J Neurosci 2002;22(11):4709–19.
46. Bassareo V, Di Chiara G. Differential influence of associative and nonassociative learning mechanisms on the responsiveness of prefrontal and accumbal dopamine transmission to food stimuli in rats fed ad libitum. J Neurosci 1997;17(2):851–61.
47. Richardson NR, Gratton A. Changes in medial prefrontal cortical dopamine levels associated with response-contingent food reward: an electrochemical study in rat. J Neurosci 1998;18(21):9130–8.
48. Salamone JD, Correa M, Farrar A, Mingote SM. Effort-related functions of nucleus accumbens dopamine and associated forebrain circuits. Psychopharmacology (Berl) 2007;191(3):461–82.
49. Watanabe M, Kodama T, Hikosaka K. Increase of extracellular dopamine in primate prefrontal cortex during a working memory task. J Neurophysiol 1997;78(5):2795–8.
50. Rossetti ZL, Carboni S. Noradrenaline and dopamine elevations in the rat prefrontal cortex in spatial working memory. J Neurosci 2005;25(9):2322–9.
51. Phillips AG, Ahn S, Floresco SB. Magnitude of dopamine release in medial prefrontal cortex predicts accuracy of memory on a delayed response task. J Neurosci 2004;24(2):547–53.
52. van der Meulen JA, Joosten RN, de Bruin JP, Feenstra MG. Dopamine and noradrenaline efflux in the medial prefrontal cortex during serial reversals and extinction of instrumental goal-directed behavior. Cereb Cortex 2007;17(6):1444–53.

53. Stefani MR, Moghaddam B. Rule learning and reward contingency are associated with dissociable patterns of dopamine activation in the rat prefrontal cortex, nucleus accumbens, and dorsal striatum. J Neurosci 2006;26(34):8810–8.
54. Chudasama Y, Robbins TW. Dopaminergic modulation of visual attention and working memory in the rodent prefrontal cortex. Neuropsychopharmacology 2004;29(9):1628–36.
55. Dalley JW, Theobald DE, Eagle DM, Passetti F, Robbins TW. Deficits in impulse control associated with tonically-elevated serotonergic function in rat prefrontal cortex. Neuropsychopharmacology 2002;26(6):716–28.
56. Winstanley CA, Theobald DE, Dalley JW, Cardinal RN, Robbins TW. Double dissociation between serotonergic and dopaminergic modulation of medial prefrontal and orbitofrontal cortex during a test of impulsive choice. Cereb Cortex 2006;16(1):106–14.
57. Lachowicz JE, Sibley DR. Molecular characteristics of mammalian dopamine receptors. Pharmacol Toxicol 1997;81(3):105–13.
58. Missale C, Nash SR, Robinson SW, Jaber M, Caron MG. Dopamine receptors: from structure to function. Physiol Rev 1998;78(1):189–225.
59. Zhuang X, Belluscio L, Hen R. G(olf)alpha mediates dopamine D1 receptor signaling. J Neurosci 2000;20(16):RC91.
60. Jin LQ, Wang HY, Friedman E. Stimulated D(1) dopamine receptors couple to multiple Galpha proteins in different brain regions. J Neurochem 2001;78(5):981–90.
61. Sibley DR, De Lean A, Creese I. Anterior pituitary dopamine receptors. Demonstration of interconvertible high and low affinity states of the D-2 dopamine receptor. J Biol Chem 1982;257(11):6351–61.
62. Mita T, Kuno T, Nakai H, Tanaka C. Evidence for the presence of D2 and 5-HT2 receptors in the human prefrontal cortex. Jpn J Pharmacol 1982;32(6):1027–32.
63. Richfield EK, Penney JB, Young AB. Anatomical and affinity state comparisons between dopamine D1 and D2 receptors in the rat central nervous system. Neuroscience 1989;30(3):767–77.
64. Kazmi SM, Ramwani J, Srivastava LK, et al. Characterization of high-affinity dopamine D2 receptors and modulation of affinity states by guanine nucleotides in cholate-solubilized bovine striatal preparations. J Neurochem 1986;47(5):1493–502.
65. Seeman P, Weinshenker D, Quirion R, et al. Dopamine supersensitivity correlates with D2High states, implying many paths to psychosis. Proc Natl Acad Sci USA 2005;102(9):3513–8.
66. Seeman P, Schwarz J, Chen JF, et al. Psychosis pathways converge via D2high dopamine receptors. Synapse 2006;60(4):319–46.
67. Gingrich JA, Caron MG. Recent advances in the molecular biology of dopamine receptors. Annu Rev Neurosci 1993;16:299–321.
68. Seeman P, Van Tol HH. Dopamine receptor pharmacology. Trends Pharmacol Sci 1994; 15(7):264–70.
69. Lidow MS, Goldman-Rakic PS, Gallager DW, Rakic P. Distribution of dopaminergic receptors in the primate cerebral cortex: quantitative autoradiographic analysis using [3H]raclopride, [3H]spiperone and [3H]SCH23390. Neuroscience 1991;40(3):657–71.
70. Goldman-Rakic PS, Lidow MS, Smiley JF, Williams MS. The anatomy of dopamine in monkey and human prefrontal cortex. J Neural Transm Suppl 1992;36:163–77.
71. Farde L, Halldin C, Stone-Elander S, Sedvall G. PET analysis of human dopamine receptor subtypes using 11C-SCH 23390 and 11C-raclopride. Psychopharmacology (Berl) 1987;92(3):278–84.
72. Gaspar P, Bloch B, Le Moine C. D1 and D2 receptor gene expression in the rat frontal cortex: cellular localization in different classes of efferent neurons. Eur J Neurosci 1995;7(5): 1050–63.
73. Goldman-Rakic PS, Leranth C, Williams SM, Mons N, Geffard M. Dopamine synaptic complex with pyramidal neurons in primate cerebral cortex. Proc Natl Acad Sci USA 1989;86(22):9015–9.

74. Verney C, Alvarez C, Geffard M, Berger B. Ultrastructural double-labelling study of dopamine terminals and GABA-containing neurons in rat anteromedial cerebral cortex. Eur J Neurosci 1990;2(11):960–72.
75. Sesack SR, Snyder CL, Lewis DA. Axon terminals immunolabeled for dopamine or tyrosine hydroxylase synapse on GABA-immunoreactive dendrites in rat and monkey cortex. J Comp Neurol 1995;363(2):264–80.
76. Sesack SR, Hawrylak VA, Melchitzky DS, Lewis DA. Dopamine innervation of a subclass of local circuit neurons in monkey prefrontal cortex: ultrastructural analysis of tyrosine hydroxylase and parvalbumin immunoreactive structures. Cereb Cortex 1998;8(7):614–22.
77. Gorelova N, Seamans JK, Yang CR. Mechanisms of dopamine activation of fast-spiking interneurons that exert inhibition in rat prefrontal cortex. J Neurophysiol 2002;88(6): 3150–66.
78. Vincent SL, Khan Y, Benes FM. Cellular colocalization of dopamine D1 and D2 receptors in rat medial prefrontal cortex. Synapse 1995;19(2):112–20.
79. Benes FM, Berretta S. GABAergic interneurons: implications for understanding schizophrenia and bipolar disorder. Neuropsychopharmacology 2001;25(1):1–27.
80. Bergson C, Mrzljak L, Smiley JF, Pappy M, Levenson R, Goldman-Rakic PS. Regional, cellular, and subcellular variations in the distribution of D1 and D5 dopamine receptors in primate brain. J Neurosci 1995;15(12):7821–36.
81. Muly EC, 3rd, Szigeti K, Goldman-Rakic PS. D1 receptor in interneurons of macaque prefrontal cortex: distribution and subcellular localization. J Neurosci 1998;18(24): 10553–65.
82. Paspalas CD, Goldman-Rakic PS. Microdomains for dopamine volume neurotransmission in primate prefrontal cortex. J Neurosci 2004;24(23):5292–300.
83. Negyessy L, Goldman-Rakic PS. Subcellular localization of the dopamine D2 receptor and coexistence with the calcium-binding protein neuronal calcium sensor-1 in the primate prefrontal cortex. J Comp Neurol 2005;488(4):464–75.
84. Sesack SR, Aoki C, Pickel VM. Ultrastructural localization of D2 receptor-like immunoreactivity in midbrain dopamine neurons and their striatal targets. J Neurosci 1994;14(1): 88–106.
85. Seamans JK, Yang CR. The principal features and mechanisms of dopamine modulation in the prefrontal cortex. Prog Neurobiol 2004;74(1):1–58.
86. Lapish CC, Kroener S, Durstewitz D, Lavin A, Seamans JK. The ability of the mesocortical dopamine system to operate in distinct temporal modes. Psychopharmacology (Berl) 2007;191(3):609–25.
87. Durstewitz D, Seamans JK. The dual-state theory of prefrontal cortex dopamine function with relevance to catechol-o-methyltransferase genotypes and schizophrenia. Biol Psychiatry 2008;64(9):739–49.
88. Trantham-Davidson H, Neely LC, Lavin A, Seamans JK. Mechanisms underlying differential D1 versus D2 dopamine receptor regulation of inhibition in prefrontal cortex. J Neurosci 2004;24(47):10652–9.
89. MacLennan AJ, Pelleymounter MA, Atmadja S, Jakubovic A, Maier SF, Fibiger HC. D2 dopamine receptors in the rat prefrontal cortex: characterization and alteration by stress. Brain Res 1989;477(1–2):300–7.
90. Arnsten AF, Murphy B, Merchant K. The selective dopamine D4 receptor antagonist, PNU-101387G, prevents stress-induced cognitive deficits in monkeys. Neuropsychopharmacology 2000;23(4):405–10.
91. Espejo EF. Selective dopamine depletion within the medial prefrontal cortex induces anxiogenic-like effects in rats placed on the elevated plus maze. Brain Res 1997;762 (1–2):281–4.
92. Falzone TL, Gelman DM, Young JI, Grandy DK, Low MJ, Rubinstein M. Absence of dopamine D4 receptors results in enhanced reactivity to unconditioned, but not conditioned, fear. Eur J Neurosci 2002;15(1):158–64.

93. Funk D, Stewart J. Role of catecholamines in the frontal cortex in the modulation of basal and stress-induced autonomic output in rats. Brain Res 1996;741(1–2):220–9.
94. Morrow BA, Elsworth JD, Rasmusson AM, Roth RH. The role of mesoprefrontal dopamine neurons in the acquisition and expression of conditioned fear in the rat. Neuroscience 1999;92(2):553–64.
95. Espejo EF. Prefrontocortical dopamine loss in rats delays long-term extinction of contextual conditioned fear, and reduces social interaction without affecting short-term social interaction memory. Neuropsychopharmacology 2003;28(3):490–8.
96. Pfeiffer UJ, Fendt M. Prefrontal dopamine D4 receptors are involved in encoding fear extinction. Neuroreport 2006;17(8):847–50.
97. Mello EST, Vianna MR, Rodrigues C, Quevedo J, Moleta BA, Izquierdo I. Involvement of the medial precentral prefrontal cortex in memory consolidation for inhibitory avoidance learning in rats. Pharmacol Biochem Behav 2000;66(3):615–22.
98. Brozoski TJ, Brown RM, Rosvold HE, Goldman PS. Cognitive deficit caused by regional depletion of dopamine in prefrontal cortex of rhesus monkey. Science 1979;205(4409): 929–32.
99. Bubser M, Schmidt WJ. 6-Hydroxydopamine lesion of the rat prefrontal cortex increases locomotor activity, impairs acquisition of delayed alternation tasks, but does not affect uninterrupted tasks in the radial maze. Behav Brain Res 1990;37(2):157–68.
100. Sawaguchi T. The effects of dopamine and its antagonists on directional delay-period activity of prefrontal neurons in monkeys during an oculomotor delayed-response task. Neurosci Res 2001;41(2):115–28
101. Sawaguchi T, Goldman-Rakic PS. The role of D1-dopamine receptor in working memory: local injections of dopamine antagonists into the prefrontal cortex of rhesus monkeys performing an oculomotor delayed-response task. J Neurophysiol 1994;71(2):515–28.
102. Seamans JK, Floresco SB, Phillips AG. D1 receptor modulation of hippocampal-prefrontal cortical circuits integrating spatial memory with executive functions in the rat. J Neurosci 1998;18(4):1613–21.
103. Zahrt J, Taylor JR, Mathew RG, Arnsten AF. Supranormal stimulation of D1 dopamine receptors in the rodent prefrontal cortex impairs spatial working memory performance. J Neurosci 1997;17(21):8528–35.
104. Aujla H, Beninger RJ. Hippocampal-prefrontocortical circuits: PKA inhibition in the prefrontal cortex impairs delayed nonmatching in the radial maze in rats. Behav Neurosci 2001;115(6):1204–11.
105. Williams GV, Goldman-Rakic PS. Modulation of memory fields by dopamine D1 receptors in prefrontal cortex. Nature 1995;376(6541):572–5.
106. Arnsten AF. Catecholamine regulation of the prefrontal cortex. J Psychopharmacol 1997;11(2):151–62.
107. Arnsten AF, Cai JX, Murphy BL, Goldman-Rakic PS. Dopamine D1 receptor mechanisms in the cognitive performance of young adult and aged monkeys. Psychopharmacology (Berl) 1994;116(2):143–51.
108. Cai JX, Arnsten AF. Dose-dependent effects of the dopamine D1 receptor agonists A77636 or SKF81297 on spatial working memory in aged monkeys. J Pharmacol Exp Ther 1997;283(1):183–9.
109. Aultman JM, Moghaddam B. Distinct contributions of glutamate and dopamine receptors to temporal aspects of rodent working memory using a clinically relevant task. Psychopharmacology (Berl) 2001;153(3):353–64.
110. Shoblock JR, Maisonneuve IM, Glick SD. Differences between d-methamphetamine and d-amphetamine in rats: working memory, tolerance, and extinction. Psychopharmacology (Berl) 2003;170(2):150–6.
111. Romanides AJ, Duffy P, Kalivas PW. Glutamatergic and dopaminergic afferents to the prefrontal cortex regulate spatial working memory in rats. Neuroscience 1999;92(1): 97–106.

112. Mizoguchi K, Yuzurihara M, Ishige A, Sasaki H, Chui DH, Tabira T. Chronic stress induces impairment of spatial working memory because of prefrontal dopaminergic dysfunction. J Neurosci 2000;20(4):1568–74.
113. Floresco SB, Phillips AG. Delay-dependent modulation of memory retrieval by infusion of a dopamine D1 agonist into the rat medial prefrontal cortex. Behav Neurosci 2001;115(4):934–9.
114. Granon S, Passetti F, Thomas KL, Dalley JW, Everitt BJ, Robbins TW. Enhanced and impaired attentional performance after infusion of D1 dopaminergic receptor agents into rat prefrontal cortex. J Neurosci 2000;20(3):1208–15.
115. Chudasama Y, Robbins TW. Psychopharmacological approaches to modulating attention in the five-choice serial reaction time task: implications for schizophrenia. Psychopharmacology (Berl) 2004;174(1):86–98.
116. Lachman HM, Papolos DF, Saito T, Yu YM, Szumlanski CL, Weinshilboum RM. Human catechol-O-methyltransferase pharmacogenetics: description of a functional polymorphism and its potential application to neuropsychiatric disorders. Pharmacogenetics 1996;6(3):243–50.
117. Weinberger DR, Egan MF, Bertolino A, et al. Prefrontal neurons and the genetics of schizophrenia. Biol Psychiatry 2001;50(11):825–44.
118. Meyer-Lindenberg A, Weinberger DR. Intermediate phenotypes and genetic mechanisms of psychiatric disorders. Nat Rev Neurosci 2006;7(10):818–27.
119. Egan MF, Goldberg TE, Kolachana BS, et al. Effect of COMT Val108/158 Met genotype on frontal lobe function and risk for schizophrenia. Proc Natl Acad Sci USA 2001;98(12): 6917–22.
120. Mattay VS, Goldberg TE, Fera F, et al. Catechol O-methyltransferase val158-met genotype and individual variation in the brain response to amphetamine. Proc Natl Acad Sci USA 2003;100(10):6186–91.
121. Apud JA, Mattay V, Chen J, et al. Tolcapone improves cognition and cortical information processing in normal human subjects. Neuropsychopharmacology 2007;32(5):1011–20.
122. Vijayraghavan S, Wang M, Birnbaum SG, Williams GV, Arnsten AF. Inverted-U dopamine D1 receptor actions on prefrontal neurons engaged in working memory. Nat Neurosci 2007;10(3):376–84.
123. Rolls ET, Thorpe SJ, Boytim M, Szabo I, Perrett DI. Responses of striatal neurons in the behaving monkey. 3. Effects of iontophoretically applied dopamine on normal responsiveness. Neuroscience 1984;12(4):1201–12.
124. DeFrance JF, Sikes RW, Chronister RB. Dopamine action in the nucleus accumbens. J Neurophysiol 1985;54(6):1568–77.
125. Servan-Schreiber D, Printz H, Cohen JD. A network model of catecholamine effects: gain, signal-to-noise ratio, and behavior. Science 1990;249(4971):892–5.
126. Winterer G, Ziller M, Dorn H, et al. Cortical activation, signal-to-noise ratio and stochastic resonance during information processing in man. Clin Neurophysiol 1999;110(7): 1193–203.
127. Winterer G, Ziller M, Dorn H, et al. Schizophrenia: reduced signal-to-noise ratio and impaired phase-locking during information processing. Clin Neurophysiol 2000; 111(5):837–49.
128. Winterer G. Cortical microcircuits in schizophrenia – the dopamine hypothesis revisited. Pharmacopsychiatry 2006;39(Suppl 1):S68–71.
129. Winterer G, Egan MF, Kolachana BS, Goldberg TE, Coppola R, Weinberger DR. Prefrontal electrophysiologic “noise” and catechol-O-methyltransferase genotype in schizophrenia. Biol Psychiatry 2006;60(6):578–84.
130. Winterer G, Musso F, Beckmann C, et al. Instability of prefrontal signal processing in schizophrenia. Am J Psychiatry 2006;163(11):1960–8.
131. Winterer G, Musso F, Vucurevic G, et al. COMT genotype predicts BOLD signal and noise characteristics in prefrontal circuits. Neuroimage 2006;32(4):1722–32.

132. Winterer G, Weinberger DR. Genes, dopamine and cortical signal-to-noise ratio in schizophrenia. Trends Neurosci 2004;27(11):683–90.
133. Gallinat J, Bajbouj M, Sander T, et al. Association of the G1947A COMT (Val(108/158)Met) gene polymorphism with prefrontal P300 during information processing. Biol Psychiatry 2003;54(1):40–8.
134. Winterer G, Coppola R, Goldberg TE, et al. Prefrontal broadband noise, working memory, and genetic risk for schizophrenia. Am J Psychiatry 2004;161(3):490–500.
135. Yang CR, Seamans JK. Dopamine D1 receptor actions in layers V-VI rat prefrontal cortex neurons in vitro: modulation of dendritic-somatic signal integration. J Neurosci 1996;16(5):1922–35.
136. Zheng P, Zhang XX, Bunney BS, Shi WX. Opposite modulation of cortical N-methyl-D-aspartate receptor-mediated responses by low and high concentrations of dopamine. Neuroscience 1999;91(2):527–35.
137. Nicola SM, Surmeier J, Malenka RC. Dopaminergic modulation of neuronal excitability in the striatum and nucleus accumbens. Annu Rev Neurosci 2000;23:185–215.
138. Gao WJ, Goldman-Rakic PS. Selective modulation of excitatory and inhibitory microcircuits by dopamine. Proc Natl Acad Sci USA 2003;100(5):2836–41.
139. Gao WJ, Krimer LS, Goldman-Rakic PS. Presynaptic regulation of recurrent excitation by D1 receptors in prefrontal circuits. Proc Natl Acad Sci USA 2001;98(1):295–300.
140. Seamans JK, Durstewitz D, Christie BR, Stevens CF, Sejnowski TJ. Dopamine D1/D5 receptor modulation of excitatory synaptic inputs to layer V prefrontal cortex neurons. Proc Natl Acad Sci USA 2001;98(1):301–6.
141. Seamans JK, Gorelova N, Durstewitz D, Yang CR. Bidirectional dopamine modulation of GABAergic inhibition in prefrontal cortical pyramidal neurons. J Neurosci 2001;21(10):3628–38.
142. Neve KA, Seamans JK, Trantham-Davidson H. Dopamine receptor signaling. J Recept Signal Transduct Res 2004;24(3):165–205.
143. Rosenkranz JA, Johnston D. Dopaminergic regulation of neuronal excitability through modulation of Ih in layer V entorhinal cortex. J Neurosci 2006;26(12):3229–44.
144. Durstewitz D, Kelc M, Gunturkun O. A neurocomputational theory of the dopaminergic modulation of working memory functions. J Neurosci 1999;19(7):2807–22.
145. Durstewitz D, Seamans JK, Sejnowski TJ. Dopamine-mediated stabilization of delay-period activity in a network model of prefrontal cortex. J Neurophysiol 2000;83(3):1733–50.
146. Compte A, Brunel N, Goldman-Rakic PS, Wang XJ. Synaptic mechanisms and network dynamics underlying spatial working memory in a cortical network model. Cereb Cortex 2000;10(9):910–23.
147. Brunel N, Wang XJ. Effects of neuromodulation in a cortical network model of object working memory dominated by recurrent inhibition. J Comput Neurosci 2001;11(1):63–85.
148. Durstewitz D, Seamans JK. The computational role of dopamine D1 receptors in working memory. Neural Netw 2002;15(4–6):561–72.
149. Fuster JM. Unit activity in prefrontal cortex during delayed-response performance: neuronal correlates of transient memory. J Neurophysiol 1973;36(1):61–78.
150. Funahashi S, Bruce CJ, Goldman-Rakic PS. Mnemonic coding of visual space in the monkey's dorsolateral prefrontal cortex. J Neurophysiol 1989;61(2):331–49.
151. Thurley K, Senn W, Luscher HR. Dopamine increases the gain of the input-output response of rat prefrontal pyramidal neurons. J Neurophysiol 2008;99(6):2985–97
152. Crofts HS, Dalley JW, Collins P, et al. Differential effects of 6-OHDA lesions of the frontal cortex and caudate nucleus on the ability to acquire an attentional set. Cereb Cortex 2001;11(11):1015–26.
153. Roberts AC, De Salvia MA, Wilkinson LS, et al. 6-Hydroxydopamine lesions of the prefrontal cortex in monkeys enhance performance on an analog of the Wisconsin Card Sort Test: possible interactions with subcortical dopamine. J Neurosci 1994;14(5 Pt 1): 2531–44.

154. Chudasama Y, Robbins TW. Psychopharmacological approaches to modulating attention in the five-choice serial reaction time task: implications for schizophrenia. Psychopharmacology (Berl) 2004;174:86–98.
155. Floresco SB, Magyar O, Ghods-Sharifi S, Vexelman C, Tse MT. Multiple dopamine receptor subtypes in the medial prefrontal cortex of the rat regulate set-shifting. Neuropsycho\-pharmacology 2006;31(2):297–309.
156. Nolan KA, Bilder RM, Lachman HM, Volavka J. Catechol O-methyltransferase Val158Met polymorphism in schizophrenia: differential effects of Val and Met alleles on cognitive stability and flexibility. Am J Psychiatry 2004;161(2):359–61.
157. Drabant EM, Hariri AR, Meyer-Lindenberg A, et al. Catechol O-methyltransferase val158met genotype and neural mechanisms related to affective arousal and regulation. Arch Gen Psychiatry 2006;63(12):1396–406.
158. Dreisbach G, Muller J, Goschke T, et al. Dopamine and cognitive control: the influence of spontaneous eyeblink rate and dopamine gene polymorphisms on perseveration and distractibility. Behav Neurosci 2005;119(2):483–90.
159. Lee B, Groman S, London ED, Jentsch JD. Dopamine D2/D3 receptors play a specific role in the reversal of a learned visual discrimination in monkeys. Neuropsychopharmacology 2007;32(10):2125–34.
160. Tost H, Meyer-Lindenberg A, Klein S, et al. D2 antidopaminergic modulation of frontal lobe function in healthy human subjects. Biol Psychiatry 2006;60(11):1196–205.
161. Mehta MA, Manes FF, Magnolfi G, Sahakian BJ, Robbins TW. Impaired set-shifting and dissociable effects on tests of spatial working memory following the dopamine D2 receptor antagonist sulpiride in human volunteers. Psychopharmacology (Berl) 2004;176(3–4): 331–42.
162. McDowell S, Whyte J, D'Esposito M. Differential effect of a dopaminergic agonist on prefrontal function in traumatic brain injury patients. Brain 1998;121(Pt 6):1155–64.
163. Mehta MA, Sahakian BJ, McKenna PJ, Robbins TW. Systemic sulpiride in young adult volunteers simulates the profile of cognitive deficits in Parkinson's disease. Psychopharmacology (Berl) 1999;146(2):162–74.
164. Mehta MA, Swainson R, Ogilvie AD, Sahakian J, Robbins TW. Improved short-term spatial memory but impaired reversal learning following the dopamine D(2) agonist bromocriptine in human volunteers. Psychopharmacology (Berl) 2001;159(1):10–20.
165. Luciana M, Collins PF. Dopaminergic modulation of working memory for spatial but not object cues in normal volunteers. J Cognit Neurosci 1997;9:330–347.
166. Kimberg DY, D'Esposito M, Farah MJ. Effects of bromocriptine on human subjects depend on working memory capacity. Neuroreport 1997;8(16):3581–5.
167. Frank MJ, O'Reilly RC. A mechanistic account of striatal dopamine function in human cognition: psychopharmacological studies with cabergoline and haloperidol. Behav Neurosci 2006;120(3):497–517.
168. Cools R, Sheridan M, Jacobs E, D'Esposito M. Impulsive personality predicts dopamine-dependent changes in frontostriatal activity during component processes of working memory. J Neurosci 2007;27(20):5506–14.
169. Reuter M, Peters K, Schroeter K, et al. The influence of the dopaminergic system on cognitive functioning: a molecular genetic approach. Behav Brain Res 2005;164(1):93–9.
170. Forbes EE, Brown SM, Kimak M, Ferrell RE, Manuck SB, Hariri AR. Genetic variation in components of dopamine neurotransmission impacts ventral striatal reactivity associated with impulsivity. Mol Psychiatry 2009;14(1):60–70.
171. Dalley JW, Fryer TD, Brichard L, et al. Nucleus accumbens D2/3 receptors predict trait impulsivity and cocaine reinforcement. Science 2007;315(5816):1267–70.
172. Frank MJ, Moustafa AA, Haughey HM, Curran T, Hutchison KE. Genetic triple dissociation reveals multiple roles for dopamine in reinforcement learning. Proc Natl Acad Sci USA 2007;104(41):16311–6.

173. Mehta MA, Montgomery AJ, Kitamura Y, Grasby PM. Dopamine D2 receptor occupancy levels of acute sulpiride challenges that produce working memory and learning impairments in healthy volunteers. Psychopharmacology (Berl) 2008;196(1):157–65.
174. Frank MJ, Santamaria A, O'Reilly RC, Willcutt E. Testing computational models of dopamine and noradrenaline dysfunction in attention deficit/hyperactivity disorder. Neuropsychopharmacology 2007;32(7):1583–99.
175. Wang X, Zhong P, Gu Z, Yan Z. Regulation of NMDA receptors by dopamine D4 signaling in prefrontal cortex. J Neurosci 2003;23(30):9852–61.
176. Wang X, Zhong P, Yan Z. Dopamine D4 receptors modulate GABAergic signaling in pyramidal neurons of prefrontal cortex. J Neurosci 2002;22(21):9185–93.

Chapter 15
In Vivo Imaging of Dopamine Receptors

Anissa Abi-Dargham and Marc Laruelle

Abstract Positron emission tomography (PET) and single photon emission computed tomography (SPECT) have been used to study indices of dopamine transmission in schizophrenia, mood disorders, anxiety, dimensions of personality, attention deficit and hyperactivity disorder (ADHD), and addiction. This is due not only to suspected dopamine alterations in these disorders, but also to the availability of a wide array of probes to image the dopaminergic system. In this review we will summarize some of the findings that have emerged from these studies and highlight controversies and future needs.

Keywords PET imaging · Dopaminergic receptors · Schizophrenia · Occupancy measures · Addiction

15.1 Introduction

In vivo molecular imaging with positron emission tomography (PET) and single photon emission computed tomography (SPECT) can be used to measure protein molecules such as receptors, transporters, and enzymes, as well as cellular processes such as transmitter synthesis and release. An important property of PET and SPECT is that they can measure molecules present in the brain in the nanomolar to picomolar range of concentrations [1]. To date, the neurochemical system most widely studied in humans with imaging is the dopamine system. This relates to multiple factors: (1) the availability of probes for many dopaminergic targets, including $D_{1/5}$ receptors, $D_{2/3}$ receptors, dopamine transporters (DAT), and vesicular monoamine transporters (VMAT), (2) the recent availability of probes that are agonists, such as [^{11}C]PHNO, which label the high-affinity sites of $D_{2/3}$ receptors, (3) the

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availability of probes with various levels of affinity revealing receptors in areas of the brain with low receptor density, such as [^{18}F]Fallypride or [^{11}C]FLB457, in addition to areas of high receptor density, such as [^{11}C]raclopride, (4) and most importantly the relevance of dopamine to many basic mental processes (cognition, reward) and many psychiatric disorders: schizophrenia, mood disorders, anxiety, dimensions of personality, attention deficit and hyperactivity disorder (ADHD), addiction. Studies using in vivo imaging of dopaminergic receptors and other transmission indices have been conducted comparing patients in these categories to healthy subjects. Furthermore, dopaminergic interventions have a prominent role in our treatment of psychosis, depression, ADHD, and others. For this reason, PET and SPECT have been used not only to assess the involvement of various dopaminergic indices in mental activity and psychiatric disease states, but also to assess the roles of dopaminergic receptors in the treatments for these psychiatric conditions.

In this chapter we will describe the contributions of imaging dopaminergic receptors, at baseline, after manipulation of dopaminergic levels in vivo, and after treatment, to the understanding of psychiatric disease and therapeutic effects.

15.2 Imaging Dopamine Receptors in Schizophrenia

Molecular imaging became available in the 1980s at a time when the dopaminergic hypothesis of schizophrenia had been a focus of research interest for decades, initially because of the early observations that dopamine receptors are activated by psychostimulants, that non-reserpine neuroleptics are dopamine antagonists, and that dopamine plays an important role in the extrapyramidal motor system. This interest was strengthened later by the discovery of the correlation between clinical doses of antipsychotic drugs and their potency to block DA D_2 receptors [2, 3] and studies confirming the psychotogenic effects of DA-enhancing drugs [4, 5]. These led to the classical dopamine hypothesis of schizophrenia, postulating an increase in dopamine transmission in this condition.

Over the years, the awareness of negative symptoms (flattening of affect, apathy, poverty of speech, anhedonia, and social withdrawal) and cognitive symptoms (deficits in attention, working memory, and executive functions) in this illness and of their resistance to D_2 receptor antagonism led to a reformulation of the classical DA hypothesis. Functional brain imaging studies suggested that these symptoms might arise from altered prefrontal cortex (PFC) functions [6]. A wealth of preclinical studies emerged documenting the importance of prefrontal DA transmission at D_1 receptors (the main DA receptor in the neocortex) for optimal PFC performance [7]. Together, these observations led to the hypothesis that a deficit in DA transmission at D_1 receptors in the PFC might be implicated in the cognitive impairments and negative symptoms of schizophrenia [8, 9] while the excess DA transmission may be related only to the core or “positive” symptoms (hallucinations, delusions).

The advent in the early 1980s of techniques based on PET and SPECT to measure indices of DA activity in the living human brain opened the possibility of direct investigation of these hypotheses. We will review these below.

Studies of striatal DA transmission in schizophrenia examined both post-synaptic ($D_{2/3}$ receptors and $D_{1/5}$ receptors, although for simplicity of notation we will refer to these as D_2 receptors and D_1 receptors) and presynaptic (DOPA decarboxylase activity, stimulant-induced DA release, baseline DA release, and DAT) functions.

15.2.1 Striatal DA Transmission and Receptors

15.2.1.1 Dopamine Receptors

Striatal D_2 receptor density in schizophrenia has been extensively studied with PET and SPECT imaging. Studies comparing parameters of D_2 receptor binding in patients with schizophrenia and healthy controls ($n = 17$ studies) included a total of 245 patients (112 were neuroleptic naïve and 133 were neuroleptic free for variable periods of time) [10–26]. These patients were compared to 231 controls, matched for age and sex. Eleven studies used PET and six studies used SPECT. Radiotracers included butyrophenones ([^{11}C]N-methyl-spiperone, [^{11}C]NMSP, $n = 4$, and [^{76}Br]bromospiperone, $n = 3$), benzamides ([^{11}C]raclopride, $n = 3$, and [^{123}I]IBZM, $n = 5$) or the ergot derivative [^{76}Br]lisuride, $n = 2$.

Only 2 out of 17 studies detected a significant elevation of D_2 receptor density parameters at a level $p < 0.05$. However, meta-analysis of the 17 studies reveals a small but significant elevation of D_2 receptors in patients with schizophrenia [27]. If D_2 receptor density did not differ between patients and controls (null hypothesis), one would expect approximately 50% of the studies to report lower D_2 receptor levels in schizophrenics compared to controls. Instead, 13 out of 17 studies reported an increase (although not significant in 11 out of 13 cases), 2 reported no change, and only 2 studies reported a decrease in patients compared to controls. This distribution is unlikely ($p < 0.05$, sign test) under the null hypothesis. The average effect size (mean value in schizophrenic group – mean value in control group/SD in control group) of the 17 studies was 0.51 ± 0.76 (SD), and the probability to yield such effect size under the null hypothesis is again lower than 0.05. The aggregate magnitude of this elevation is thus 51% of the SD of controls. Given an average control SD of 23%, the effect is about 12%. To detect an effect size of 0.51 at 0.05 significance level with a power of 80%, a sample of 64 patients and 64 controls would be needed. Clearly, none of the studies included enough patients to detect this small effect with appropriate power.

No clinical correlates of increased D_2 receptor binding parameters have been reliably identified. Thus, the simplest conclusion from these studies is that untreated or never treated patients with schizophrenia show a modest elevation in D_2 receptor density parameters (of about 12%) of undetermined clinical significance, that all studies were underpowered, and that positive results occasionally reported [10, 11] are due to a sampling effect. A similar conclusion was reached by Kestler et al. [28]

in their own meta-analysis. This conclusion is reached under the assumptions that all studies measured parameters from the "same" D_2 receptors population. Clearly, the aggregate D_2 receptor increase reported in vivo in drug-free patients is lower than that the increase reported in postmortem studies, supporting the idea that postmortem results were significantly affected by antemortem medications. A study in unaffected monozygotic twins of patients with schizophrenia also suggested that a modest elevation of D_2 receptors in the caudate might be associated with genetic vulnerability to schizophrenia [29].

Studies performed with butyrophenones ($n = 7$) have an effect size of 0.96 ± 1.05, while studies performed with other ligands (benzamides and lisuride, $n = 10$) have an effect size of 0.20 ± 0.26, a difference that is significant ($p = 0.04$). This observation suggests that the in vivo increase in butyrophenone binding might be larger than the increase in benzamide binding. Unfortunately, no studies have been reported in which the same subjects were scanned with both ligands. Such a study is warranted to directly test this proposition. Several hypotheses have been advanced to account for the existence of a differential increase in [^{11}C]NMSP in vivo binding in patients with schizophrenia in the face of normal in vivo benzamide binding. Since [^{11}C]raclopride and [^{123}I]IBZM bind to D_2 and D_3 receptors while [^{11}C]NMSP binds to D_2, D_3 and D_4 receptors, this difference could reflect a selective elevation of D_4 receptors in schizophrenia [30]. However, this hypothesis has not been substantiated [31]. Another hypothesis derives from the observation that D_2 receptors, like several G-protein-coupled receptors, exist in monomers, dimers, and other oligomeric forms [32–35]. Photoaffinity-labeling experiments suggested that butyrophenones detect only monomers, while benzamides detect both monomers and dimers. Thus, increased butyrophenone binding and normal benzamide binding might reflect a higher monomer/dimer ratio in schizophrenia. This interesting hypothesis deserves further exploration. A third proposition evolved around the idea that the binding of these ligands would display different vulnerability to competition by endogenous DA [36, 37]. This proposition was based on two assumptions: (1) the concentration of DA in the proximity of D_2 receptors might be higher in patients compared to controls and (2) [^{11}C]NMSP might be less affected than [^{11}C]raclopride or [^{123}I]IBZM binding by endogenous DA competition. It follows that D_2 receptor density measured in vivo with [^{11}C]raclopride and [^{123}I]IBZM would be "underestimated" to a greater extent in patients with schizophrenia than in control subjects. This hypothesis played an important role in bringing the endogenous competition concept to the attention of the imaging field (see below).

Regarding striatal D_1 receptors, several imaging studies [16, 38, 39] have confirmed the results of postmortem studies of unaltered levels of these receptors in the striatum as a whole in patients with schizophrenia.

Several lines of evidence suggest that D_3 receptors might play an important role in the pathophysiology and treatment of schizophrenia [40, 41]. Until recently, imaging D_3 receptors was not feasible: PET radiotracers commonly used to study D_2 and D_3 receptors exhibit similar affinities for both receptors and the concentration of D_3 receptors in the human striatum is lower than that of D_2 receptors.

[^{11}C]PHNO is an agonist at $D_{2/3}$ receptors with higher affinity for D_3 versus D_2 [42]. A study comparing [^{11}C]PHNO in patients with schizophrenia to healthy controls found no differences showing no alterations in the affinity for the receptors or in the D_3 composition [43], although a more selective tracer is needed to confirm these results.

15.2.1.2 Dopamine Transporter

Three imaging studies have confirmed the in vitro observation of normal striatal DAT density in schizophrenia [44, 45]. In addition, no association between amphetamine-induced DA release and DAT density was found [44], suggesting that the increased presynaptic output revealed by the studies reviewed above is not due to higher terminal density.

15.2.1.3 Vesicular Monoamine Transporter

Using the radiotracer [^{11}C]DTBZ [46] Taylor et al. were not able to show any difference in vesicular monoamine transporter BP in patients with schizophrenia compared to healthy subjects.

15.2.1.4 Striatal Amphetamine-Induced DA Release

In addition to the small increase in striatal D_2 receptors, studies have shown increased rates of dopamine synthesis and release. The decrease in [^{11}C]raclopride and [^{123}I]IBZM in vivo binding following acute amphetamine challenge has been well validated as a measure of the change in D_2 receptor stimulation by DA due to amphetamine-induced DA release [47–49].

Three studies showed that the amphetamine-induced decrease in [^{11}C]raclopride or [^{123}I]IBZM binding is elevated in untreated patients with schizophrenia compared to well-matched controls [50, 51] [49]. A significant relationship was observed between the magnitude of this effect and the transient induction or worsening of positive symptoms. This exaggerated response of the DA system to amphetamine was observed in both first-episode/drug-naïve patients and previously treated patients [52], but was larger in patients experiencing an episode of illness exacerbation than in patients in remission at the time of the scan [52]. This exaggerated DA reactivity did not appear to be a nonspecific effect of stress, as higher self-reports of anxiety before the experiments were not associated with larger effect of amphetamine on [^{123}I]IBZM binding. Furthermore, non-psychotic subjects with unipolar depression, who reported levels of anxiety similar to the schizophrenic patients at the time of the scan, showed normal amphetamine-induced displacement of [^{123}I]IBZM [53].

15.2.1.5 Baseline Occupancy of Striatal D_2 Receptors by DA

In rodents, acute depletion of synaptic DA is associated with an acute increase in the in vivo binding of [^{11}C]raclopride or [^{123}I]IBZM to D_2 receptors [54]. The increased binding is observed in vivo but not in vitro, indicating that it is not due to receptor upregulation [55], but to removal of endogenous DA and unmasking of D_2 receptors previously occupied by DA. A similar acute DA depletion technique paired with D_2 receptor imaging in humans using alpha-methyl-para-tyrosine, αMPT, an irreversible inhibitor of tyrosine hydroxylase, the rate-limiting step in dopamine synthesis, has been developed to assess the degree of occupancy of D_2 receptors by DA [55]. In schizophrenia, there was a higher occupancy of D_2 receptors by DA in patients experiencing an episode of illness exacerbation, compared to healthy controls [24]. Higher synaptic DA levels in patients with schizophrenia were predictive of good therapeutic response of these symptoms following 6 weeks of treatment with atypical antipsychotic medications [24].

Both sets of findings, stimulated and baseline DA release, shown to be higher in drug naïve patients with schizophrenia than in controls [56], have generally been interpreted as reflecting an increase in synaptic DA in the schizophrenic group. Another interpretation of these observations would be that schizophrenia is associated with increased affinity of D_2 receptors for DA, but this was shown not to be the case recently by using a $D_{2/3}$ agonist radiotracer [43]. Furthermore, the increased release is consistent with the findings of presynaptic increased synthesis as shown by [^{18}F]DOPA or [^{11}C]DOPA studies.

15.2.1.6 Striatal Aromatic Amino Acid Decarboxylase Activity

Eight studies have reported rates of DOPA decarboxylase in patients with schizophrenia, using [^{18}F]DOPA or [^{11}C]DOPA. Six out of the eight studies reported increased accumulation of DOPA in the striatum of patients with schizophrenia [57–64], one reported no change [57], and one study reported reduced [^{18}F]DOPA striatal uptake [58]. All three studies that involved first-episode schizophrenia showed an increase of DOPA in the striatum [59–61]. Increased uptake of radiolabeled DOPA reflects both increased DOPA decarboxylase activity and increased vesicular accumulation of the radiolabeled DOPA product, dopamine. Interestingly, a recent study observed a relationship between poor prefrontal activation during the Wisconsin Card Sorting task and elevated [^{18}F]DOPA accumulation in the striatum, suggesting a link between alteration of the dorsolateral prefrontal cortex function and increased striatal DA activity in schizophrenia [63]. In rats as in anesthetized pigs, increases in AADC activity in vitro and in vivo have been reported following acute treatment with dopamine antagonists [65–67]. Conversely acute treatment with the dopamine agonist apomorphine decreases ^{11}C-DOPA influx in monkeys [68]. Evidence for such effects in humans, however, is extremely limited. Thus, in the only comprehensive study to date Grunder et al. recently reported a decrease in [^{18}F]DOPA uptake in nine patients with schizophrenia following subchronic treatment with haloperidol [69], suggesting that chronic neuroleptic

administration will tend to decrease AADC activity and hence dopamine synthesis. Interestingly, acute administration of antipsychotics increases DA neurons firing whereas chronic administration decreases the number of spontaneously active DA neurons in the rat substantia nigra [70], suggesting that the different effects of antipsychotics on AADC activity in the living brain could reflect such phenomena.

15.2.2 Extrastriatal D_2 Receptors

The recent availability of high-affinity D_2 radiotracers allowed the study of D_2 receptors in low-density regions such as the substantia nigra, thalamus, and temporal cortex in patients with schizophrenia compared to controls (Fig. 15.1). Lower D_2 receptor density has been described in untreated schizophrenia in the thalamus [71–75], as well as in the midbrain [76], temporal cortex [71], and cingulate cortex [75, 77]. One study showed an increase in D_2 receptors in the substantia nigra [78]. A very recent large study using similar methodology did not confirm any of these alterations in extrastriatal D_2 receptors [79]. Additional studies are needed to resolve the discrepancies and expand beyond measuring levels of D_2 receptors to assess alterations of the transmitter itself in extrastriatal areas. As in striatum,

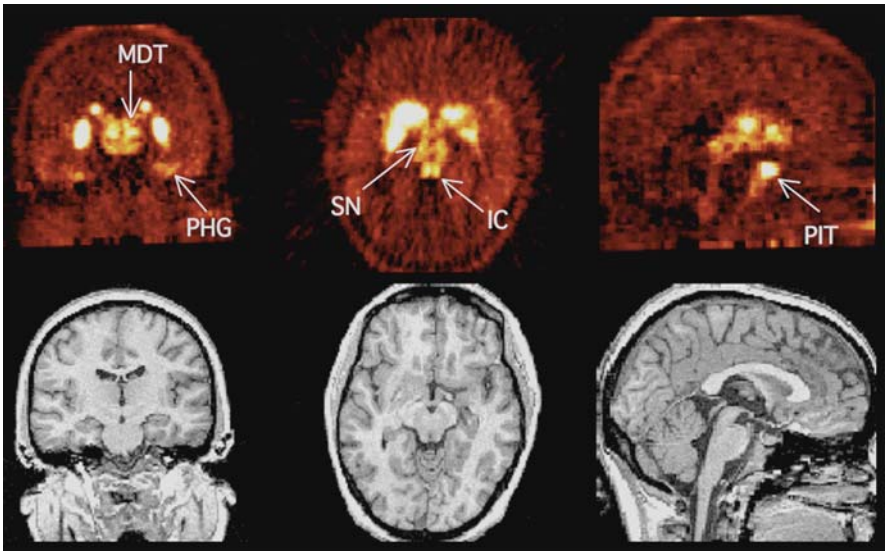

Fig. 15.1 Coronal, transaxial, and sagittal views of PET images acquired 50–80 min following injection of 2.5 mCi [^{18}F]Fallypride, a high-affinity $D_{2/3}$ radiotracer, in a 30-year-old female, with coregistered MRI. Slices were chosen to illustrate extrastriatal regions with detectable specific-binding signal, such as the thalamus including the medio-dorsal nucleus (MDT), the parahippocampal gyrus (PHG), the substantia nigra (SN), the inferior colliculi (IC), and the pituitary (PIT)

alterations in levels of neurotransmitter may mask potential differences in receptor density between patients and controls.

15.2.3 Prefrontal DA Receptors

The majority of DA receptors in the PFC are of the D_1 subtype [80, 81]. Cortical D_1 receptors have been studied in schizophrenia using [^{11}C]SCH 23390 [82] and [^{11}C]NNC 112 [83, 84]. In humans, [^{11}C]NNC 112 provides higher specific to non-specific ratios compared to [^{11}C]SCH 23390 [84, 85], a property that is important for quantification of cortical D_1 receptors. It should be noted that both ligands display only moderate in vivo selectivity for D_1 relative to 5-HT_{2A} receptors, and that 20–30% of cortical binding of both radiotracers correspond to binding to 5-HT_{2A} receptors [86, 87].

PET studies with [^{11}C]SCH 23390 reported decreased [16] or unchanged [88] prefrontal D_1 receptor availability in untreated patients with schizophrenia. In contrast, a study using [^{11}C]NNC 112 reported increased D_1 receptor availability in the dorsolateral PFC (DLPFC) of patients with schizophrenia [39]. Interestingly, increased [^{11}C]NNC 112 binding was associated with poor performance on the "n-back" test of working memory [39]. The reason for the discrepancy in the results obtained with [^{11}C]SCH 23390 and [^{11}C]NNC 112 remains to be elucidated, but it is interesting to note that the binding of both radiotracers is differentially affected by endogenous DA competition and receptor trafficking [54]. For example, chronic DA depletion in rodents is associated with decreased and increased in vivo binding of [^{11}C]SCH 23390 and [^{11}C]NNC 112, respectively [89]. Thus, the contradictory observations of decreased [^{11}C]SCH 23390 binding [16] and increased [^{11}C]NNC 112 binding [39] observed in the PFC in patients with schizophrenia might in fact both represent consequences of sustained deficit in prefrontal DA function. Much work remains to be done to validate this hypothesis. This point illustrates that the in vivo binding of radiotracers is affected by several factors that are not present in the typical in vitro situation, such as the impact of receptor trafficking on ligand affinity [54]. This situation represents both a challenge, because the interpretation of the results is less straightforward, and an opportunity, because more information can be gained on the functions of the living neurons. However, more selective tracers are needed to pursue this line of investigation.

15.2.4 Antipsychotic Drug Occupancy Studies

An important use of neuroreceptor imaging in schizophrenia over the last two decades has been the assessment of receptor occupancy achieved by typical and atypical antipsychotic drugs [90, 91]. The main focus has been on D_2 receptor occupancy, but $5HT_{2A}$ and D_1 receptors have also been investigated. Studies have

repeatedly confirmed the existence of a threshold of occupancy of striatal D_2 receptors (about 80%) above which extrapyramidal side effects (EPS) are likely to occur [92] with the exception of the most recently approved antipsychotic, aripiprazole, which functions as a partial agonist [93]. A relationship between the degree of D_2 receptor occupancy and clinical response has not been observed [94, 95] at doses achieving more than 50% occupancy; however, two recent studies that included a wider range of receptor occupancies showed a relationship between striatal D_2 receptor occupancy and treatment of positive symptoms [93, 96]. Two studies performed with low doses of relatively selective D_2 receptor antagonists (haloperidol and raclopride) suggested that 50–60% occupancy was required to observe a rapid clinical response [97, 98]. Clozapine, at clinically therapeutic doses, has been found to achieve only 40–60% D_2 receptor occupancy [92, 95, 99], which, in conjunction with its anticholinergic properties, may account for its low liability for EPS. Occupancy of 5-HT_{2A} receptors by "5-HT_{2A}/D_2 balanced antagonists" such as risperidone does not confer protection against EPS, since the threshold of D_2 receptor occupancy associated with EPS is not markedly different between these drugs and drugs devoid of $5HT_{2A}$ antagonism [100–103]. Studies with quetiapine suggest that, at least with this agent, transient high occupancy of D_2 receptors might be sufficient to elicit clinical response [104, 105].

An interesting question relates to putative differences in the degree of occupancy achieved by antipsychotic drugs in striatal and extrastriatal areas. Pilowsky et al. [106] initially reported lower occupancy of striatal D_2 receptors compared to temporal cortex D_2 receptors in seven patients treated with the atypical antipsychotic drug clozapine, using the high-affinity SPECT ligand [^{123}I]epidepride. In contrast, typical antipsychotics were reported to achieve similar occupancy in striatal and extrastriatal areas, as measured with [^{11}C]FLB 457 [107] or [^{123}I]epidepride [108]. It should be noted, however, that these very high-affinity ligands do not allow accurate determination of D_2 receptor availability in the striatum [109]. Conversely, [^{18}F]fallypride enables accurate determination of D_2 receptor availability in both striatal and extrastriatal areas [110]. Occupancy studies using [^{18}F]fallypride confirmed that clozapine and quetiapine , but not olanzapine or haloperidol, achieved higher D_2 receptor occupancy in temporal compared to striatal regions [111–113]. Occupancy studies performed with [^{76}Br]FLB457 also reported higher occupancies in cortex compared to striatum for a number of antipsychotic drugs, including typical antipsychotic drugs [114]. Conversely, a study combining [^{11}C]FLB 457 imaging for extrastriatal D_2 receptor receptors and [^{11}C]raclopride imaging for striatal D_2 receptors suggested similar occupancy of D_2 receptors in both regions for both typical and atypical antipsychotic drugs [115]. Our own study showed a small difference with higher EC50 for aripiprazole in extrastriatal regions compared to striatal regions [93]. This difference may have been too small to detect consistently, conferring discrepant findings across studies. Thus, at this point in time, there is a strong suggestion that many, if not most, antipsychotic drugs achieve higher occupancies in extrastriatal regions compared to striatum, although this phenomenon has not been universally observed. Factors underlying this difference remain to be elucidated.

Finally, it is important to point out that D_2 receptor occupancy levels in striatum has been showed to be more predictive of therapeutic response than in temporal cortex [93, 96]. Thus, the observation that, in a restricted dose range, D_2 receptor occupancy by antipsychotic drugs is higher in temporal cortex than in striatum does not necessarily imply that the temporal cortex is the therapeutic site of actions of these agents.

15.3 Dopamine Receptors in Affective Disorders

15.3.1 Major Depressive Disorder

The critical role of DA in brain reward systems, the reports of low cerebrospinal fluid homovanillic acid levels in depressed patients, the association of major depression with Parkinson's disease, and the enhancement of dopaminergic activity by several antidepressant treatments suggest that a deficiency of dopaminergic function might be associated with major depression [116–119]. Five studies compared striatal D_2 receptor availability with [^{123}I]IBZM and SPECT in patients with major depression and control subjects. Two of the five studies reported higher [^{123}I]IBZM-specific binding in the striatum of depressed subjects compared to controls [120, 121], whereas three studies reported no changes [122–124]. Using [^{11}C]raclopride and PET, one study reported elevated D_2 receptor availability in putamen in patients with depression with motor retardation [125]. Amphetamine-induced DA release was also assessed in patients with major depression and found to be unchanged [124].

Two studies examined [^{123}I]β-CIT striatal binding to the dopamine transporter (DAT) in patients with major depression and yielded conflicting results: one study reported normal levels of striatal DAT in patients with major depression [126], while the other one reported increased DAT levels [127]. One study reported decreased DAT density in depression using [^{11}C]RTI-32 [128]. SPECT studies conducted with [^{99m} Tc]TRODAT-1 also reported conflicting results, with studies reported increased [129] or unchanged [130] striatal [^{99m} Tc]TRODAT-1 uptake in patients with major depression.

Finally, [^{18}F]DOPA uptake in the left caudate was observed to be significantly lower in depressed patients with psychomotor retardation than in depressed patients with high impulsivity and in comparison subjects [131]. Thus, major depression per se does not appear to be consistently associated with alteration of the dopaminergic parameters at the level of the whole striatum. However, DA might play a role in the neurobiology underlying some clinical features of depression, such as psychomotor retardation.

15.3.2 Bipolar Disorder

Because of the relationship between mania and psychosis, a number of PET studies have investigated the DA system in bipolar disorders. D_1 receptor binding in

the frontal cortex was reported to be decreased in a study of ten symptomatically heterogeneous, drug-free bipolar patients [132]. Increases in D_2-like (i.e., D_2, D_3, and D_4) receptor density in the striatum were found in 7 psychotic patients with bipolar disorder when compared to 7 non-psychotic patients with bipolar disorder and 24 control subjects. The authors concluded that an increase in D_2-like receptors is associated with the state of psychosis rather than with a diagnosis of bipolar disorder [133]. As part of the same studies, Gjedde and Wong also reported findings consistent with an elevated concentration of synaptic DA in bipolar patients with psychosis, but not in non-psychotic bipolar patients [134]. On the other hand, amphetamine-induced DA release was reported to be normal in euthymic patients with bipolar disorders [135].

In conclusion, few investigations have been reported using PET molecular imaging techniques in patients with bipolar disorders, and the findings reported so far might be related to clinical states (depression, mania with psychosis) rather than to the bipolar condition per se.

15.4 Social Phobia (Social Anxiety Disorder)

Neurobiological mechanisms underlying social phobia, including neuroimaging findings, have been reviewed recently [136–138]. One SPECT study using [^{123}I]β-CIT to label DAT in the striatum reported that densities were markedly lower in patients with social phobia than in age- and gender-matched controls [139]. Another study using [^{123}I]IBZM reported a significant decrease in D_2 receptor binding potential in patients with social phobia compared to controls [140]. However, this was not replicated by the same group using PET and examining D_2 receptor and DA release as well as DAT with a SPECT tracer, [^{123}I]β-CIT [141]. These inconsistencies, often described in the imaging literature, may result from heterogeneity of the disorders.

15.5 Personality Disorders and Traits

Personality disorders (PD) are characterized by stable patterns of maladaptive behavior. Some, such as paranoid, schizoid, schizotypal, avoidant, and obsessive-compulsive PD, have stable patterns of behavior reminiscent of their corresponding clinical disorders but do not reach a sufficient severity and their response to medication is generally poor. It might therefore be expected that some personality disorders might be associated with neurobiological abnormalities similar to, but less marked than, the disorders described in this chapter. Functional imaging studies have now begun to address the issue of how neurochemical brain functions may be associated with normal and pathological personality traits.

A number of studies have investigated differences within the normal range of personality traits or temperaments in healthy subjects. Most receptor PET studies have

so far investigated dopaminergic neurotransmission. Studies using [^{11}C]raclopride report that the traits of depression and personal detachment are related to low D_2 receptor density in the striatum [142, 143]. However, the relationship is not evident on all measures of detachment [143, 144]. Detachment was also found to be associated with low DAT binding in the putamen [145]. These findings are also interesting in view of the association between social phobia and low DAT and D_2 receptors [139, 140], and it has been argued that these neurobiological findings might underlie a commonality between detachment and social phobia [146].

In schizotypal personality disorder, similar alterations in dopamine transmission have been reported as in schizophrenia, albeit in a smaller magnitude [147].

15.6 Attention Deficit Hyperactivity Disorder

Interesting findings have been reported in the study of attention deficit hyperactivity disorder (ADHD), a condition treated with psychostimulants. In a preliminary study of six adult subjects with ADHD, Dougherty et al. [148] observed a large increase in DAT availability (70%) compared to controls. This finding was replicated in a larger samples of adults with ADHD by Dresel et al. [149], using [^{99m}Tc]TRODAT-1, by Larish et al. [150], using [^{123}I]FP-CIT, and by Spencer et al. [151], using [^{18}F]altropane. In these subsequent studies, the magnitude of DAT elevation was considerably lower than in the original study. Elevated DAT availability was also replicated in children with ADHD [152]. On the other hand, this finding has not been replicated in several studies carried out by other groups [153–155] using [^{123}I]β-CIT SPECT, [^{11}C]PE2I PET, and [^{11}C]cocaine PET, respectively. Here again, the heterogeneity of this condition might account for the discrepant results.

The DAT blocker methylphenidate is the treatment of choice of ADHD, and oral therapeutic doses induce significantly decreased [^{11}C]raclopride binding [156], presumably due to increased synaptic DA levels. This method might provide a tool to monitor the biological effectiveness (increased synaptic DA) of the treatment and be useful in the evaluation of non-responders.

15.7 Substance Abuse

Molecular imaging investigations yielded important information about the mode of action of addictive substances and neurochemical abnormalities associated with addictions. Given the central role of DA in mediating the rewarding effects of drugs of abuse [157–162], it is not surprising that many imaging studies focused on this transmitter system. In general, studies demonstrated that acute administration of addictive drugs is associated with increased DA transmission in the limbic striatum, and that the pleasurable effects of these drugs are associated with the magnitude of DA system stimulation. In patients suffering from addiction, studies demonstrated

deficits of both pre- and post-synaptic DA function, a finding not readily predicted from animal data.

15.7.1 Cocaine

Cocaine abuse has been extensively studied using molecular imaging techniques. Most of the work has focused on changes in striatal DA that occur with chronic cocaine use. The studies have generated a remarkably consistent set of data and illustrate admirably the ability of molecular imaging to unravel neurochemical abnormalities in the human brain associated with pathological conditions.

15.7.1.1 D_2 Receptors

A reduction in striatal DA D_2 receptors has been demonstrated by Volkow et al. using both [^{18}F] N-methylspiroperidol and [^{11}C] raclopride [163–166], a finding that has been independently replicated [167]. Volkow et al. [164] also noted that this decrease in striatal D_2 receptor availability correlated with years of use. This deficit appears to be long lasting in a group of subjects re-scanned after 3 months of inpatient rehabilitation [164]. PET studies also revealed reduction in striatal D_2 receptor availability in heroin abuse [168], methamphetamine abuse [169], and alcoholism [170–172], suggesting that decreased striatal D_2 receptor availability might be a general feature of the addicted brain.

The results of these studies raise the question of whether a decrease in D_2 receptors is the result of years of drug abuse or represents a neurochemical risk factor for developing substance abuse. Two observations suggested that low D_2 receptor availability might constitute a risk factor for the development of addiction. First, studies in healthy subjects suggested that low striatal D_2 receptor availability was predictive of a pleasurable experience following administration of the psychostimulant methylphenidate [173, 174]. Second, studies in nonhuman primates demonstrated that low striatal D_2 receptor availability was predictive of increased propensity to self-administer cocaine [175]. It should be noted, however, that low D_2 receptor availability is not predictive of a pleasurable experience following amphetamine in healthy subjects [176–178] nor in cocaine abusers [167]. Thus, while the association between low striatal D_2 receptor availability and history of chronic cocaine abuse is well established, the direction of causality in this relationship remains to be clarified.

15.7.1.2 Stimulant-Induced DA Release

As described above, PET and SPECT studies can be used to measure changes in subcortical DA transmission in the human brain following psychostimulant administration [54]. In healthy subjects, a number of independent studies have shown that the percentage decrease in radioligand binding (i.e., the increase in DA release) is

positively correlated with the pleasurable subjective effects induced by psychostimulant administration [176, 178–182]. This observation is consistent with the preclinical body of evidence indicating that DA transmission in the ventral striatum mediates the reinforcing effects of drugs of abuse [183, 184]. PET studies showed a greater decrease in [^{11}C]raclopride binding in the ventral versus dorsal striatum in healthy controls in response to an amphetamine challenge [167, 180], and in response to a monetary reward [185]. Collectively, these studies suggest that strong DA response would correlate with increased reward value, might mediate the reinforcing effects of drugs of abuse, and might therefore constitute a risk factor toward the development of addiction.

However, cocaine abusers have been shown to have a blunted DA response to psychostimulants. A study of Volkow et al. [166] used [^{11}C]raclopride to measure the change in D_2 receptor availability before and after an i.v. dose of 0.5 mg/kg methylphenidate in healthy controls and cocaine abusers who had been abstinent for 3–6 weeks. The authors reported a 9% decrease in [^{11}C]raclopride binding in the cocaine abusers compared to a 21% decrease in healthy controls. Malison et al. [186] performed a similar study in abstinent cocaine abusers and controls using [^{123}I]IBZM and an amphetamine challenge (0.3 mg/kg i.v.) and reported a 1% change in binding in the cocaine abusers compared to a 10% decrease in controls. Similarly, Martinez et al. [167] showed that the effect of amphetamine on (0.3 mg/kg i.v.) on [^{11}C]raclopride binding is markedly blunted in cocaine abusers in all striatal subregions. In the ventral striatum, this response was completely blunted. Furthermore, blunted dopamine transmission in the ventral striatum and anterior caudate was predictive of the choice for cocaine over money, suggesting that this deficit might confer vulnerability to relapse [167].

15.7.1.3 DOPA Decarboxylase

The findings above of blunted presynaptic DA function in cocaine abusers are supported by the study of Wu et al. [187] showing a reduction in the rate of uptake of [^{18}F]6-FDOPA in abstinent cocaine abusers.

15.7.1.4 DAT

Imaging studies of the DAT in cocaine abusers have been published, and this body of work has failed to provide a clear picture of the status of the DAT in cocaine abusers. Using [^{11}C]cocaine, no changes in DAT were observed in detoxified (>1 month) cocaine abusers [188]. In contrast, Malison et al. [189] showed a significant upregulation of DA transporters, measured with SPECT and [^{123}I]β-CIT, in the striatum of recently detoxified (<96 h) cocaine abusers. Such upregulation was not observed following prolonged abstinence.

DAT occupancy by cocaine. Studies of DAT occupancy by cocaine have generated valuable information. Volkow et al. [190] reported that a DAT occupancy of about 50% or more is needed to produce the subjective effects of cocaine. These data suggest that any treatment approach to cocaine abuse in which the transporter

is blocked would need to produce somewhere between 60 and 90% occupancy of the transporters. This issue was addressed in an occupancy study of mazindol, a non-selective catecholamine reuptake inhibitor [191]. This study showed that the clinical dosage generally used produced only a modest occupancy of 16–23% and would therefore not be expected to have sufficient efficacy to block the reinforcing effects of cocaine.

While the magnitude of DAT blockade is important for the experience of the rewarding effect, the rate of occupancy is an important feature of the addictive process [192, 193]. Thus, cocaine exhibits very rapid association and dissociation from the DAT and is highly addictive. The reinforcing effects of methylphenidate are stronger after i.v. compared to p.o. administration, due to faster access to the DAT after i.v. administration. Antidepressants like bupropion or radafaxine associated with moderated and sustained DAT occupancy due to slow peripheral clearance are devoid of abuse liability [194].

Cue-induced cocaine craving. Two studies showed that cue-induced cocaine craving is associated with acute decrease in [^{11}C]raclopride availability, presumably related to acute changes in synaptic DA [195, 196]. Interestingly, craving intensity is associated with DA release in the dorsal rather than the ventral striatum suggesting the involvement of this region in the habituation process associated with craving and addiction.

Overall, the studies in cocaine abuse demonstrate a clear and pronounced dysregulation of the DA system in this disorder. The findings of decreased [^{18}F]DOPA accumulation, decreased amphetamine- and methylphenidate-induced DA release, and decreased D_2 receptor density suggest a functional deficit in D_2 receptor transmission at the level of the whole striatum in this population. This alteration in DA transmission might contribute to the addictive process and the relapse risk.

15.7.2 Methamphetamine

Two imaging studies in methamphetamine abusers have demonstrated a significant decrease in DAT density using PET [197, 198]. The authors also found that the decrease in DAT availability correlated with years of abuse and with impairment in motor and memory tasks. Both studies are in agreement with a postmortem report of reduced DA transporter density in the striata of chronic methamphetamine abusers, as well as decreases in DA and tyrosine hydroxylase [199]. Evidence from studies in Parkinson's disease support the hypothesis that the reduction in DAT availability reflects a loss of DA neurons which is detectable with functional imaging [200–202]. Based on this interpretation, these studies raise the issue of whether this decrease is reversible or whether methamphetamine abuse results in neurotoxicity to the dopaminergic neurons. PET and postmortem studies in nonhuman primates have shown that methamphetamine exposure results in decreased DAT and other markers of dopaminergic transmission, suggesting a frank loss of dopaminergic neurons [203, 204]. However, one study suggested that this reduction might be reversible

after prolonged abstinence [205]. Overall, the PET data demonstrate that methamphetamine abuse in humans results in a reduction in the DAT and raise concerns about the DA neurotoxicity associated with this addiction.

15.7.3 Nicotine

Relative to the impact of smoking on public health, relatively few molecular imaging studies have been carried out to understand the impact of smoking on brain chemistry.

Molecular imaging of nicotinic receptors demonstrated that typical cigarette smoking results in rapid and sustained near saturation of the alpha2 beta4 nicotinic receptors measured with 2-[^{18}F]85380 [206]. One SPECT study in nonhuman primates suggested that this was associated with significant and prolonged upregulation of these receptors [207].

Several studies evaluated the effect of nicotine on striatal DA release, as measured with the [^{11}C]raclopride-binding reduction method. In rhesus monkeys, i.v. nicotine doses ranging from 0.01 to 0.06 mg/kg caused a significant albeit small reduction (5%) in [^{11}C]raclopride availability [208]. In humans, a first study [209] failed to detect a significant effect of cigarette smoking in regular smokers on striatal [^{11}C]raclopride binding, but detected a relationship between the hedonic response to nicotine and decreased [^{11}C]raclopride binding. Another study in nicotine-dependent subjects demonstrated a 30% reduction in [^{11}C]raclopride binding in the ventral striatum following one cigarette [210]. The magnitude of this effect was surprising, as it exceeded the magnitude of the [^{11}C]raclopride reduction observed in the same region following i.v. amphetamine 0.3 mg/kg i.v. (15–20%) [180, 181]. In a subsequent study in a larger group of subjects, this group reported a much lower decrease in [^{11}C]raclopride striatal binding following cigarette smoking (8%) [211]. Finally, Montgomery et al. [212] failed to detect changes in [^{11}C]raclopride binding in regular smokers following intranasal nicotine administration, while relationships were reported between positive subjective effects of the drug and decrease [^{11}C]raclopride binding in striatal subregions. Together, these studies demonstrated that nicotine exposure in human smokers is associated with increases in synaptic DA and that this effect is generally of lower magnitude than the one observed with psychostimulants [54] and close to the detection limit associated with this imaging method. As previously described for stimulants [176, 178, 180–182, 213], the subjective effects of the drugs are associated with the extent of DA release.

Fowler et al. [214–217] investigated levels of monoamine oxidase (MAO) A and B in smokers and showed marked and global decreases in both enzymes. MAO A and B exist in neurons and glial cells and both enzymes degrade DA. MAO B activity was measured using [^{11}C]L-deprenyl [218]. Smokers were found to have a 42% decrease in global MAO B activity compared to controls [214, 217]. Interestingly, a study in former smokers showed that levels of MAO B activity returned to baseline after smoking cessation [216]. In a later study, this same group demonstrated a

decrease in MAO A activity in the brains of cigarette smokers using [^{11}C]clorgyline [215]. In this study smokers had an average reduction of 28% in MAO A activity across brain regions, with a 22% decrease in the basal ganglia [215]. Decreased activities of MAO A and B are expected to be associated with increased DA availability.

Salokongas et al. [219] used [^{18}F]fluorodopa to measure presynaptic DA and reported higher uptake in the striatum in smokers, a finding which could be explained by an increase in DOPA decarboxylase activity or a decrease in MAO activity. A study by Dagher et al. [220] reported a reduction in D_1 receptor availability using [^{11}C]SCH23390 in the striatum. Lastly, Staley et al. [221] investigated DAT and SERT density in the striatum and midbrain, respectively, in smokers and healthy controls using [^{123}I]β-CIT. No difference was seen in DAT availability between these groups but there was a trend toward an increase in [^{123}I]β-CIT binding in the midbrain.

Overall, these findings are consistent with the hypothesis of alterations of the DA system in nicotine smokers, but much work remains to be done to better understand the potential role of this dysregulation in the maintenance of nicotine addiction.

15.7.4 Alcohol

The DA system has been the most investigated neurochemical system using SPECT and PET in alcohol research, due to the wealth of preclinical data suggesting a role for DA in the reward system and clinical data suggesting alterations in DA function in alcoholic patients.

Acute effects of alcohol on DA release in humans have been studied with the [^{11}C]raclopride reporting method. Similarly to results reported with nicotine, alcohol administration in humans is not associated with major changes in [^{11}C]raclopride binding, but the pleasurable effects of the drugs are associated with reduced [^{11}C]raclopride binding [222].

A number of well-controlled and independent studies have consistently demonstrated a reduction in D_2 receptor availability in recently detoxified alcoholics [170, 172, 223, 224], an alteration that does not appear to be reversible following early sustained abstinence for 4 months [225]. The majority of preclinical data do not indicate that chronic alcohol exposure affects D_2 receptor density [226–230], but conflicting results have been published suggesting that the effects of chronic alcohol on DA receptors might vary according to the dose and duration of exposure [231–234]. Such differences in duration of exposure, as well as inter-species differences in the response of D_2 receptors to alcohol, may undermine the relevance of rodent studies in answering the question of whether decreased D_2 receptor binding potential (BP) measured with PET in chronic alcoholics is a risk factor for, or an effect of, chronic alcohol intake. Another important question is whether the alterations in D_2 receptor density in recently detoxified alcoholics are transient or permanent, i.e., if this abnormality persists with a prolonged period of abstinence

even beyond the initial 4 months of abstinence. Interestingly, recent data suggest that low D_2 receptor availability might be associated with high relapse risk [235]. Studies reporting DAT measurements in chronic alcoholics have failed to detect consistent alterations of DAT binding in alcoholism [223, 236–239].

Alcoholism is also associated with reduced amphetamine-induced DA release in the ventral striatum [172]. Together with the evidence of reduced D_2 receptor availability, these findings suggest that alcoholism, like cocaine and maybe other addictions, is associated with a significant decrease in DA transmission at D_2 receptors. Interestingly, a recent study [240] in nonalcoholic offspring from families with a positive history of alcohol dependence failed to detect alteration of DA transmission (D_2 receptor availability and amphetamine-induced DA release) compared to nonalcoholic subjects without a family history of alcohol dependence. Therefore, one might conclude that these alterations are a consequence rather than a risk factor of alcoholism. However, it could also be argued that a normal DA transmission in these subjects might provide protective effects, counterbalancing other genetically mediated risks. Short of a long-term prospective study, the status of these DA alterations as risk factors or consequences of addiction is likely to remain unsolved. A recent study assessing D_2 receptors and amphetamine-induced dopamine release in family history positive versus family history negative subjects showed no difference between groups [240].

15.8 Conclusions

This chapter has reviewed key findings from PET and SPECT molecular imaging of dopaminergic receptors, transporters, enzymes, and the transmitter that have contributed to our understanding of the pathophysiology and treatment of psychiatric disorders. Since 1986, the year of publication of the seminal paper of Wong et al. [10] describing the use of PET imaging to detect elevated D_2 receptor density in antipsychotic drug-naïve schizophrenics, this field has undergone a major expansion. So far, it is clear that these techniques have already provided unique insights into the neurochemical imbalances underlying some of these conditions and the pharmacological mechanisms involved in their treatment. It is foreseeable that this contribution will continue to expand in the near future.

Psychiatric conditions are generally characterized by clinical heterogeneity. It is likely that a number of illnesses with different etiologies and neurobiological mechanisms are currently subsumed under the same name by our diagnostic classifications. Despite this, a number of findings have been remarkably consistent and replicated across studies, suggesting that the clinical commonality underlying our diagnostic syndromes might be associated with unique and perhaps specific final common pathophysiological pathways. Furthermore, the examination of the biological processes involved in clinical conditions with nuclear medicine techniques also provides an opportunity for redefining illnesses [241].

For example, one study reported that elevated DA synaptic levels in acute schizophrenia were predictive of rapid symptomatic response to antipsychotic (i.e., antidopaminergic) treatment [24]. A subgroup of patients showed no detectable abnormality of striatal DA function despite frank psychotic symptoms and failed to respond to treatment. It is possible that, in these patients, the psychotic state is not driven by excess DA activity, and that the antidopaminergic treatment fails because the problem being treated does not exist in these patients. This result has led to the concept of dopaminergic versus non-dopaminergic driven psychotic states in schizophrenia [24]. This biological, rather than clinical, classification might prove to be useful in evaluation of non-dopaminergic antipsychotic pharmacological strategies.

Another example is the constellation of conditions that have been reliably shown to be associated with low D_2 receptor availability in the striatum. This finding has been associated with a personality trait (detachment), anxiety disorder (social phobia), and addiction to a variety of substances, including cocaine, heroin, alcohol, and even food. These conditions are not similar but are frequently overlapping or comorbid. Therefore, imaging studies might reveal common biological processes across conditions that were hitherto unsuspected and might help to delineate psychopathological features more directly related to altered biological brain functions than our current diagnostic classifications.

Despite these successes, a substantial number of studies yielded discordant results, and it is important to examine potential sources of discrepancies. An important drawback of this literature is the generally low number of subjects included in studies (typically less than 20 per group). In conditions characterized by marked heterogeneity, such as major depressive disorders, this factor is bound to yield divergent results across studies. Small samples are obviously due to the cost of these investigations, but also, in some instances, to the difficulty in recruiting appropriate clinical subjects (such as drug-free patients with schizophrenia). Another source of discrepancy is the variety of technical approaches to data acquisition and analysis. For example, analytical methods range from "empirical" or "semi-quantitative" methods (typically a region of interest to a region of reference ratio measured at one time point) to model-based methods using an arterial input function. The limitations associated with empirical analytical methods have been discussed elsewhere [242] and might account for artifactual results, especially when the effect size of the between-group difference and the number of subjects are small [243].

In addressing these limitations it will be important to increase the availability of these techniques beyond a few academic centers, to promote multi-center studies in well-characterized populations, and to standardize analytical methods. Until recently, SPECT was the only widely available technique, and SPECT studies have so far provided a substantial contribution to this field. With the current increase in PET camera availability, the development of ^{18}F-based molecular imaging probes will provide unique opportunities for further dissemination of these techniques.

The greatest challenge facing this field is to develop molecular imaging probes suitable for imaging neurochemical processes beyond neurotransmission itself, to

examine growth factors or intracellular signaling pathways. A sustained collaboration between industry and academic institutions will be required to expand the study of brain biomolecular processes beyond our current, and still relatively limited, arsenal.

In conclusion, the chapter has reviewed seminal findings obtained with PET and SPECT molecular imaging of DA transmission in psychiatric conditions. These techniques do not yet play a major role in the diagnosis and treatment of these disorders, and at present remain essentially as research tools. However, the results produced by this field so far suggest that PET will significantly contribute to unraveling the biological bases of these conditions and might play an increasing role in their clinical management. Moreover, it is foreseeable that PET will become more and more involved in the development of new psychiatric medications. Expanding the availability of PET and the current radiopharmaceutical portfolio will be critical for these predictions to become reality.

References

1. Talbot PS, Laruelle M. The role of in vivo molecular imaging with PET and SPECT in the elucidation of psychiatric drug action and new drug development. Eur Neuropsychopharmacol 2002;12:503–11.
2. Seeman P, Chau-Wong M, Tedesco J, Wong K. Brain receptors for antipsychotic drugs and dopamine: direct binding assays. Proc Natl Acad Sci USA 1975;72:4376–80.
3. Creese I, Burt DR, Snyder SH. Dopamine receptor binding predicts clinical and pharmacological potencies of antischizophrenic drugs. Science 1976;19:481–3.
4. Lieberman JA, Kane JM, Alvir J. Provocative tests with psychostimulant drugs in schizophrenia. Psychopharmacology 1987;91:415–33.
5. Angrist B, van Kammen DP. CNS stimulants as a tool in the study of schizophrenia. Trends Neurosci 1984;7:388–90.
6. Knable MB, Weinberger DR. Dopamine, the prefrontal cortex and schizophrenia. J Psychopharmacol 1997;11:123–31.
7. Goldman-Rakic PS, Muly EC, 3rd, Williams GV. D(1) receptors in prefrontal cells and circuits. Brain Res Brain Res Rev 2000;31:295–301.
8. Davis KL, Kahn RS, Ko G, Davidson M. Dopamine in schizophrenia: a review and reconceptualization. Am J Psychiatry 1991;148:1474–86.
9. Weinberger DR. Implications of the normal brain development for the pathogenesis of schizophrenia. Arch Gen Psychiatry 1987;44:660–9.
10. Wong DF, Wagner HN, Tune LE, et al. Positron Emission Tomography reveals elevated D_2 dopamine receptors in drug-naive schizophrenics. Science 1986;234:1558–63.
11. Crawley JC, Owens DG, Crow TJ, et al. Dopamine D2 receptors in schizophrenia studied in vivo. Lancet 1986;2:224–5.
12. Blin J, Baron JC, Cambon H, et al. Striatal dopamine D2 receptors in tardive dyskinesia: PET study. J Neurol Neurosurg Psychiatry 1989;52:1248–52.
13. Martinot J-L, Peron-Magnan P, Huret J-D, et al. Striatal D2 dopaminergic receptors assessed with positron emission tomography and 76-Br-bromospiperone in untreated patients. Am J Psychiatry 1990;147:346–50.
14. Tune LE, Wong DF, Pearlson G, et al. Dopamine D2 receptor density estimates in schizophrenia: a positron emission tomography study with 11C-N-methylspiperone. Psychiatry Res 1993;49:219–37.

15. Nordstrom AL, Farde L, Eriksson L, Halldin C. No elevated D2 dopamine receptors in neuroleptic-naive schizophrenic patients revealed by positron emission tomography and [11C]N- methylspiperone [see comments]. Psychiatry Res 1995;61:67–83.
16. Okubo Y, Suhara T, Suzuki K, et al. Decreased prefrontal dopamine D1 receptors in schizophrenia revealed by PET. Nature 1997;385:634–6.
17. Farde L, Wiesel F, Stone-Elander S, et al. D2 dopamine receptors in neuroleptic-naive schizophrenic patients. A positron emission tomography study with [^{11}C]raclopride. Arch Gen Psychiatry 1990;47:213–9.
18. Hietala J, Syvälahti E, Vuorio K, et al. Striatal D2 receptor characteristics in neuroleptic-naive schizophrenic patients studied with Positron Emission Tomography. Arch Gen Psychiatry 1994;51:116–23.
19. Pilowsky LS, Costa DC, Ell PJ, Verhoeff NPLG, Murray RM, Kerwin RW. D2 dopamine receptor binding in the basal ganglia of antipsychotic-free schizophrenic patients. An I-123-IBZM single photon emission computerized tomography study. Br J Psychiatry 1994;164:16–26.
20. Laruelle M, Abi-Dargham A, van Dyck CH, et al. Single photon emission computerized tomography imaging of amphetamine-induced dopamine release in drug free schizophrenic subjects. Proc Natl Acad Sci USA 1996;93:9235–40.
21. Knable MB, Egan MF, Heinz A, et al. Altered dopaminergic function and negative symptoms in drug-free patients with schizophrenia. [123I]-iodobenzamide SPECT study. Br J Psychiatry 1997;171:574–7.
22. Breier A, Su TP, Saunders R, et al. Schizophrenia is associated with elevated amphetamine-induced synaptic dopamine concentrations: evidence from a novel positron emission tomography method. Proc Natl Acad Sci USA 1997;94:2569–74.
23. Abi-Dargham A, Gil R, Krystal J, et al. Increased striatal dopamine transmission in schizophrenia: confirmation in a second cohort. Am J Psychiatry 1998;155:761–7.
24. Abi-Dargham A, Rodenhiser J, Printz D, et al. Increased baseline occupancy of D2 receptors by dopamine in schizophrenia. Proc Natl Acad Sci USA 2000;97:8104–9.
25. Martinot Jl, Paillère-Martinot ML, Loc'h C, et al. The estimated density of D2 striatal receptors in schizophrenia. A study with positron Emission tomography and 76Br-bromolisuride. Br J Psychiatry 1991;158:346–50.
26. Martinot JL, Paillère-Martinot ML, Loch'H C, et al. Central D2 receptors and negative symptoms of schizophrenia. Br J Pharmacol 1994;164:27–34.
27. Laruelle M. Imaging dopamine transmission in schizophrenia. A review and meta-analysis. Q J Nucl Med 1998;42:211–21.
28. Kestler LP, Walker E, Vega EM. Dopamine receptors in the brains of schizophrenia patients: a meta-analysis of the findings. Behav pharmacol 2001;12:355–71.
29. Hirvonen J, van Erp TG, Huttunen J, et al. Increased caudate dopamine D2 receptor availability as a genetic marker for schizophrenia. Arch Gen Psychiatry 2005;62: 371–8.
30. Seeman P, Guan HC, Van Tol HHM. Dopamine D4 receptors elevated in schizophrenia. Nature 1993;365:411–45.
31. Lahti RA, Roberts RC, Cochrane EV, et al. Direct determination of dopamine D-4 receptors in normal and schizophrenic postmortem brain tissue: a [H-3]NGD-94-1 study. Mol Psychiatr 1998;3:528–33.
32. Ng GY, O'Dowd BF, Caron M, Dennis M, Brann MR, George SR. Phosphorylation and palmitoylation of the human D2L dopamine receptor in Sf9 cells. J Neurochem 1994;63:1589–95.
33. Ng GY, O'Dowd BF, Lee SP, et al. Dopamine D2 receptor dimers and receptor-blocking peptides. Biochem Biophys Res Commun 1996;227:200–4.
34. Zawarynski P, Tallerico T, Seeman P, Lee SP, O'Dowd BF, George SR. Dopamine D2 receptor dimers in human and rat brain. FEBS Lett 1998;441:383–6.

35. Lee SP, O'Dowd BF, Ng GY, et al. Inhibition of cell surface expression by mutant receptors demonstrates that D2 dopamine receptors exist as oligomers in the cell [In Process Citation]. Mol Pharmacol 2000;58:120–8.
36. Seeman P, Guan H-C, Niznik HB. Endogenous dopamine lowers the dopamine D2 receptor density as measured by [^{3}H]raclopride: Implications for positron emission tomography of the human brain. Synapse 1989;3:96–7.
37. Seeman P. Brain dopamine receptors in schizophrenia: PET problems. Arch Gen Psychiatry 1988;45:598–60.
38. Karlsson P, Farde L, Halldin C, Sedvall G. D1-dopamine receptors in schizophrenia examined by PET. Schizophrenia Res 1997;24:179.
39. Abi-Dargham A, Gil R, Mawlawi O, et al. Selective alteration in D1 receptors in schizophrenia: a PET in vivo study. J Nuc Med 2001;42:17P.
40. Sokoloff P, Diaz J, Le Foll B, et al. The dopamine D3 receptor: a therapeutic target for the treatment of neuropsychiatric disorders. CNS Neurol Disord Drug Targets 2006;5:25–43.
41. Gurevich EV, Bordelon Y, Shapiro RM, Arnold SE, Gur RE, Joyce JN. Mesolimbic dopamine D3 receptors and use of antipsychotics in patients with schizophrenia. A postmortem study. Arch Gen Psychiatry 1997;54:225–32.
42. Narendran R, Slifstein M, Guillin O, et al. Dopamine (D2/3) receptor agonist positron emission tomography radiotracer [11C]-(+)-PHNO is a D3 receptor preferring agonist in vivo. Synapse 2006;60:485–95.
43. Graff-Guerrero A, Mizrahi R, Agid O, et al. The Dopamine D(2) Receptors in High-Affinity State and D(3) Receptors in Schizophrenia: A Clinical [(11)C]-(+)-PHNO PET Study. Neuropsychopharmacology 2008.
44. Laruelle M, Abi-Dargham A, van Dyck C, et al. Dopamine and serotonin transporters in patients with schizophrenia: an imaging study with [(123)I]beta-CIT. Biol Psychiatry 2000;47:371–9.
45. Laakso A, Vilkman H, Alakare B, et al. Striatal dopamine transporter binding in neuroleptic-naive patients with schizophrenia studied with positron emission tomography. Am J Psychiatry 2000;157:269–71.
46. Taylor SF, Koeppe RA, Tandon R, Zubieta JK, Frey KA. In vivo measurement of the vesicular monoamine transporter in schizophrenia. Neuropsychopharmacology 2000;23:667–75.
47. Laruelle M, Iyer RN, al-Tikriti MS, et al. Microdialysis and SPECT measurements of amphetamine-induced dopamine release in nonhuman primates. Synapse 1997;25:1–14.
48. Villemagne VL, Wong DF, Yokoi F, et al. GBR12909 attenuates amphetamine-induced striatal dopamine release as measured by [(11)C]raclopride continuous infusion PET scans. Synapse 1999;33:268–73.
49. Breier A, Su TP, Saunders R, et al. Schizophrenia is associated with elevated amphetamine-induced synaptic dopamine concentrations: evidence from a novel positron emission tomography method. Proc Natl Acad Sci USA 1997;94:2569–74.
50. Laruelle M, Abi-Dargham A, van Dyck CH, et al. Single photon emission computerized tomography imaging of amphetamine-induced dopamine release in drug-free schizophrenic subjects. Proc Natl Acad Sci USA 1996;93:9235–40.
51. Abi-Dargham A, Gil R, Krystal J, et al. Increased striatal dopamine transmission in schizophrenia: confirmation in a second cohort. Am J Psychiatry 1998;155:761–7.
52. Laruelle M, Abi-Dargham A, Gil R, Kegeles L, Innis R. Increased dopamine transmission in schizophrenia: relationship to illness phases. Biol Psychiatry 1999;46:56–72.
53. Parsey RV, Oquendo MA, Zea-Ponce Y, et al. Dopamine D(2) receptor availability and amphetamine-induced dopamine release in unipolar depression. Biol Psychiatry 2001;50:313–22.
54. Laruelle M. Imaging synaptic neurotransmission with in vivo binding competition techniques: a critical review. J Cereb Blood Flow Metab 2000;20:423–51.
55. Laruelle M, DSouza CD, Baldwin RM, et al. Imaging D-2 receptor occupancy by endogenous dopamine in humans. Neuropsychopharmacology 1997;17:162–74.

56. Abi-Dargham A, Giessen EV, Slifstein M, Kegeles LS, Laruelle M. Baseline and amphetamine-stimulated dopamine activity are related in drug-naive schizophrenic subjects. Biol Psychiatry 2009.
57. Dao-Castellana MH, Paillere-Martinot ML, Hantraye P, et al. Presynaptic dopaminergic function in the striatum of schizophrenic patients. Schizophr Res 1997;23:167–74.
58. Elkashef AM, Doudet D, Bryant T, Cohen RM, Li SH, Wyatt RJ. 6-(18)F-DOPA PET study in patients with schizophrenia. Positron emission tomography. Psychiatry Res 2000;100: 1–11.
59. Hietala J, Syvalahti E, Vilkman H, et al. Depressive symptoms and presynaptic dopamine function in neuroleptic-naive schizophrenia. Schizophr Res 1999;35:41–50.
60. Hietala J, Syvalahti E, Vuorio K, et al. Presynaptic dopamine function in striatum of neuroleptic-naive schizophrenic patients. Lancet 1995;346:1130–1.
61. Lindstrom LH, Gefvert O, Hagberg G, et al. Increased dopamine synthesis rate in medial prefrontal cortex and striatum in schizophrenia indicated by L-(beta-11C) DOPA and PET. Biol Psychiatry 1999;46:681–8.
62. McGowan SW, Lawrence A, Sale T, Quested D, Grasby PM. Presynaptic dopaminergic dysfunction in medicated schizophrenic patients. Arch Gen Psychiatry 2004;61:134–42.
63. Meyer-Lindenberg A, Miletich RS, Kohn PD, et al. Reduced prefrontal activity predicts exaggerated striatal dopaminergic function in schizophrenia. Nat Neurosci 2002;5: 267–71.
64. Reith J, Benkelfat C, Sherwin A, et al. Elevated dopa decarboxylase activity in living brain of patients with psychosis. Proc Natl Acad Sci USA 1994;91:11651–4.
65. Zhu MY, Juorio AV, Paterson IA, Boulton AA. Regulation of striatal aromatic L-amino acid decarboxylase: effects of blockade or activation of dopamine receptors. Eur J Pharmacol 1993;238:157–64.
66. Cho S, Neff NH, Hadjiconstantinou M. Regulation of tyrosine hydroxylase and aromatic L-amino acid decarboxylase by dopaminergic drugs. Eur J Pharmacol 1997;323:149–57.
67. Danielsen EH, Smith D, Hermansen F, Gjedde A, Cumming P. Acute neuroleptic stimulates DOPA decarboxylase in porcine brain in vivo. Synapse 2001;41:172–5.
68. Torstenson R, Hartvig P, Langstrom B, Bastami S, Antoni G, Tedroff J. Effect of apomorphine infusion on dopamine synthesis rate relates to dopaminergic tone. Neuropharmacology 1998;37:989–95.
69. Grunder G, Vernaleken I, Muller MJ, et al. Subchronic haloperidol downregulates dopamine synthesis capacity in the brain of schizophrenic patients in vivo. Neuropsychopharmacology 2003;28:787–94.
70. Grace AA. Phasic versus tonic dopamine release and the modulation of dopamine system responsivity: a hypothesis for the etiology of schizophrenia. Neuroscience 1991;41:1–24.
71. Tuppurainen H, Kuikka J, Viinamaki H, Husso-Saastamoinen M, Bergstrom K, Tiihonen J. Extrastriatal dopamine D 2/3 receptor density and distribution in drug-naive schizophrenic patients. Mol Psychiatry 2003;8:453–5.
72. Talvik M, Nordstrom AL, Olsson H, Halldin C, Farde L. Decreased thalamic D2/D3 receptor binding in drug-naive patients with schizophrenia: a PET study with [11C]FLB 457. Int J Neuropsychopharmacol 2003;6:361–70.
73. Talvik M, Nordstrom AL, Okubo Y, et al. Dopamine D2 receptor binding in drug-naive patients with schizophrenia examined with raclopride-C11 and positron emission tomography. Psychiatry Res 2006;148:165–73.
74. Yasuno F, Suhara T, Okubo Y, et al. Low dopamine d(2) receptor binding in subregions of the thalamus in schizophrenia. Am J Psychiatry 2004;161:1016–22.
75. Suhara T, Okubo Y, Yasuno F, et al. Decreased dopamine D2 receptor binding in the anterior cingulate cortex in schizophrenia. Arch Gen Psychiatry 2002;59:25–30.
76. Tuppurainen H, Kuikka JT, Laakso MP, Viinamaki H, Husso M, Tiihonen J. Midbrain dopamine D2/3 receptor binding in schizophrenia. Eur Arch Psychiatry Clin Neurosci 2006;256:382–7.

77. Glenthoj BY, Mackeprang T, Svarer C, et al. Frontal dopamine D(2/3) receptor binding in drug-naive first-episode schizophrenic patients correlates with positive psychotic symptoms and gender. Biol Psychiatry 2006;60:621–9.
78. Kessler RC, Woodward N, Riccardi P, et al. Dopamine D2 Receptor Levels in Striatum, Thalamus, Substantia Nigra, Limbic Regions, and Cortex in Schizophrenic Subjects. Biol Psych 2009;65(12):1024–1031.
79. Kegeles LS, Slifstein M, Xu X, et al. [18F]Fallypride PET assessment of D2/D3 receptor binding in schizophrenia. J Nucl Med 2008;49:36P.
80. De Keyser J, Ebinger G, Vauquelin G. Evidence for a widespread dopaminergic innervation of the human cerebral neocortex. Neurosci Lett 1989;104:281–5.
81. Hall H, Sedvall G, Magnusson O, Kopp J, Halldin C, Farde L. Distribution of D1- and D2-dopamine receptors, and dopamine and its metabolites in the human brain. Neuropsychopharmacology 1994;11:245–56.
82. Halldin C, Stone-Elander S, Farde L, et al. Preparation of 11C-labelled SCH 23390 for the in vivo study of dopamine D1 receptors using positron emission tomography. Appl Radiat Isot 1986;37:1039–43.
83. Andersen PH, Gronvald FC, Hohlweg R, et al. NNC-112, NNC-687 and NNC-756, new selective and highly potent dopamine D1 receptor antagonists. Eur J Pharmacol 1992;219:45–52.
84. Halldin C, Foged C, Chou YH, et al. Carbon-11-NNC 112: a radioligand for PET examination of striatal and neocortical D1-dopamine receptors. J Nucl Med 1998;39:2061–8.
85. Abi-Dargham A, Simpson N, Kegeles L, et al. PET studies of binding competition between endogenous dopamine and the D1 radiotracer [11C]NNC 756. Synapse 1999;32: 93–109.
86. Ekelund J, Slifstein M, Narendran R, et al. In Vivo DA D(1) Receptor Selectivity of NNC 112 and SCH 23390. Mol Imaging Biol 2007;9:117–25.
87. Slifstein M, Kegeles LS, Gonzales R, et al. [(11)C]NNC 112 selectivity for dopamine D(1) and serotonin 5-HT(2A) receptors: a PET study in healthy human subjects. J Cereb Blood Flow Metab 2007;27:1733–1741.
88. Karlsson P, Farde L, Halldin C, Sedvall G. PET study of D(1) dopamine receptor binding in neuroleptic-naive patients with schizophrenia. Am J Psychiatry 2002;159:761–7.
89. Guo N, Hwang DR, Lo ES, Huang YY, Laruelle M, Abi-Dargham A. Dopamine depletion and in vivo binding of PET D1 receptor radioligands: implications for imaging studies in schizophrenia. Neuropsychopharmacology 2003;28:1703–11.
90. Kapur S, Zipursky RB, Remington G. Clinical and theoretical implications of 5-HT2 and D2 receptor occupancy of clozapine, risperidone, and olanzapine in schizophrenia. Am J Psychiatry 1999;156:286–93.
91. Nyberg S, Nilsson U, Okubo Y, Halldin C, Farde L. Implications of brain imaging for the management of schizophrenia. Int Clin Psychopharmacol 1998;13(Suppl 3):S15–20.
92. Farde L, Nordström AL, Wiesel FA, Pauli S, Halldin C, Sedvall G. Positron emission tomography analysis of central D1 and D2 dopamine receptor occupancy in patients treated with classical neuroleptics and clozapine. Arch Gen Psychiatry 1992;49:538–44.
93. Kegeles LS, Slifstein M, Frankle WG, et al. Dose-Occupancy Study of Striatal and Extrastriatal Dopamine D(2) Receptors by Aripiprazole in Schizophrenia with PET and [(18)F]Fallypride. Neuropsychopharmacology 2008;33:3111–3125.
94. Wolkin A, Barouche F, Wolf AP, et al. Dopamine blockade and clinical response: evidence for two biological subgroups of schizophrenia. Am J Psychiatry 1989;146:905–8.
95. Pilowsky LS, Costa DC, Ell PJ, Murray RM, Verhoeff NPLG, Kerwin RW. Clozapine, single photon emission tomography, and the D2 dopamine receptor blockade hypothesis of schizophrenia. Lancet 1992;340:199–202.
96. Agid O, Mamo D, Ginovart N, et al. Striatal vs Extrastriatal dopamine D(2) receptors in antipsychotic response – a double-blind PET study in schizophrenia. Neuropsychopharmacology 2007;32:1209–15.

97. Nordstrom AL, Farde L, Wiesel FA, et al. Central D2-dopamine receptor occupancy in relation to antipsychotic drug effects: a double-blind PET study of schizophrenic patients. Biol Psychiatry 1993;33:227–35.
98. Kapur S, Zipursky R, Jones C, Remington G, Houle S. Relationship between dopamine D(2) occupancy, clinical response, and side effects: a double-blind PET study of first-episode schizophrenia. Am J Psychiatry 2000;157:514–20.
99. Nordstrom AL, Farde L, Nyberg S, Karlsson P, Halldin C, Sedvall G. D1, D2, and 5-HT2 receptor occupancy in relation to clozapine serum concentration: a PET study of schizophrenic patients. Am J Psychiatry 1995;152:1444–9.
100. Nyberg S, Farde L, Eriksson L, Halldin C, Eriksson B. 5-HT2 and D2 dopamine receptor occupancy in the living human brain. A PET study with risperidone. Psychopharmacology 1993;110:265–672.
101. Kapur S, Remington G, Zipursky RB, Wilson AA, Houle S. The D2 dopamine receptor occupancy of risperidone and its relationship to extrapyramidal symptoms: a PET study. Life Sci 1995;57:L103–7.
102. Knable MB, Heinz A, Raedler T, Weinberger DR. Extrapyramidal side effects with risperidone and haloperidol at comparable D2 receptor occupancy levels. Psychiatr Res Neuroimag 1997;75:91–101.
103. Kapur S, Zipursky RB, Remington G, et al. 5-HT2 and D2 receptor occupancy of olanzapine in schizophrenia: a PET investigation. Am J Psychiatry 1998;155:921–8.
104. Gefvert O, Bergstrom M, Langstrom B, Lundberg T, Lindstrom L, Yates R. Time course of central nervous dopamine-D2 and 5-HT2 receptor blockade and plasma drug concentrations after discontinuation of quetiapine (Seroquel) in patients with schizophrenia. Psychopharmacology (Berl) 1998;135:119–26.
105. Kapur S, Zipursky R, Jones C, Shammi CS, Remington G, Seeman P. A positron emission tomography study of quetiapine in schizophrenia: a preliminary finding of an antipsychotic effect with only transiently high dopamine D2 receptor occupancy. Arch Gen Psychiatry 2000;57:553–9.
106. Pilowsky LS, Mulligan RS, Acton PD, Ell PJ, Costa DC, Kerwin RW. Limbic selectivity of clozapine. Lancet 1997;350:490–1.
107. Farde L, Suhara T, Nyberg S, et al. A PET study of [C-11]FLB 457 binding to extrastriatal D-2-dopamine receptors in healthy subjects and antipsychotic drug-treated patients. Psychopharmacology 1997;133:396–404.
108. Bigliani V, Mulligan RS, Acton PD, et al. In vivo occupancy of striatal and temporal cortical D2/D3 dopamine receptors by typical antipsychotic drugs. [123I]epidepride single photon emission tomography (SPET) study. Br J Psychiatry 1999;175:231–8.
109. Olsson H, Farde L. Potentials and pitfalls using high affinity radioligands in pet and spet determinations on regional drug induced d2 receptor occupancy–a simulation study based on experimental data. Neuroimage 2001;14:936–45.
110. Abi-Dargham A, Hwang DR, Huang Y, et al. Reliable quantification of both striatal and extrastriatal D2 receptors in humans with [18F]fallypride. J Nucl Med 2000;41: 139p.
111. Kessler RM, Ansari MS, Riccardi P, et al. Occupancy of striatal and extrastriatal dopamine D2 receptors by clozapine and quetiapine. Neuropsychopharmacology 2006;31:1991–2001.
112. Grunder G, Landvogt C, Vernaleken I, et al. The striatal and extrastriatal D2/D3 receptor-binding profile of clozapine in patients with schizophrenia. Neuropsychopharmacology 2006;31:1027–35.
113. Kessler RM, Ansari MS, Riccardi P, et al. Occupancy of striatal and extrastriatal dopamine D2/D3 receptors by olanzapine and haloperidol. Neuropsychopharmacology 2005;30: 2283–9.
114. Xiberas X, Martinot JL, Mallet L, et al. Extrastriatal and striatal D(2) dopamine receptor blockade with haloperidol or new antipsychotic drugs in patients with schizophrenia. Br J Psychiatry 2001;179:503–8.

115. Talvik M, Nordstrom AL, Nyberg S, Olsson H, Halldin C, Farde L. No support for regional selectivity in clozapine-treated patients: a PET study with [(11)C]raclopride and [(11)C]FLB 457. Am J Psychiatry 2001;158:926–30.
116. Kapur S, Mann JJ. Role of the dopaminergic system in depression. Biol Psychiatry 1992;32:1–17.
117. Brown AS, Gershon S. Dopamine and depression. J Neural Transm Gen Sect 1993;91: 75–109.
118. Diehl DJ, Gershon S. The role of dopamine in mood disorders. Compr Psychiatry 1992;33:115–20.
119. Willner P, Muscat R, Papp M. Chronic mild stress-induced anhedonia: a realistic animal model of depression. Neurosci Biobehav Rev 1992;16:525–34.
120. D'Haenen HA, Bossuyt A. Dopamine D2 receptors in depression measured with single photon emission computed tomography. Biol Psychiatry 1994;35:128–32.
121. Shah PJ, Ogilvie AD, Goodwin GM, Ebmeier KP. Clinical and psychometric correlates of dopamine D2 binding in depression. Psychol Med 1997;27:1247–56.
122. Ebert D, Feistel H, Loew T, Pirner A. Dopamine and depression – striatal dopamine D2 receptor SPECT before and after antidepressant therapy. Psychopharmacology (Berl) 1996;126:91–4.
123. Klimke A, Larisch R, Janz A, Vosberg H, Muller-Gartner HW, Gaebel W. Dopamine D2 receptor binding before and after treatment of major depression measured by [123I]IBZM SPECT. Psychiatry Res 1999;90:91–101.
124. Parsey RV, Oquendo MA, Zea-Ponce Y, et al. Dopamine D(2) receptor availability and amphetamine-induced dopamine release in unipolar depression. Biol Psychiatry 2001;50:313–22.
125. Meyer JH, McNeely HE, Sagrati S, et al. Elevated putamen D(2) receptor binding potential in major depression with motor retardation: an [11C]raclopride positron emission tomography study. Am J Psychiatry 2006;163:1594–602.
126. Malison RT, Price LH, Berman R, et al. Reduced brain serotonin transporter availability in major depression as measured by [123I]-2 beta-carbomethoxy-3 beta-(4-iodophenyl)tropane and single photon emission computed tomography. Biol Psychiatry 1998;44: 1090–8.
127. Laasonen-Balk T, Kuikka J, Viinamaki H, Husso-Saastamoinen M, Lehtonen J, Tiihonen J. Striatal dopamine transporter density in major depression. Psychopharmacology (Berl) 1999;144:282–5.
128. Meyer JH, Kruger S, Wilson AA, et al. Lower dopamine transporter binding potential in striatum during depression. Neuroreport 2001;12:4121–5.
129. Brunswick DJ, Amsterdam JD, Mozley PD, Newberg A. Greater availability of brain dopamine transporters in major depression shown by [99m Tc]TRODAT-1 SPECT imaging. Am J Psychiatry 2003;160:1836–41.
130. Argyelan M, Szabo Z, Kanyo B, et al. Dopamine transporter availability in medication free and in bupropion treated depression: a 99mTc-TRODAT-1 SPECT study. J Affect Disord 2005;89:115–23.
131. Martinot M, Bragulat V, Artiges E, et al. Decreased presynaptic dopamine function in the left caudate of depressed patients with affective flattening and psychomotor retardation. Am J Psychiatry 2001;158:314–6.
132. Suhara T, Nakayama K, Inoue O, et al. D1 dopamine receptor binding in mood disorders measured by positron emission tomography. Psychopharmacology 1992;106:14–8.
133. Wong WF, Pearlson GD, Tune LE, et al. Quantification of neuroreceptors in the living human brain: IV. Effect of aging and elevations of D2-like receptors in schizophrenia and bipolar illness. J Cerebral Blood Flow Metab 1997;17:331–42.
134. Gjedde A, Wong DF. Quantification of neuroreceptors in living human brain. V. Endogenous neurotransmitter inhibition of haloperidol binding in psychosis. J Cerebral Blood Flow Metab 2001;21:982–94.

135. Anand A, Verhoeff P, Seneca N, et al. Brain SPECT imaging of amphetamine-induced dopamine release in euthymic bipolar disorder patients. Am J Psychiatry 2000;157: 1108–14.
136. Dewar KM, Stravynski A. The quest for biological correlates of social phobia: An interim assessment. Acta Psychiatrica Scandinavica 2001;103:244–51.
137. Bell CJ, Malizia AL, Nutt DJ. The neurobiology of social phobia. [Review]. Eur Arch Psychiatry Clin Neurosci 1999;249(Suppl 1):S11–S8.
138. Nutt DJ, Bell CJ, Malizia AL. Brain mechanisms of social anxiety disorder. [Review]. J Clin Psychiatry 1998;59(Suppl 17):4–11.
139. Tiihonen J, Kuikka J, Bergstrom K, Lepola U, Koponen H, Leinonen E. Dopamine reuptake site densities in patients with social phobia. Am J Psychiatry 1997;154:239–42.
140. Schneier FR, Liebowitz MR, Abi-Dargham A, Zea-Ponce Y, Lin SH, Laruelle M. Low dopamine D(2) receptor binding potential in social phobia. Am J Psychiatry 2000;157: 457–9.
141. Schneier FR, Abi-Dargham A, Martinez D, et al. Dopamine transporters, D(2) receptors, and dopamine release in generalized social anxiety disorder. Depress Anxiety 2009:26(5): 411–8.
142. Farde L, Gustavsson JP, Jonsson E. D2 dopamine receptors and personality traits. Nature 1997;385:590.
143. Breier A, Kestler L, Adler C, et al. Dopamine D2 receptor density and personal detachment in healthy subjects. Am J Psychiatry 1998;155:1440–2.
144. Kestler LP, Malhotra AK, Finch C, Adler C, Breier A. The relation between dopamine D2 receptor density and personality: preliminary evidence from the NEO personality inventory-revised. Neuropsychiatry Neuropsychol Behav Neurol 2000;13:48–52.
145. Laakso A, Vilkman H, Kajander J, et al. Prediction of detached personality in healthy subjects by low dopamine transporter binding. Am J Psychiatry 2000;157:290–2.
146. Schneier FR, Liebowitz MR, Laruelle M. Detachment and Generalized Social Phobia. Am J Psychiatry 2001;158:327.
147. Abi-Dargham A, Kegeles L, Zea-Ponce Y, et al. Striatal amphetamine-induced dopamine release in patients with schizotypal personality disorders studied by SPECT and [123I]IBZM. Biol Psych 2004;55(10):1001–1006.
148. Dougherty DD, Bonab AA, Spencer TJ, Rauch SL, Madras BK, Fischman AJ. Dopamine transporter density in patients with attention deficit hyperactivity disorder. Lancet 1999;354:2132–3.
149. Dresel S, Krause J, Krause KH, et al. Attention deficit hyperactivity disorder: binding of [99mTc]TRODAT-1 to the dopamine transporter before and after methylphenidate treatment. Eur J Nucl Med 2000;27:1518–24.
150. Larisch R, Sitte W, Antke C, et al. Striatal dopamine transporter density in drug naive patients with attention-deficit/hyperactivity disorder. Nucl Med Commun 2006;27:267–70.
151. Spencer TJ, Biederman J, Madras BK, et al. Further evidence of dopamine transporter dysregulation in ADHD: a controlled PET imaging study using altropane. Biol Psychiatry 2007;62(9):1059–1061.
152. Cheon KA, Ryu YH, Kim YK, Namkoong K, Kim CH, Lee JD. Dopamine transporter density in the basal ganglia assessed with [123I]IPT SPET in children with attention deficit hyperactivity disorder. Eur J Nucl Med Mol Imaging 2003;30:306–11.
153. Jucaite A, Fernell E, Halldin C, Forssberg H, Farde L. Reduced midbrain dopamine transporter binding in male adolescents with attention-deficit/hyperactivity disorder: association between striatal dopamine markers and motor hyperactivity. Biol Psychiatry 2005;57: 229–38.
154. van Dyck CH, Quinlan DM, Cretella LM, et al. Unaltered dopamine transporter availability in adult attention deficit hyperactivity disorder. Am J Psychiatry 2002;159:309–12.
155. Volkow ND, Wang GJ, Newcorn J, et al. Brain dopamine transporter levels in treatment and drug naive adults with ADHD. Neuroimage 2007;34:1182–90.

156. Volkow ND, Wang GJ, Fowler JS, et al. Therapeutic Doses of Oral Methylphenidate Significantly Increase Extracellular Dopamine in the Human Brain. J Neurosci 2001;21:RC121.
157. Wise R, Romprè P. Brain dopamine and reward. Ann Rev Psychol 1989;40:191–225.
158. Kuhar MJ, Ritz MC, Boja JW. The dopamine hypothesis of the reinforcing properties of cocaine. Trends Neurosci 1991;14:299–302.
159. Di Chiara G. The role of dopamine in drug abuse viewed from the perspective of its role in motivation. Drug Alcohol Depend 1995;38:95–137.
160. Nestler EJ, Berhow MT, Brodkin ES. Molecular mechanisms of drug addiction: adaptations in signal transduction pathways. Mol Psychiatry 1996;1:190–9.
161. Self DW, Nestler EJ. Molecular mechanisms of drug reinforcement and addiction. Annu Rev Neurosci 1995;18:463–95.
162. Koob GF. Drugs of abuse: anatomy, pharmacology and function of reward pathways. Trends Pharmacol Sci 1992;13:177–84.
163. Volkow ND, Fowler JS, Wolf AP, et al. Effects of chronic cocaine abuse on postsynaptic dopamine receptors. Am J Psychiatry 1990;147:719–24.
164. Volkow ND, Fowler JS, Wang GJ, et al. Decreased dopamine D2 receptor availability is associated with reduced frontal metabolism in cocaine abusers. Synapse 1993;14:169–77.
165. Volkow ND, Wang GJ, Fowler JS, et al. Cocaine uptake is decreased in the brain of detoxified cocaine abusers. Neuropsychopharmacology 1996;14:159–68.
166. Volkow ND, Wang GJ, Fowler JS, et al. Decreased striatal dopaminergic responsiveness in detoxified cocaine-dependent subjects. Nature 1997;386:830–3.
167. Martinez D, Broft A, Foltin RW, et al. Cocaine dependence and D2 receptor availability in the functional subdivisions of the striatum: relationship with cocaine-seeking behavior. Neuropsychopharmacology 2004;29:1190–202.
168. Wang GJ, Volkow ND, Fowler JS, et al. Dopamine D2 receptor availability in opiate-dependent subjects before and after naloxone-precipitated withdrawal. Neuropsychopharmacology 1997;16:174–82.
169. Volkow ND, Chang L, Wang GJ, et al. Low level of brain dopamine D2 receptors in methamphetamine abusers: association with metabolism in the orbitofrontal cortex. Am J Psychiatry 2001;158:2015–21.
170. Hietala J, West C, Syvälahti E, et al. Striatal D2 dopamine receptor binding characteristics in vivo in patients with alcohol dependence. Psychopharmacology 1994;116:285–90.
171. Volkow ND, Wang JG, Fowler JS, et al. Decreases in dopamine receptors but not in dopamine transporters in alcoholics. J Nucl Med 1996;37:33p.
172. Martinez D, Gil R, Slifstein M, et al. Alcohol dependence is associated with blunted dopamine transmission in the ventral striatum. Biol Psychiatry 2005;58:779–86.
173. Volkow ND, Wang GJ, Fowler JS, et al. Prediction of reinforcing responses to psychostimulants in humans by brain dopamine D2 receptor levels. Am J Psychiatry 1999;156: 1440–3.
174. Volkow ND, Wang GJ, Fowler JS, et al. Brain DA D2 receptors predict reinforcing effects of stimulants in humans: replication study. Synapse 2002;46:79–82.
175. Morgan D, Grant KA, Gage HD, et al. Social dominance in monkeys: dopamine D2 receptors and cocaine self-administration. Nat Neurosci 2002;5:169–74.
176. Laruelle M, Abi-Dargham A, van Dyck CH, et al. SPECT imaging of striatal dopamine release after amphetamine challenge. J Nucl Med 1995;36:1182–90.
177. Martinez D, Slifstein M, Broft A, et al. Imaging human mesolimbic dopamine transmission with positron emission tomography. Part II: amphetamine-induced dopamine release in the functional subdivisions of the striatum. J Cereb Blood Flow Metab 2003;23: 285–300.
178. Abi-Dargham A, Kegeles LS, Martinez D, Innis RB, Laruelle M. Dopamine mediation of positive reinforcing effects of amphetamine in stimulant naive healthy volunteers: results from a large cohort. Eur Neuropsychopharmacol 2003;13:459–68.

179. Volkow ND, Wang GJ, Fowler JS, et al. Reinforcing effects of psychostimulants in humans are associated with increases in brain dopamine and occupancy of D(2) receptors. J Pharmacol Exp Ther 1999;291:409–15.
180. Drevets WC, Gautier C, Price JC, et al. Amphetamine-induced dopamine release in human ventral striatum correlates with euphoria. Biol Psychiatry 2001;49:81–96.
181. Martinez D, Mawlawi O, Simpson N, et al. Comparison of amphetamine-induced endogenous dopamine release in striatal substructures in humans using PET. Soc Neurosc Abst 2000;26:1327.
182. Oswald LM, Wong DF, McCaul M, et al. Relationships among ventral striatal dopamine release, cortisol secretion, and subjective responses to amphetamine. Neuropsychopharmacology 2005;30:821–32.
183. Di Chiara G, Imperato A. Drugs abused by humans preferentially increase synaptic dopamine concentrations in the mesolimbic system of freely moving rats. Proc Natl Acad Sci USA 1988;85:5274–8.
184. Le Moal M, Simon H. Mesocorticolimbic dopamine network: functional and regulatory role. Physiol Rev 1991;71:155–324.
185. Koepp MJ, Gunn RN, Lawrence AD, et al. Evidence for striatal dopamine release during a video game. Nature 1998;393:266–8.
186. Malison RT, Mechanic KY, Klummp H, et al. Reduced amphetamine-stimulated dopamine release in cocaine addicts as measured by [123I]IBZM SPECT. J Nucl Med 1999;40: 110p.
187. Wu JC, Bell K, Najafi A, et al. Decreasing striatal 6-FDOPA uptake with increasing duration of cocaine withdrawal. Neuropsychopharmacology 1997;17:402–9.
188. Volkow ND, Wang GJ, Fowler JS, et al. Cocaine uptake is decreased in the brain of detoxified cocaine abusers. Neuropsychopharmacology 1996;14:159–68.
189. Malison RT, Best SE, van Dyck CH, et al. Elevated striatal dopamine transporters during acute cocaine abstinence as measured by [123I] beta-CIT SPECT. Am J Psychiatry 1998;155:832–4.
190. Volkow ND, Wang GJ, Fischman MW, et al. Relationship between subjective effects of cocaine and dopamine transporter occupancy. Nature 1997;386:827–30.
191. Malison RT, McCance E, Carpenter LL, et al. [123I]beta-CIT SPECT imaging of dopamine transporter availability after mazindol administration in human cocaine addicts. Psychopharmacology (Berl) 1998;137:321–5.
192. Volkow N, Wang G, Fischman M, et al. Relationship between subjective effects of cocaine and dopamine transporter occupancy. Nature 1997;386:827–30.
193. Volkow ND, Ding YS, Fowler JS, et al. Is methylphenidate like cocaine? Studies on their pharmacokinetics and distribution in the human brain. Arch Gen Psychiatry 1995;52: 456–63.
194. Volkow ND, Wang GJ, Fowler JS, et al. The slow and long-lasting blockade of dopamine transporters in human brain induced by the new antidepressant drug radafaxine predict poor reinforcing effects. Biol Psychiatry 2005;57:640–6.
195. Wong DF, Kuwabara H, Schretlen DJ, et al. Increased occupancy of dopamine receptors in human striatum during cue-elicited cocaine craving. Neuropsychopharmacology 2006;31:2716–27.
196. Volkow ND, Wang GJ, Telang F, et al. Cocaine cues and dopamine in dorsal striatum: mechanism of craving in cocaine addiction. J Neurosci 2006;26:6583–8.
197. McCann UD, Wong DF, Yokoi F, Villemagne V, Dannals RF, Ricaurte GA. Reduced striatal dopamine transporter density in abstinent methamphetamine and methcathinone users: evidence from positron emission tomography studies with [11C]WIN-35,428. J Neurosci 1998;18:8417–22.
198. Volkow ND, Wang G, Fowler JS, et al. Therapeutic doses of oral methylphenidate significantly increase extracellular dopamine in the human brain. J Neurosci 2001;21: RC121.

199. Wilson JM, Kalasinsky KS, Levey AI, et al. Striatal dopamine nerve terminal markers in human, chronic methamphetamine users. Nat Med 1996;2:699–703.
200. Wilson JM, Levey AI, Rajput A, et al. Differential changes in neurochemical markers of striatal dopamine nerve terminals in idiopathic Parkinson's disease. Neurology 1996;47:718–26.
201. Seibyl JP, Marek K, Sheff K, et al. Test/retest reproducibility of iodine-123-betaCIT SPECT brain measurement of dopamine transporters in Parkinson's patients. J Nucl Med 1997;38:1453–9.
202. Guttman M, Burkholder J, Kish SJ, et al. [11C]RTI-32 PET studies of the dopamine transporter in early dopa-naive Parkinson's disease: implications for the symptomatic threshold. Neurology 1997;48:1578–83.
203. Villemagne V, Yuan J, Wong DF, et al. Brain dopamine neurotoxicity in baboons treated with doses of methamphetamine comparable to those recreationally abused by humans: evidence from [11C]WIN-35,428 positron emission tomography studies and direct in vitro determinations. J Neurosci 1998;18:419–27.
204. Melega WP, Lacan G, Harvey DC, Huang SC, Phelps ME. Dizocilpine and reduced body temperature do not prevent methamphetamine- induced neurotoxicity in the vervet monkey: [11C]WIN 35,428 – positron emission tomography studies. Neurosci Lett 1998;258: 17–20.
205. Harvey DC, Lacan G, Tanious SP, Melega WP. Recovery from methamphetamine induced long-term nigrostriatal dopaminergic deficits without substantia nigra cell loss. Brain Res 2000;871:259–70.
206. Brody AL, Mandelkern MA, London ED, et al. Cigarette smoking saturates brain alpha 4 beta 2 nicotinic acetylcholine receptors. Arch Gen Psychiatry 2006;63:907–15.
207. Kassiou M, Eberl S, Meikle SR, et al. In vivo imaging of nicotinic receptor upregulation following chronic (-)-nicotine treatment in baboon using SPECT. Nucl Med Biol 2001;28:165–75.
208. Marenco S, Carson RE, Berman KF, Herscovitch P, Weinberger DR. Nicotine-induced dopamine release in primates measured with [11C]raclopride PET. Neuropsychopharmacology 2004;29:259–68.
209. Barrett SP, Boileau I, Okker J, Pihl RO, Dagher A. The hedonic response to cigarette smoking is proportional to dopamine release in the human striatum as measured by positron emission tomography and [11C]raclopride. Synapse 2004;54:65–71.
210. Brody AL, Olmstead RE, London ED, et al. Smoking-induced ventral striatum dopamine release. Am J Psychiatry 2004;161:1211–8.
211. Brody AL, Mandelkern MA, Olmstead RE, et al. Gene variants of brain dopamine pathways and smoking-induced dopamine release in the ventral caudate/nucleus accumbens. Arch Gen Psychiatry 2006;63:808–16.
212. Montgomery AJ, Lingford-Hughes AR, Egerton A, Nutt DJ, Grasby PM. The effect of nicotine on striatal dopamine release in man: a [(11)C]raclopride PET study. Synapse 2007;61:637–45.
213. Volkow ND, Wang GJ, Fowler JS, et al. Reinforcing effects of psychostimulants in humans are associated with increases in brain dopamine and occupancy of D(2) receptors. J Pharmacol Exp Ther 1999;291:409–15.
214. Fowler JS, Volkow ND, Wang GJ, et al. Inhibition of monoamine oxidase B in the brains of smokers. Nature 1996;379:733–6.
215. Fowler JS, Volkow ND, Wang GJ, et al. Brain monoamine oxidase A inhibition in cigarette smokers. Proc Natl Acad Sci USA 1996;93:14065–9.
216. Fowler JS, Volkow ND, Wang GJ, et al. Neuropharmacological actions of cigarette smoke: brain monoamine oxidase B (MAO B) inhibition. J Addict Dis 1998;17:23–34.
217. Fowler JS, Wang GJ, Volkow ND, et al. Maintenance of brain monoamine oxidase B inhibition in smokers after overnight cigarette abstinence. Am J Psychiatry 2000;157: 1864–6.

218. Logan J, Fowler JS, Volkow ND, Wang GJ, MacGregor RR, Shea C. Reproducibility of repeated measures of deuterium substituted [11C]L- deprenyl ([11C]L-deprenyl-D2) binding in the human brain. Nucl Med Biol 2000;27:43–9.
219. Salokangas RK, Vilkman H, Ilonen T, et al. High levels of dopamine activity in the basal ganglia of cigarette smokers. Am J Psychiatry 2000;157:632–4.
220. Dagher A, Bleicher C, Aston JA, Gunn RN, Clarke PB, Cumming P. Reduced dopamine D1 receptor binding in the ventral striatum of cigarette smokers. Synapse 2001;42:48–53.
221. Staley JK, Krishnan-Sarin S, Zoghbi S, et al. Sex differences in [123I]beta-CIT SPECT measures of dopamine and serotonin transporter availability in healthy smokers and nonsmokers. Synapse 2001;41:275–84.
222. Yoder KK, Constantinescu CC, Kareken DA, et al. Heterogeneous effects of alcohol on dopamine release in the striatum: a PET study. Alcohol Clin Exp Res 2007;31:965–73.
223. Volkow ND, Wang GJ, Fowler JS, et al. Decreases in dopamine receptors but not in dopamine transporters in alcoholics. Alcohol Clin Exp Res 1996;20:1594–8.
224. Heinz A, Siessmeier T, Wrase J, et al. Correlation between dopamine D(2) receptors in the ventral striatum and central processing of alcohol cues and craving. Am J Psychiatry 2004;161:1783–9.
225. Volkow ND, Wang GJ, Maynard L, et al. Effects of alcohol detoxification on dopamine D2 receptors in alcoholics: a preliminary study. Psychiatry Res 2002;116:163–72.
226. Tabakoff B, Hoffman P. Development of functional dependence on ethanol in dopaminergic systems. J Pharmacol Exp Ther 1979;208:216–22.
227. Muller P, Britton R, Seman P. The effects of long term ethanol on brain receptors for dopamine, acetylcholine, serotonin and noradrenaline. Eur J Pharmacol 1980;65:31–7.
228. Rabin RA, Wolfe BB, Dibner MD, Zahniser NR, Melchior C, Molinoff PB. Effects of ethanol administration and withdrawal on neurotransmitter receptor systems in C57 mice. J Pharmacol Exp Ther 1983;213:491–6.
229. Fuchs V, Coper H, Rommelspacher H. The effects of ethanol and haloperidol on dopamine receptors (D2) density. Neuropharmacology 1987;26:1231–3.
230. Hietala J, Salonen I, Lappalainen J, Syvälahti E. Ethanol administration does not alter dopamine D1 and D2 receptor characteristic in rat brain. Neurosci Lett 1990;108:289–94.
231. Lai H, Carino MA, Hrita A. Effects of ethanol on central dopamine function. Life Sci 1980;27:299–304.
232. Hruska RE. Effects of ethanol administration on striatal D1 and D2 receptors. J Neurochem 1988;50:1929–33.
233. Lucchi L, Moresco RM, Govoni S, Trabucchi M. Effect of chronic ethanol treatment on dopamine receptor subtypes in rat striatum. Brain Res 1988;449:347–51.
234. Hamdi A, Prasad C. Bidirectional changes in striatal D2-dopamine receptor density during chronic ethanol intake. Alcohol 1993;93:203–6.
235. Heinz A, Siessmeier T, Wrase J, et al. Correlation of alcohol craving with striatal dopamine synthesis capacity and D2/3 receptor availability: a combined [18F]DOPA and [18F]DMFP PET study in detoxified alcoholic patients. Am J Psychiatry 2005;162:1515–20.
236. Tiihonen J, Kuikka J, Bergström K, et al. Altered striatal dopamine re-uptake site densities in habitually violent and non-violent alcoholics. Nat Med 1995;1:654–7.
237. Laine TP, Ahonen A, Rasanen P, Tiihonen J. Dopamine transporter availability and depressive symptoms during alcohol withdrawal [In Process Citation]. Psychiatry Res 1999;90:153–7.
238. Heinz A, Knable MB, Wolf SS, et al. Tourette's syndrome: [I-123]beta-CIT SPECT correlates of vocal tic severity. Neurology 1998;51:1069–74.
239. Laine TP, Ahonen A, Torniainen P, et al. Dopamine transporters increase in human brain after alcohol withdrawal. Mol Psychiatry 1999;4:189–91, 104–5.
240. Munro CA, McCaul ME, Oswald LM, et al. Striatal dopamine release and family history of alcoholism. Alcohol Clin Exp Res 2006;30:1143–51.
241. Wagner HW, Jr. Highlights 2001 lecture. J Nuc Med 2001;42:12 N–30 N.

242. Slifstein M. Revisiting an old issue: the discrepancy between tissue ratio-derived binding parameters and kinetic modeling-derived parameters after a bolus of the serotonin transporter radioligand 123I-ADAM. J Nucl Med 2008;49:176–8.
243. Laruelle M. The role of model-based methods in the development of single scan techniques. Nucl Med Biol 2000;27:637–42.

Chapter 16
Dopamine Receptors and the Treatment of Schizophrenia

Nathalie Ginovart and Shitij Kapur

Abstract Schizophrenia is a most disabling psychiatric disorder characterized by a myriad of symptoms. While the delusions and hallucinations are the most iconic symptoms of schizophrenia, patients also exhibit negative and cognitive symptoms. It is thought that these symptoms arise, at least in part, through a cortical–subcortical imbalance of dopamine function and pharmacological approaches that reduce dopaminergic neurotransmission through dopamine receptor blockade, and in particular through the D_2 receptor, have antipsychotic action in humans. However, D_2 antagonists are not optimally effective against the full spectrum of schizophrenia symptoms and induce side effects that limit their use. Research to enhance the therapeutic benefits of antipsychotics while diminishing their side effects has led to the development of atypical antipsychotics (D_2 antagonists with activity at other receptors) and, more recently, a new strategy using dopamine partial agonists to reduce dopaminergic neurotransmission has proven to be successful. This chapter reviews the pharmacological effects of typical and atypical antipsychotics on the different dopamine receptor subtypes, as well as on non-dopaminergic receptor targets, and on the prominent role of D_2 receptor blockade as the primary site of their action in brain. In addition, we discuss current theories on the mechanisms of antipsychotic action, including the role of combined action at the dopamine and serotonin receptors, transient dopamine D_2 blockade, preferential blockade of limbic D_2 receptors, or combined blockade of D_1 and D_2 receptors. Some critical clinical considerations with regard to the speed of onset action and the occurrence of relapse and supersensitivity psychosis on withdrawal are discussed with special relevance to their relationship to the dopamine system. While the D2 receptor-based treatments seem to have dominated the field till now, drugs that reduce dopamine-mediated transmission through action at presynaptic sites and of drugs providing D_1 signaling augmentation in prefrontal cortex may provide novel therapeutic avenues for the treatment of schizophrenia.

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16.1 Schizophrenia

Schizophrenia is a chronic disabling disease that afflicts 0.5–0.8% of the world's population [1]. Symptoms usually begin in late adolescence or early adulthood and are typically classified as positive (hallucinations and delusions), negative (amotivation, flattened affect, and social withdrawal), and cognitive (disorganized thoughts, deficits in attention, working and verbal memory, social cognition, and executive function) [2, 3].

The course of schizophrenia is characterized by periods of symptom exacerbation (i.e., relapse) alternating with periods of relative remission, with a different pattern of exacerbation/remission episodes between the three psychopathology dimensions and between different individuals [4, 5]. Such findings have led several researchers to theorize that positive, negative, and cognitive symptoms reflect separate pathophysiological processes [6, 7], though that still remains to be proven beyond doubt. Nonetheless, there is little doubt that schizophrenia is a disorder with strong biological underpinnings, some of it genetically determined (though the precise genes have not as yet been confirmed) and the rest influenced by the environment and interactions.

Not surprisingly then, there are several hypotheses that explain different aspects of the illness. The major thinking in the field regarding the development of the illness is that it is a neurodevelopmental disorder. In other words, the biological determinants of the disorder manifest themselves often years in advance of the cardinal symptoms. While neurodevelopmental deficits may lead to the disorder, there is little doubt that when the disease manifests itself, there is a significant neurochemical aberration. And it is this aspect of the illness, with special attention to the dopamine receptors, that is the focus of this chapter.

While the major focus of this chapter is the role of dopamine receptors vis-à-vis treatment, to provide the appropriate background for this we first review the dominant neurochemical hypotheses regarding schizophrenia. We then explain the classification of typical vs. atypical antipsychotics and then focus on the different dopamine receptors and their roles in antipsychotic action.

16.1.1 The Dopamine Hypothesis

The "classical" dopamine (DA) hypothesis of schizophrenia proposed that subcortical hyperactivity of DA transmission in brain is responsible for the positive symptoms of the illness [8]. This hypothesis was initially based on several lines of indirect evidence. First, exposure to DA-enhancing drugs, such as amphetamine, induces

psychosis in normal individuals and worsens psychotic symptoms in schizophrenia patients [9]. Second, drugs alleviating psychotic symptoms of schizophrenia were suspected to act through blockade of central DA receptors. Indeed, in 1963, Carlsson and Lindqvist [10] reported an increase in DA metabolites following chlorpromazine and haloperidol administration and proposed that this increase was the result of dopaminergic blockade, resulting in an increased rate of turnover. Further evidence for a central role of a DA dysregulation in schizophrenia came from research on the mechanism of antipsychotic action. Indeed, all drugs with a proven efficacy at relieving the positive symptoms of schizophrenia block DA D_2 receptors to some extent. Moreover, there is a tight correlation between the clinical doses of antipsychotic drugs needed to achieve therapeutic efficacy and their potency at blocking D_2 receptors [11]. This led to the dominant theory that the abnormal psychotic ideation observed in schizophrenia is largely due to an abnormally high DA transmission. Extensive research efforts have been made to determine whether the expression of DA receptors, and in particular D_2 receptors, is altered in schizophrenia. Early postmortem studies showed increased D_2 receptor levels in the striatum of patients with schizophrenia [12, 13]. However, the finding that antipsychotic drug treatment increased D_2 receptor density in experimental animals [14, 15] raised concerns that the D_2 elevation observed in schizophrenia could be related to prior drug treatment. In support of this view, some reports showed that D_2 receptors were only increased in patients treated with antipsychotic drugs up until death [16–18]. However, other postmortem studies in patients who were apparently drug free still showed elevated D_2 receptor density [19, 20]. The interpretation of D_2 elevation obtained postmortem thus remained controversial and imaging studies in never-medicated patients were critical to determine whether or not D_2 receptors were increased in schizophrenia. Initial in vivo brain imaging studies in drug-naïve patients yielded conflicting results, with some studies showing elevation [21, 22] and others no change in striatal D_2 receptors [23, 24]. Many factors have been proposed to account for this discrepancy, most of all being related to the chemical class and different pharmacokinetic properties of the radioligands employed between studies (review in [25]). Since these initial reports, there have been many other studies in this field and those generally failed to detect significant increase in striatal D_2 receptor in drug-naïve schizophrenic patients [26–31]. It has been proposed that while there may not be an absolute change in D_2 receptor number in schizophrenia, a shift toward a greater population in the D_2^{High} state (D_2 receptors with functional high affinity for DA) may explain hyperdopaminergia [32]. However, a recent clinical study to investigate levels of D_2^{High} found no difference between schizophrenic patients and controls [33]. In vivo studies of D_1 receptors in never-medicated patients showed unaltered receptor density in striatum [34, 35] but are conflicting with regard to their density in frontal cortex, with studies showing decreased [36], unaltered [34], or increased [35] D_1 receptor binding in this brain region. Thus far, in vivo study of D_3 receptors has been hampered by the lack of radioligands with sufficient selectivity for D_3 over D_2 receptors and by the partially overlapping distribution of these two receptor subtypes in brain. The only study performed in the field was postmortem and reported elevated striatal density of D_3 receptor in drug-free

patients with schizophrenia [37], a finding that remains to be confirmed in other studies. As for the D_3, in vivo measures of the D_4 receptor have not been possible yet due to the lack of specific radioligand for this receptor subtype. Using an indirect subtraction binding method, elevated striatal density of D_4 receptors has been reported in schizophrenic patients postmortem [38–40], but this finding was not replicated in other studies [41–44]. Thus despite extensive efforts over the past 40 years, no convincing evidence has emerged yet that unequivocally points to a DA receptor abnormality in schizophrenia. In contrast, converging lines of evidence point to an elevated presynaptic DA function in schizophrenia. An accumulating body of in vivo imaging studies indicates that schizophrenia is associated with an increased subcortical capacity of DA synthesis [45–47], an increased DA turnover [48], and increased subcortical levels of endogenous DA at baseline [49] together with an increased subcortical release of DA following an amphetamine challenge [50–53]. Moreover, it seems that some of these DA alterations may confer susceptibility to schizophrenia as increased striatal presynaptic DA synthesis capacity is correlated to the risk of developing schizophrenia and other psychoses [54]. All together, these data suggest that psychosis is related, at least in part, to an excessive subcortical DA presynaptic function rather than to abnormalities in the levels of postsynaptic DA receptors.

Since its first formulation, the DA hypothesis of schizophrenia has been revised to account for both the positive and the negative symptoms of the disease [55]. Indeed, while administration of amphetamine worsens the positive symptoms of the illness, it may partly improve negative symptoms [56]. The revised theory proposes that the positive and negative symptoms of schizophrenia arise from an imbalance between the brain DA pathways mediating D_2 and D_1 receptor signaling. A subcortical excess of DA, leading to hyperstimulation of D_2 receptors, would give rise to the positive symptoms, while a concomitant cortical deficit of DA, leading to hypostimulation of D_1 receptors, would give rise to the negative and cognitive symptoms. There is indeed robust evidence that DA hypofunction and altered D_1 receptor signaling within the prefrontal cortex (PFC) play a central role in the induction of working memory deficits, suggesting that a reduced D_1 receptor neurotransmission might cause cognitive impairments in schizophrenia (review in [57]). Interestingly, reduced prefrontal activity has been shown to predict exaggerated striatal DA function in schizophrenia [58]. Both hypofunctionning and hyperfunctionning DA systems thus likely coexist in schizophrenia, albeit in different brain regions.

16.1.2 The Glutamate Hypothesis

The second dominant pathophysiological hypothesis of schizophrenia postulates a hypofunctional glutamate system in this disorder and, more specifically, a decreased neurotransmission at the *N*-methyl-D-aspartate (NMDA) glutamate receptor. Glutamate is the major excitatory neurotransmitter in the brain. It acts on two families of receptors: the metabotropic receptors, which include the mGlu1

to mGlu8 subtypes, and the ionotropic receptors, which include the kainate, the AMPA, and the NMDA subtypes. Some mGlu receptors, in particular the $mGlu_{2/3}$ and $mGlu_5$ subtypes, interact closely with NMDA receptors and may directly modulate the function of the NMDA receptor channel [59]. The idea of a glutamatergic abnormality in schizophrenia was first proposed by Kim and colleagues in 1980 [60] based on their findings of low cerebrospinal fluid (CSF) glutamate levels in patients with schizophrenia. Moreover, subanesthetic doses of NMDA receptor antagonists, such as ketamine and phencyclidine (PCP), can produce a spectrum of responses in normal subjects that partially resemble the positive, negative, and cognitive symptoms of schizophrenia [61, 62]. Ketamine and PCP can also precipitate psychoses in schizophrenic patients [63, 64]. Finally, a number of postmortem studies indicate abnormalities in NMDA receptor expression in the temporal cortex, cingulate cortex, hippocampus, and thalamus in schizophrenia [65]. Recent in vivo imaging studies confirmed reduced NMDA receptor binding in hippocampus [66, 67] and abnormal concentrations of glutamate in hippocampus and PFC of schizophrenic patients [68–70]. These observations thus give support to the theory that a NMDA receptor hypofunction might be involved in the pathophysiology of schizophrenia [71, 72].

16.1.3 Integration of the Dopamine and Glutamate Hypotheses

There is substantial evidence of reciprocal interactions between the DA and the glutamatergic systems. The acute administration of NMDA antagonists such as PCP and ketamine has consistently been reported to increase the firing rate of midbrain DA neurons [73–76] and to selectively increase DA release in PFC and in nucleus accumbens [76–79]. Subchronic administration of NMDA antagonists also leads to a profound dysregulation of the mesocorticolimbic DA system. Specifically, mesocortical DA neurons which largely project to the PFC show a profound loss of burst firing with decreased DA release, whereas mesolimbic DA neurons, which mainly project to the ventral striatum, show an increased firing with increased DA release [80, 81]. One mechanism to account for the subcortical DA hyperfunction proposes that the corticostriatal glutamate pathway may indirectly (via γ-aminobutyric acid [GABA]) inhibit DA function in the ventral striatum [82]. Therefore, protracted NMDA receptor hypofunction can produce the cortical DA hypoactivity and limbic DA hyperactivity postulated in schizophrenia and associated with the negative and positive symptoms, respectively.

16.2 Classification of Antipsychotic Drugs

16.2.1 Typical Antipsychotics

Antipsychotic medications are the cornerstone treatments for reducing psychotic symptoms and relapse rates in schizophrenia. The first antipsychotic drug used for schizophrenia, chlorpromazine, was introduced in 1952 [83] and, in the late 1950s,

several other antipsychotics were subsequently introduced, including haloperidol, thioridazine, trifluoperazine, and loxapine. These first-generation antipsychotics, termed typical antipsychotics, are effective against the positive symptoms but have limited efficacy and may even exacerbate the negative and cognitive symptoms of schizophrenia. Moreover, some 30% of patients with schizophrenia show little or no response of their positive symptoms to typical antipsychotic therapy [84, 85]. Besides this, typical drugs are associated with a wide spectrum of side effects, including sedation, acute extrapyramidal symptoms (EPS), hyperprolactinemia and, although rarely, the neuromalignant syndrome. Acute EPS are dose-dependent and manifest as dystonia, akathisia, and pseudoparkinsonism [86]. The most worrisome form of EPS, tardive dyskinesia (TD), develops on long-term utilization with an incidence of about 5% a year and can be irreversible [3]. These side effects are often severe and disabling and are a leading cause of patient noncompliance [87, 88] which, in turn, leads to relapse [89]. Efforts to minimize EPS have revealed that lowering the dose indeed decreases side effects and still achieves therapeutic efficacy in many patients with schizophrenia [90, 91]. However, lower doses also carry additional risk of relapse as the doses required for efficacy are only slightly lower than the doses that cause side effects [92, 93]. Despite their clear benefits for the treatment of schizophrenia, the limited tolerability and narrow therapeutic index of typical antipsychotic drugs pointed out the need for better treatment options.

16.2.2 Atypical Antipsychotics

Research to enhance the therapeutic benefits of antipsychotics while diminishing their side effects has led to the development of a new class of antipsychotic agents, the atypical antipsychotics. Clozapine was introduced in Europe in 1975 and marked a turning point in schizophrenia therapy. Clozapine appeared to be effective with a minimal incidence of EPS, thus challenging the dogma that antipsychotic efficacy required high levels of EPS (review in [94]). Interestingly, this drug demonstrated benefits in cases of patients refractory to typical antipsychotics and was found to be somewhat more effective against the negative and cognitive symptoms [95]. However, its use is limited because of major side effects including potentially lethal agranulocytosis [96]. It was not until the late 1980s that a second atypical drug, amisulpride, was introduced. The following decade saw the introduction of a multiplicity of new drugs, including olanzapine, risperidone, sertindole, and quetiapine, with ziprasidone and aripiprazole following in the early 2000s.

Although atypical drugs improve positive symptoms with a similar efficacy [97–99], they differ from typical ones by their lower incidence of EPS and TD [100–102] and with the exception of risperidone and amisulpride have only transient effect on serum prolactin levels [103, 104]. These reduced side effects result in better tolerance and may enhance compliance [3, 97] and decrease relapse rates [89, 105, 106] when compared with high-dose typical drugs. Atypical drugs are, however, associated with other distressing non-EPS side effects which may limit their

use in clinical settings. Those include sedation, weight gain, and severe metabolic disturbances [107, 108].

Rather than their efficacy against the positive symptoms, it is thus their side effect profile and efficacy against the negative and cognitive symptoms that are generally considered to differentiate typical and atypical antipsychotics. Such a clear dichotomy between the two classes of drugs may not be as absolute as originally thought. For instance, while clozapine and quetiapine do not produce significant EPS, risperidone, olanzapine, and ziprasidone can produce them when used at higher therapeutic doses [109, 110]. Similarly, recent studies comparing atypical drugs to low doses of typical antipsychotics showed clinical benefits on negative and cognitive symptoms for typical drugs, although perhaps not as pronounced as that found after treatment with atypical drugs [111–114]. It has therefore been suggested that the superior motor side effect profile of atypicals is largely owing to excessive dosing of the typical drug used as comparator as well as to their wide therapeutic index [91, 115]. Clearly though, the newer atypical agents have emerged as a good long-term treatment option because of their wide therapeutic index and improved control of motor side effects.

16.3 Neuropharmacology of Antipsychotics

Antipsychotic drugs have a multitude of effects on various physiological variables through their actions on different neurotransmitter systems. Antipsychotics interact mainly at four neurotransmitter receptor systems in brain: the DA type 1 (D_1 and D_5) and type 2 receptor (D_2, D_3, and D_4) families, serotonin receptors ($5HT_{1A}$, $5HT_{2A}$, $5HT_{2c}$), muscarinic cholinergic receptors (m_1 and m_2), and α-adrenergic receptors (α_1 and α_2).

There have been a number of hypotheses on the mechanisms underlying antipsychotic atypicality. Most of the hypotheses postulate that the different side effect profile of typical and atypical drugs mainly results from differences in their receptor-binding profile. Indeed, while typical neuroleptics are usually preferential for D_2-like receptors, atypical neuroleptics usually bind to a larger spectrum of receptor types (Table 16.1). For instance clozapine exerts its action through D_2 receptors but also through D_1, serotonin, muscarinic, and adrenergic receptors and this multireceptor action has been proposed to be the main determinant for atypicality. However, if this concept of multireceptor action stands for clozapine, it does not for other atypical drugs such as amisulpride, which is a highly selective D_2-like blocker with no other receptor interaction. Moreover, chlorpromazine displays affinity for D_2 like but also for serotonin and adrenergic receptor subtypes and still is a typical agent with high EPS liability. D_2 receptor affinity seems to be the only common denominator among all the presently available antipsychotics. Action at this receptor thus appears the most relevant marker for understanding some key actions of these drugs in humans. As developed in the following paragraphs, the weight of the evidence indicates that DA receptor blockade is essential to clinical antipsychotic

Table 16.1 In vitro receptor-binding profile (K_I values, nM) of various typical and atypical antipsychotic drugs

	CPZ	HDL	CZ	AMS	RIS	OLZ	QUE	ZIP	ARI
D_1	112	83	189	>1,000 [a]	60.6	58	712	30	387
D_2	2	2	431	2.8 [a]	4.9	72	567	4.0	0.95
D_3	5	12	646	3.2 [a]	12.2	63	483	17	4.5
D_4*	24	15	39	>1,000 [a]	18.6	19	1,202	105	514
D_5	133	147	235	na	16	90	1,738	152	1,676
5-HT_{1A}	3,115	1,202	105	>10,000[a]	427	2,063	431	76	5.6
5-HT_{2A}*	3.2	73	13	2,000[a]	0.19	3	366	2.8	4.6
5-HT_{2C}*	26	>10,000	29	>10,000[a]	94.9	24	1,500	68	181
α_1*	2.6[c]	7.3[b]	3.7[b]	na	0.7[b]	7.3[b]	4.5[b]	1.9[b]	na
α_2*	na	1,600[b]	51[b]	1,600[a]	1.8[b]	140[b]	1,100[b]	390*[b]	na
m_1Ach	47	>10,000	14	na	>10,000	79	858	>10,000	6,776
m_2Ach	433	>10,000	14	na	>10,000	24	1,339	>10,000	3,507

Data represent mean K_I values obtained from the National Institute of Mental Health's Psychoactive Drug Screening Program (PSPD) K_I database except where indicated. PSPD K_I values are for cloned human or cloned rat (as indicated by *) receptors. PSPD-binding assays were performed using [^{3}H]SCH23390 for the D_1- and D_5-receptors, [^{3}H]NMSP for D_2-, D_3-, and D_4-receptors, [^{3}H]-8-OH-DPAT for the 5HT_{1A}-receptor, [^{3}H]ketanserin for the 5HT_{2A}-receptor, [^{3}H]Mesulergine for the 5HT_{2C}-receptor, and [^{3}H]QNB for the muscarinic acetylcholine m_1 and m_2 receptors.

AMS: amisulpride; ARI: aripiprazole; CPZ: chlorpromazine; CZ: clozapine; HDL: haloperidol; OLZ: olanzapine; QUE: quetiapine; RIS: risperidone; ZIP: ziprasidone;
na = not available

[a] adapted from [331].
[b] adapted from [378].
[c] adapted from [379].

activity, especially for controlling hallucinations and delusions, while interaction with serotonin receptors may rather be involved in alleviation of EPS.

16.4 Dopamine Receptors Involved in Antipsychotic Drug Action

DA exerts its pharmacological action through multiple membrane-spanning proteins of the G protein-coupled receptor (GPCR) superfamily. These may be divided into two subfamilies of receptor subtypes based on their structures, their linkage to adenylate cyclase, and their pharmacological properties: the D_1-like (D_1, D_5) and the D_2-like receptor subtypes (D_2, D_3, D_4).

D_1 receptors are positively coupled to adenylate cyclase. They are mainly located postsynaptically on the primary dopaminergic projections of the ventral tegmental area (VTA) and substantia nigra (SN) such as the nucleus accumbens (NAcc), cortex, and striatum. In addition, D_1 receptors have also been detected in amygdala, globus pallidus, and hippocampal formation [116]. The D_5 receptors are poorly

expressed in the human brain when compared to D_1 receptors and are mainly localized in the SN pars compacta, hypothalamus, striatum, cerebral cortex, NAcc, and olfactory tubercle [117]. D_2 receptors are located both presynaptically and postsynaptically. Their expression largely overlaps with D_1 receptor expression in DA-rich regions such as the striatum, NAcc, SN, and VTA [116]. D_3 receptors are expressed in high levels in limbic brain regions such as the NAcc, the ventral putamen, and the Islands of Calleja but also to some extent in the striatum, SN, and thalamus [118]. D_4 receptors are located mainly in cortical regions (temporal, entorhinal, cingulate) and hippocampus with low-density levels being found in the caudate/putamen [119].

16.4.1 Role of D_2 Receptor Blockade

Antipsychotic drugs have a multitude of effects on various physiological variables through their antagonistic actions on different neurotransmitter systems. The antipsychotic effects of these agents are believed to occur primarily through antagonism of D_2-type DA receptors. Historically, blockade of D_2 receptors was believed to be indispensable for the treatment of psychosis, although the efficacy of weak D_2 blockers such as clozapine called this theory into question. The major therapeutic as well as adverse effects of D_2 antagonism have been conceptualized in the context of the major DA tracts in brain, which include the mesocortical, mesolimbic (A10), nigrostriatal (A9), and tuberoinfundibular (A12) tracts. Although the effects of D_2 blockade on the mesocortical and mesolimbic systems are believed to represent the putative mechanism of action of antipsychotics, excessive blocking of these tracts is also believed to result in their adverse cognitive and behavioral side effects.

16.4.1.1 In Vitro Evidence for an Antipsychotic Action at the D_2 Receptors

A central role of DA receptor blockade in the mechanism of action of antipsychotics was first anticipated by Carlsson and Lindqvist [10] and later studies of the neuropharmacology of antipsychotics revealed their specifically high affinity for dopamine D_2 receptors and provided strong support for the dopamine hypothesis of schizophrenia [120]. The antipsychotic potency of a compound was found to be directly related to its D_2 receptor affinity. That is, a highly significant correlation exists between the dissociation constant of the D_2 receptor–antipsychotic complex and the average clinically effective dosage [121]. No such correlation exists between dosage and dissociation constant for the D_1, D_3, or D_4 receptors [122].

16.4.1.2 Preclinical Evidence for an Antipsychotic Action at the D_2 Receptors

A number of animal models have been used to explore antipsychotic action and to investigate the mechanisms underlying the differences between the actions of typical and atypical drugs. Some models are used to predict antipsychotic efficacy (i.e., inhibition of amphetamine-induced hyperactivity, disruption of conditioned avoidance responding, and induction of c-fos expression in the shell region of the NAcc), while

others are used to predict their motor side effect liability (i.e., induction of catalepsy, blockade of apomorphine-induced sniffing, and induction of c-fos expression in the dorsolateral striatum).

Antipsychotic Effect on Indirect DA Agonist-Induced Behavior

Reversal of amphetamine-induced hyperactivity in rodents is one classical animal model used to screen antipsychotic drugs. Indirect DA agonists such as amphetamine or methylphenidate induce a strong increase in locomotor activity when injected into rodents. There is evidence that this increased locomotor activity is due to an increased DAergic activity in the mesolimbic system, mainly within the NAcc [123]. For instance, rats with lesion of this structure do not show hyperactivity after amphetamine administration [124]. While lacking in face validity, blockade of amphetamine-induced hyperactivity is considered as one of the better preclinical predictors of antipsychotic activity. Accordingly, both typical and atypical antipsychotic drugs reduce the hyperactivity produced by amphetamine and this effect has been linked to D_2 receptor blockade [125]. Here again, the potency of antipsychotics to antagonize amphetamine-induced hyperlocomotion correlated with their affinity at D_2 receptors [126]. The similar action of typical and atypical antipsychotics in the amphetamine model is consistent with their similar blockade of D_2 receptors and their similar antipsychotic effect in schizophrenia patients.

Antipsychotic Effect on Direct DA Agonist-Induced Behaviors

In addition to characterize antipsychotic action on the effects of indirect DA agonists, another classical model consists of studying antipsychotic action on the behavioral effects of direct DA agonists. The most studied compound in this regard is the mixed D_1/D_2 receptor agonist, apomorphine, which binds directly to each DA receptor subtype. When administered to rodents, apomorphine induces climbing behavior (i.e., the mice climb on the wire lids of their cages for extended periods of time) as well as stereotyped sniffing. The climbing behavior produced by apomorphine is believed to be mediated by excessive DA activity in the mesolimbic pathway, while the stereotyped sniffing is believed to reflect excessive DA activity in the nigrostriatal pathway. Reversal of apomorphine-induced climbing is predictive of efficacy against the positive symptoms of psychosis [127], while reversal of sniffing is predictive of EPS liability ([128]; see also [129] for review). As with the amphetamine model, both typical and atypical antipsychotics such as clozapine, olanzapine, and risperidone are very effective in these models [130], largely to the extent that they block the DA D_2 receptors. Indeed, a correlation analysis based on affinities at rat striatal D_2 sites has shown that the ability of antipsychotics to inhibit apomorphine-induced climbing and sniffing correlated positively with their affinity at D_2 receptors [131]. Typical antipsychotics though tend to be equipotent at reducing both apomorphine-induced climbing and sniffing, whereas most atypical drugs block apomorphine-induced climbing at doses lower than those blocking

apomorphine-induced sniffing (see [129] for review). These models thus demonstrate in vivo antagonist activity of all antipsychotics at D_2 receptors and suggest that a lower D_2 action of atypical drugs in the nigrostriatal system may explain their lower propensity to cause EPS when compared to typical.

Antipsychotic Effect on Conditioned Avoidance Response

Conditioned avoidance response (CAR) is an important preclinical animal model in the study of antipsychotic drugs [132]. In this paradigm, rats are placed in a two-compartment shuttle box and are trained to avoid an aversive stimulus on presentation of a neutral stimulus that immediately precedes it. All antipsychotics produce a dose-dependent suppression of the avoidance response to the neutral stimulus while the incidence of escapes to the aversive stimulus itself is relatively unaffected, indicating that the effects of antipsychotic on CAR are not due to general sedative effects. In fact, suppression of CAR represents a behavioral effect that is shared by all effective antipsychotics, including the novel compound aripiprazole [133], and potencies in the CAR test have been found to be highly predictive of therapeutic efficacy in schizophrenia [134, 135]. Since the CAR does not help to distinguish between typical and atypical drugs, it suggests that both classes of compounds exert their antipsychotic action through a common mechanism. From a neurochemical perspective, a number of receptor systems have been implicated in the CAR model, including DA D_1, adrenergic, glutaminergic, muscarinic, and serotonergic receptors. However, it has been suggested that the ability of antipsychotics to selectively suppress CAR is due to their ability to block DA D_2 neurotransmission in the mesolimbic system [136]. Indeed, local application of the DA D_2 receptor antagonist (–)sulpiride into the shell portion of the NAcc, but not into the dorsolateral striatum, suppresses CAR [136]. Furthermore, a direct dose-dependent relationship has been established between the blockade of D_2 receptors and the disruption of CAR [126, 137, 138], indicating that D_2 receptor blockade is an important mechanism of antipsychotic action.

Antipsychotic Effect on Catalepsy

The catalepsy test is a common and widely used preclinical screening test for the propensity of an antipsychotic drug to induce EPS in humans [139]. Antipsychotics such as haloperidol and risperidone, which have a dose-dependent propensity to induce EPS in humans, dose-dependently induce catalepsy in animals. In contrast, atypical drugs such as clozapine and aripiprazole, which do not produce EPS in humans do not produce catalepsy in animals [133, 140]. The cataleptic behavior seems primarily to involve D_2 antagonism in the DA nigrostriatal pathway that mediate extrapyramidal motor function and a direct relationship has been established between the blockade of striatal D_2 receptors and catalepsy [137].

Both cataleptic potential and blocking of CAR in animals are often used in combination for predicting antipsychotic action in humans. By studying the dose effects of a given antipsychotic on catalepsy (which is related to striatal function) and CAR

(which is related to limbic function), it is possible to differentiate typical and atypical antipsychotic drugs. Indeed, whereas typical antipsychotics tend to be equipotent at inducing catalepsy and at blocking CAR, most atypical drugs are more potent at blocking CAR than at producing catalepsy [141]. Presumably drugs that are more active against limbic function than striatal function will have antipsychotic activity at doses that will not produce motor side effects [128].

Antipsychotic Effect on *c-fos* Expression

Immediate early genes, such as *c-fos*, have proven to be useful markers of changes in neuronal activity, and *c-fos* immunohistochemistry has been used to examine the effects of antipsychotic drugs in the brain [142–146]. These studies have shown that typical and atypical antipsychotic drugs differentially affect *c-fos* expression in various regions of the brain. Haloperidol induces *c-fos* expression in the NAcc, the lateral septum, and the dorsolateral striatum, whereas clozapine increases *c-fos* expression in the NAcc, the lateral septal nucleus, and the medial PFC. Although both typical and atypical antipsychotics increase c-Fos expression in the NAcc, they have different effects within the two subdivisions of the nucleus: the shell, which is allied with limbic circuits, and the core, which is allied with the extrapyramidal system. Typical drugs increase c-*fos* in both the shell and the core of the NAcc, whereas atypical drugs increase c-*fos* in the shell but not in the core. This regional specificity of antipsychotic-mediated *c-Fos* expression is thought to reflect different patterns of neuronal activation and consequently differences in the clinical profile between typical and atypical drugs. Since all antipsychotics act on the shell of the NAcc and improve positive symptoms, the shell of the NAcc is probably associated with improvement of positive symptoms. *C-fos* expression in the dorsolateral striatum is associated with motor side effects, and low or no c-*fos* production in this region is predictive of a low potential for producing EPS. The exact mechanism underlying antipsychotic drug-induced c-*fos* expression is unclear but a number of findings support the view that it is the direct result of D_2 blockade. Indeed, the administration of a D_2 receptor agonist prevents the induction of c-*fos* by haloperidol in the dorsal striatum [147] and indirect DA agonists such as cocaine and *d*-amphetamine decrease antipsychotic-induced striatal c-*fos* expression [148, 149].

16.4.1.3 Clinical Evidence for an Antipsychotic Action at the D_2 Receptors

The last decades of research have produced unquestionable evidence for the central role of DA neurotransmission in the pathogenesis of schizophrenia and the mechanism of action of antipsychotic drugs. All currently available antipsychotics are antagonists at the DA D_2 receptor and the primary mechanism through which they achieve their therapeutic effect is through a blockade of these receptors in the mesolimbic DA system. Unfortunately, a concurrent blockade of the D_2 receptor in the nigrostriatal pathway can produce extrapyramidal symptoms (EPS), while D_2 receptor blockage in the tuberoinfundibular tract results in hyperprolactinemia. In

an effort to minimize EPS, positron emission tomography (PET) studies have investigated the doses of antipsychotic used to treat schizophrenics and their relations to side effects and therapeutic efficacy. A number of PET imaging studies in patients with schizophrenia showed that typical antipsychotics are most likely to be effective in a therapeutic window in which 65–78% of D_2 receptors are blocked [150]. Occupancies below 65% are usually associated with a lack of antipsychotic effect, whereas occupancies above 78% are associated with EPS [151]. On the basis of these findings, an optimal window for D_2 receptor occupancy between 65 and 78% has been suggested. D_2 occupancy is thus thought to be the central determinant for the therapeutic efficacy and propensity to cause EPS and prolactin elevation of antipsychotic medication.

However, the efficacy of weak D_2 blockers such as clozapine and quetiapine called this theory into question. Indeed, a key difference between typical and these two atypical antipsychotics is that the two latter drugs achieve robust antipsychotic activity at doses producing D_2 receptor occupancy that are clearly lower than those produced by typical agents. For example, treatment with clozapine and quetiapine induced D_2 receptor occupancies in the 20–68% and 20–64% range, respectively, whereas treatment with typical drugs such as haloperidol led to D_2 receptor occupancies that are rather in the 70–90% range [109, 152–156]. These low levels of striatal D_2 receptor blockade have been proposed as a condition for what was referred as to atypicality, that is, low incidence of EPS and TD. However, some recent evidence weakens this theory. First, some atypical drugs such as sulpiride and amisulpride do not induce EPS or TD despite inducing D_2 occupancies higher than 80% [152, 153, 157]. These studies thus argue against the view that modest D_2 antagonism alone is a valid explanation for the low incidence of EPS and TD associated with atypical drugs. Second, as observed with typical drugs, D_2 antagonism can predict therapeutic efficacy and incidence of EPS even for atypical drugs. Indeed, remoxipride, olanzapine, and risperidone induce D_2 receptor occupancies that range from modest (50–60%) to high (80–90%) depending on the dose used, but therapeutic efficacy is achieved only at doses which exceed the 65% D_2 occupancy level [109, 152, 158–161]. When higher doses are used and D_2 occupancy crosses the 80% level, "atypicality" is sometimes lost, leading to EPS and hyperprolactinemia [162, 163]. In summary, the primary mechanism through which both typical and atypical antipsychotics achieve their therapeutic effect is through a blockade of D_2 receptors.

16.4.2 Role of D_2 Receptor Partial Agonism

Differences in regional DA function and observations of DA agonists' therapeutic effects in schizophrenia [164] led to the development of D_2 partial agonists as a new class of atypical antipsychotic agents. DA D_2 partial agonists have high affinity for D_2 receptors but reduced intrinsic activity when compared to the naturally occurring full agonist ligand DA, that is, they will produce less D_2 receptor activation than DA. As a consequence, D_2 partial agonists can act as agonists or antagonists depending on the local concentration of endogenous DA: in the presence of high

DA levels, partial agonists will act as antagonists by blocking DA access to the D_2 receptor, whereas they will act as agonists in the presence of low DA levels [165]. In theory, this means that partial DA agonists could alleviate the positive and negative symptoms of schizophrenia by counteracting DA transmission in hyperdopaminergic mesolimbic regions and by enhancing it in hypodopaminergic mesocortical regions. Partial DA agonists are therefore thought to stabilize dopaminergic tone in schizophrenia and are thereby often referred as to "dopamine stabilizers." On the other hand, a low intrinsic activity at the D_2 receptors prevents complete blockade of D_2 activity in the striatum and would confer a low propensity to cause EPS and prolactin elevation.

Dopamine D_2 autoreceptors may also be involved in the therapeutic action of partial agonists in schizophrenia. D_2 autoreceptors are localized on the presynaptic aspect of DA neurons and exert a negative feedback on DA release and/or synthesis. A dysregulated control of DA release/synthesis by these autoreceptors could contribute to the postulated imbalance of cortical and subcortical DA neurotransmission in schizophrenia. For instance, a reduced activity of the D_2 autoreceptors could lead to an increased DA release and thus contribute to the subcortical DA hyperactivity postulated in this disorder. Hence, D_2 partial agonists with high affinity for the presynaptic autoreceptors are particularly valuable antipsychotics as they can reduce DA synthesis and release through an agonist action at the presynaptic autoreceptors and block the response to DA through an antagonist action at the postsynaptic receptors.

Several D_2 partial agonists have been evaluated in schizophrenia. Preclamol, also known as (–)-3-(3-hydroxyphenyl)-*N*-n-propylpiperidine or (–)-3PPP, is a D_2-like partial agonist with high selectivity for the autoreceptor [166, 167]. A placebo-controlled trial indicated that preclamol improved both the positive and the negative symptoms of schizophrenia with no occurrence of motor side effects [168]. However, tolerance occurred over the course of a week, presumably because of a D_2 autoreceptor desensitization, and thus limited its usefulness for the treatment of schizophrenia. Other DA partial agonists that have undergone trials in schizophrenia include talipexole (B-HT 920), roxindole (EMD 49980), terguride, and SDZ-HDC 912. Despite their favorable preclinical profile, these compounds were not successful antipsychotic agents as they either failed to improve positive symptoms [169–171] or were associated with significant motor side effects [172]. Differences in clinical effects of these partial agonists may be due to their differential intrinsic activity at D_2 receptors [165].

The first successful D_2 partial agonist to come into practice is aripiprazole (see [173, 174] for review). Aripiprazole has high affinity for the D_2 receptor and behaves both as a potent D_2 autoreceptor agonist and as a postsynaptic D_2 antagonist [175]. It also exhibits high affinity and partial agonism at the D_3 and serotonin $5HT_{1A}$ receptors and antagonism at the serotonin $5HT_{2A}$ receptors. In animal models, aripiprazole inhibits amphetamine-induced locomotion, CAR, and apomorphine-induced stereotypy without causing catalepsy and produces an increased expression of *c-fos* in the shell of the NAcc but not in the striatum [130, 133, 176]. Thus, aripiprazole has preclinical and receptor-binding profiles that predict therapeutic

efficacy with a low risk of side effects. Concordant with this, aripiprazole is active against both the positive and the negative symptoms of schizophrenia, has a low propensity for EPS, and produces no elevation in serum prolactin levels [177, 178]. At clinically effective doses (10–30 mg/day), aripiprazole occupies 85–95% of the striatal D_2 receptors but still has a low incidence of EPS [179, 180]. The threshold for aripiprazole antipsychotic response thus appears to be higher than the 65% conventional threshold observed for D_2 antagonists, and EPS appear to be uncommon even at occupancies that exceed the extrapyramidal side effects threshold of 78% [180]. Aripiprazole thus does not conform to the conventional relationship between D_2 receptor occupancy and occurrence of motor side effects. Based on the magnitude of the upward shift of this relationship, the intrinsic activity of aripiprazole has been estimated to be approximately 25% [180]. Such a small intrinsic activity limits excessive DA stimulation and D_2 receptor blockade and preserves dopaminergic function in presynaptic and postsynaptic neurons, thereby conferring aripiprazole its favorable clinical profile. Interestingly, the principle active metabolite of clozapine, *N*-desmethylclozapine (NDMC), was recently identified as a partial agonist at D_2 receptors [181], and higher ratios of NDMC to clozapine levels in plasma have been found to predict better improvements in clinical response [182]. The unique clinical profiles of aripiprazole and clozapine, which are both distinguished even from other atypical antipsychotics by their much lower propensity to induce EPS and TD, suggest that partial agonism at D_2 receptors is a desirable feature of an antipsychotic drug.

16.4.3 Role of D_1 Receptor Blockade

In the search for new leads for the development of novel antipsychotic drugs with atypical properties, D_1 receptor antagonists have been thought as particularly promising. Indeed, D_1 antagonists have often met criteria for antipsychotic potential and low EPS liability in both rodents and nonhuman primates. For instance, the potent and selective D_1 antagonist SCH39166 inhibits apomorphine-induced climbing at lower doses than it inhibits apomorphine-induced sniffing in mice, it inhibits CAR response in both rat and squirrel monkey, but it does not cause catalepsy or increase plasma prolactin levels [183]. The relatively high affinity of clozapine for D_1 receptors as compared to D_2 receptors points to the same direction and has been regarded as a potential reason for its unique clinical profile (high antipsychotic efficacy with low EPS). At therapeutic dose, clozapine produces stronger blockade of D_1 receptors as compared to typical antipsychotics (40–60% vs. 0–40%) and lower blockade of D_2 receptors (maximum 50% blockade vs. 70–80%) [152, 184, 185], thus providing support to the view that D_1 antagonism might be a potential ground to atypicality. However, several findings do not support a role for D_1 receptor as a primary target of antipsychotic action. First, and opposed to the predictions from animal models, efforts to improve psychosis with selective D_1 antagonists have failed [186–188]. Second, therapeutic doses of various typical and atypical drugs occupy low or negligible levels of D_1 receptors. For instance, drugs such

as haloperidol, sulpiride, perphenazine, and even quetiapine, despite being effective antipsychotic agents, occupy less than 10% of the D_1 receptors [152, 184, 189]. These data thus suggest that D_1 antagonism by itself does not significantly contribute to the therapeutic effect of antipsychotics.

However, the D_1 receptor may be an important target for improving cognitive function in schizophrenia. Indeed, DA neurotransmission in the PFC strongly modulates working memory performance. Microiontophoretic applications of DA into the PFC facilitate whereas 6-hydroxydopamine PFC lesions disrupt working memory processes in primates [190–193]. This beneficial effect of DA on working memory processes appeared to be primarily mediated by D_1 receptors as it was mimicked by local injection of D_1 agonists, while local injection of D_1, but not D_2 antagonists, disrupts working memory in primates [194–196]. Interestingly, long-term treatment with a typical antipsychotic induced working memory deficits in nonhuman primates that can be reversed by brief treatment with a D_1 full agonist [197]. D_1 receptors are thus critically important in working memory function. It seems, however, that an optimal level of D_1 receptor "tone" in the PFC is required for adequate working memory performance. If DA levels are low, increasing D_1 receptor stimulation is beneficial for improving working memory while if DA levels are high, D_1 receptor activation could have the exact opposite effects (i.e., disrupting instead of improving working memory) [198, 199]. D_1 receptor activation thus follows an inverted "U"-shape function regarding performance on working memory, with too much or too little D_1 agonist stimulation both disrupting performance [200].

Considering the central role of D_1 receptors in memory function, the cognitive deficits observed in schizophrenia have long been thought to relate, at least in part, to a reduced D_1 signaling in the PFC. PET studies have, however, produced conflicting findings in this regards, with studies showing decreased [36], unaltered [34], or increased [35] D_1 receptor binding in the PFC of unmedicated patients as compared to control subjects. Despite this, D_1 receptor agonism, when combined with D_2 antagonism, should be of importance in the clinical efficacy of an atypical antipsychotic drug. The favorable profile of clozapine on cognitive functioning in schizophrenia is of particular interest. Clozapine indeed shows D_1 agonist properties in some animal models [201–203]. When combined with D_2 blockade, D_1 agonism rather than D_1 antagonism thus seems a promising option for the treatment of schizophrenia.

16.4.4 Role of D_3 Receptor Blockade

The D_3 receptor shares a high degree of homology with the D_2 receptor subtype and both are negatively coupled to adenylate cyclase. In contrast to D_2 receptors, which are prominently localized in projection areas of the motor nigrostriatal DA system (e.g., dorsolateral striatum), the D_3 receptor is preferentially localized in projection areas of the mesocorticolimbic dopaminergic system (e.g., NAcc, ventral putamen, PFC). This preferential localization of D_3 receptors in limbic vs. motor regions of the brain has sparked great interest in the D_3 receptor as a potential

target of antipsychotic action. Antipsychotic drugs generally have similar affinities for D_2 and D_3 receptors (Table 16.1). However, the relative contribution of D_3 vs. D_2 antagonism to antipsychotic efficacy is difficult to establish in humans due to the lack of selective D_3 compounds and to the partially overlapping distribution of D_2 and D_3 receptors. Preclinical studies have produced mixed results regarding the potential antipsychotic activity of D_3 antagonism. When administered repeatedly to rodents, SB-277011-A, a high affinity and highly selective D_3 antagonist [204], has been shown to selectively decrease the number of spontaneously active DA neurons in VTA [205]. This electrophysiological model is postulated to predict antipsychotic activity as both typical and atypical agents such as haloperidol and clozapine alter the activity of midbrain DA neurons, whereas drugs with no antipsychotic activity do not have such an effect [206, 207]. According to this model, selective D_3 receptor blockade using SB-277011-A is thus predicted to be associated with antipsychotic efficacy. On the other hand, this compound was found to be ineffective for blocking the amphetamine-induced hyperlocomotion, another putative animal model for antipsychotic activity [204]. Clinical trials of selective D_3 receptor antagonists in humans have not been reported yet and are needed to better understand the significance of D_3 receptors as targets for the treatment of schizophrenia.

16.4.5 Role of D_4 Receptor Blockade

The observation that clozapine has a high affinity for D_4 receptors as compared to D_2 receptors has sparked enormous interest in the D_4 receptor as a potential target for the treatment of schizophrenia [208]. This interest was further strengthened by postmortem studies showing elevated densities of D_4 receptors in the striatum of schizophrenic patients [38–40], although this finding has not been consistently confirmed [41–44]. However, D_4 receptor blockade does not appear essential for mediating antipsychotic action or for conferring low EPS liability. Indeed, several atypical drugs (e.g., amisulpride, quetiapine, aripiprazole) have very little D_4 receptor blocking activity, and several typical (e.g., chlorpromazine, haloperidol) and some atypical drugs (i.e., olanzapine, clozapine, risperidone) have high D_4 affinities (Table 16.1; see also [209]). Finally, clinical trials with two D_4 selective antagonists, L745870 [210, 211] and sonepiprazole [212], have failed to demonstrate antipsychotic efficacy.

16.5 Considerations Critical for Understanding Receptor Involvement in Antipsychotic Action

16.5.1 Speed of Onset and Implications for Mechanism

It has long been held that onset of antipsychotic effect on psychotic symptoms is delayed by 2–3 weeks after initiation of treatment. One widely accepted hypothesis for this delayed onset of action is that of depolarization block [213]. According to

that hypothesis, the acute blockade of D_2 receptors initially leads to an increased firing of ventral tegmental DA neurons. However, over successive administrations this subsides, and after 2–3 weeks of chronic treatment antipsychotics ultimately produce a reversible cessation of firing of midbrain DA neurons, known as depolarization block. The delayed onset of antipsychotic action is thus thought to correlate with the delayed induction of depolarization blockade of mesolimbic DA neurons [206, 207]. Another hypothesis postulates that antipsychotic-induced changes in the synthesis or degradation of critical "effector" protein synthesis could also explain the delayed onset of antipsychotic action [214]. Although the molecular mechanism underlying the delayed improvement of psychotic symptoms is unclear, the idea of a "delayed onset" of antipsychotic action is at odds with the immediate effect of antipsychotics on D_2 receptor blockade, which occurs within the first few hours of antipsychotic treatment [215, 216]. This dissociation between early D_2 blockade and delayed therapeutic gain thus speaks against a central role of D_2 blockade in the mechanism of action of antipsychotic drugs. However, an emerging body of evidence suggests that antipsychotics have an earlier onset of efficacy than previously thought. In 2003, Agid et al. [217, 218] conducted a meta-analysis of 42 double-blind, comparator-controlled studies (>7000 patients) of patients treated with the common typical (haloperidol, chlorpromazine) and atypical (risperidone and olanzapine) antipsychotics. Without significant difference across antipsychotics, they found – contrary to expectations under the delayed onset hypothesis – that psychosis improved within the very first week of treatment. In fact, a larger reduction of symptoms occurs during the first 2 weeks than during the second 2 weeks of treatment. This observation of early onset of symptom response has subsequently been extended to other agents, including quetiapine [219, 220] and amisulpride [221]. Interestingly, early trials with ziprasidone [222] and aripiprazole [223] also showed detectable onset of effects on positive symptom scores within the first 2 weeks of treatment. In a double-blind placebo-controlled study comparing intramuscular olanzapine to intramuscular haloperidol in patients experiencing a psychotic exacerbation, Kapur et al. [224] reported that onset of therapeutic response occurred within the first 24 h. Abi-Dargham [49] also observed rapid improvement (i.e., within 72 h) in psychotic symptoms upon DA depletion with a-methyl-para-tyrosine. These observations thus support a direct role for DA in the mediation of psychosis and further indicate that the mechanism of antipsychotic action may be more proximally related to the blockade of D_2 receptors than originally thought.

16.5.2 Relapse on Withdrawal and Supersensitivity

Withdrawal or decreased dosing of long-term antipsychotic treatment can induce relapse of the psychotic symptoms in some schizophrenic patients. This effect, usually called supersensitivity psychosis or rebound psychosis, has been observed after withdrawal from antipsychotic drugs such as quetiapine [225], clozapine [226, 227], olanzapine [228], haloperidol [229], chlorpromazine [230], and fluphenazine

enanthate [231, 232]. As supersensitivity psychosis can be accompanied by the development or worsening of latent TD [231, 232], both clinical manifestations are thought to arise from a DAergic supersensitivity. Supports for this hypothesis come from behavioral experiments in rodents showing that withdrawal from chronic antipsychotic treatment results in an increased responsiveness to direct or indirect DA agonists, such as apomorphine [233–238], amphetamine [239–241], and DA injected into the striatum [242]. There is evidence that postsynaptic processes contribute to this antipsychotic-induced behavioral supersensitivity. Indeed, chronic treatment with typical drugs such as haloperidol and chlorpromazine as well as atypical drugs such as remoxipride, olanzapine, and risperidone has been consistently shown to increase D_2 receptor density in the dorsal striatum and NAcc [243–249]. This finding led to the proposal that long-term treatment with antipsychotic drugs in humans could lead to a compensatory D_2 receptor upregulation, causing receptor supersensitivity and resulting in the onset of TD and supersensitivity psychosis upon withdrawal [232, 250–252]. More specifically, the development of TD is thought to reflect an increased density of D_2 receptors in the striatum leading to a functional supersensitivity of the nigrostriatal system, whereas receptor supersensitivity occurring in mesolimbic system would result in the development of supersensitivity psychosis [14, 250, 251, 253, 254]. It is noteworthy that besides the D_2 receptor supersensitivity hypothesis, other hypotheses have been proposed to explain TD. These include a deficiency of the GABA neurotransmitter system or excitotoxic neuronal damages induced by dopamine release, glutamate release, or oxidative stress [255–257]. Yet, depleting DA brain levels with tetrabenazine or reserpine constitute a most effective treatment for TD [258–260], thus indicating an important role for excessive DA signaling, likely supersensitive DA D_2 system, in the pathophysiology of TD.

Although D_2 receptor upregulation seems to be a central mechanism in mediating the long-term, and potentially irreversible, consequences of chronic exposure to most antipsychotic drugs, several lines of evidence suggest that DA supersensitivity and D_2 receptor upregulation can be dissociated. Indeed, the two atypical antipsychotics most often associated with supersensitivity psychosis are quetiapine and clozapine [225–227]. Both drugs also induce a supersensitive psychomotor response to DA agonists in animals [238, 261] but still, they do not induce striatal D_2 receptor upregulation [249, 262, 263]. This lack of striatal D_2 receptor upregulation has been proposed to underlie the low propensity of clozapine to induce TD in humans [264]. Therefore, increase in D_2 receptor density is not sufficient to explain DA supersensitivity. However, a lack of D_2 upregulation does not necessarily preclude the involvement of D_2 receptor mechanisms in the DA supersensitized state seen upon clozapine and quetiapine withdrawal. For instance, alterations at the level of G proteins that couple D_2 receptors to adenylyl cyclase can also result in an increased D_2 signaling and supersensitivity despite the lack of D_2 upregulation [265–267]. Such a mechanism may be involved in the DA supersensitivity seen after clozapine and quetiapine as both drugs induce elevation in D_2^{High} (D_2 receptors with functional high affinity for DA) despite the absence of any elevation in total D_2 receptors [32]. Interestingly, a functional coupling of D_2 receptors with G proteins also appears

to be involved in the action of atypical drugs, as indicated by a larger proportion of D_2^{High} [32, 268], an increased efficacy of DA to stimulate [^{35}S]GTPγS binding [233], and an increased activity of the D_2 receptor–G_i protein system after repeated haloperidol administrations [266, 269–271]. Moreover, as observed with clozapine and quetiapine, withdrawal from low doses of typical antipsychotics also reveals a dissociation between increased DA-mediated behavior and D_2 upregulation [272, 273]. For instance, Samaha et al. [273] recently showed that chronic low doses of haloperidol enhanced amphetamine-induced locomotion with no increase in D_2 densities and only higher doses lead to concomitant D_2 upregulation. Low doses of haloperidol though increased D_2^{High}, indicating that elevation in D_2^{High} rather than elevation in the total population of D_2 sites best predicted behavioral DA supersensitivity. Thus, there may be two types of D_2 receptor-mediated supersensitivity: one associated with increased D_2^{High} and leading to supersensitivity psychosis and the other associated with increased D_2 density and leading to TD. Relatively low levels of D_2 blockade induced by either weak D_2 antagonists or low doses of potent D_2 antagonists may lead to an increased population of functionally coupled D_2 receptors (i.e., D_2^{High}) and this may be sufficient to induce DA supersensitivity. On the other hand, stronger D_2 blockade induced by typical drugs or by high doses of atypical drugs would further result in D_2 upregulation, which would in turn lead to TD.

16.5.3 Antagonist vs. Inverse Agonist

It is generally assumed that antipsychotic drugs are acting as antagonists at the D_2 receptor. In recent years, this picture of antipsychotic action has been modified to include inverse agonism. Inverse agonism at D_2-like receptors was first noted for haloperidol, which increased prolactin production and cyclic AMP formation in pituitary cell lines [274]. Subsequently, several other antipsychotic drugs were identified as D_2 inverse agonists using a variety of techniques [275–277]. In fact, with the exception of aripiprazole, all antipsychotics tested so far are inverse agonists at the D_2 receptor, independently of their typical or atypical profile [181, 278]. D_2 inverse agonist activity thus appears to be a common feature of antipsychotic drugs although different antipsychotics exhibit different degrees of inverse agonism, ranging from high (e.g., haloperidol, pimozide) to very low inverse agonist activity (e.g., quetiapine) [181]. From a mechanistic perspective, the way D_2 inverse agonists work is not clearly established. Based on the ternary complex model of G protein-coupled receptor action [279], it has been proposed that D_2 receptors may exist in an inactive state, which can isomerize to a partially active state that can ultimately couple to G proteins to form a fully active state of the receptor [280]. The fully active state of the receptor is believed to give rise to constitutive activity, leading to a tonic inhibition of adenylate cyclase even in the absence of DA. Inverse agonists would reduce this basal spontaneous D_2 receptor activity by stabilizing the uncoupled forms to the detriment of the coupled, fully active, form of the receptor

[281]. Although intrinsic constitutive D_2 receptor activity and D_2 inverse agonism properties of antipsychotic drugs have been demonstrated in vitro, it is still unclear whether this has any relevance in vivo. Inverse agonism will be relevant to the effects of antipsychotics only if there is a spontaneous agonist-independent activity of the D_2 receptors in vivo. If there is no such spontaneous activity, then an inverse agonist will be indistinguishable from an antagonist.

16.5.4 Fast Dissociation and Transient Occupancy of D_2 Receptors

In contrast to the multireceptor hypothesis, which postulates that the atypical features of antipsychotics are conferred by binding to receptors other than D_2, another theory postulates that atypicality can be produced by appropriate modulation of the D_2 receptor alone. Indeed, Kapur and collaborators [150, 282, 283] have proposed that the lower liability of atypical vs. typical drugs to induce EPS is related to their fast dissociation from D_2 receptors. Typical antipsychotics bind tightly to and dissociate slowly from D_2 receptors, whereas atypical drugs bind more loosely and dissociate fast from the receptors [284]. As a consequence, atypical drugs may block the receptors but be more responsive to phasic surges of DA transmission and hence may normalize transmission, rather than suppress it. In addition to molecular considerations (of fast or slow dissociation) is also the systemic issue of occupancy kinetics. In vivo PET studies have shown that typical drugs such as haloperidol give rise to sustained high levels of D_2 receptor blockade through the day [215], while certain atypical drugs such as quetiapine give rise to only transiently high levels of D_2 receptor blockade (up to 64% at 2 h; 0% at 12–14 h) with no incidence of EPS and prolactin elevation [156, 285]. Transient D_2 blockade may thus be sufficient to induce antipsychotic response while minimizing the risks of EPS and hyperprolactinemia. It is thus predicted that a low affinity and fast dissociation from D_2 receptors, along with administration of the drug in doses that lead to appropriate levels of D_2 blockade, are the most important requirements for atypicality [282]. A transient duration of action may also explain the lack of D_2 receptor upregulation reported following treatment with atypical drugs such as clozapine, loxapine, or thioridazine in rats [249, 263, 286–289] and the low propensity of atypical drugs to induce TD in humans [264]. Indeed, recent studies indicate that the pattern of D_2 occupancy kinetics achieved by chronic haloperidol has an important influence on D_2 upregulation and on the development of vacuous chewing movements (VCMs), an animal model for TD. While continuously high levels (>70–80% for 24 h/day) of D_2 receptor blockade produced by continuous infusion of haloperidol lead to a robust upregulation of striatal D_2 receptors and to an increased risk for the development of VCMs in experimental animals, transiently high levels (>80% for a few hours/day) of D_2 receptor blockade produced by daily bolus injections of the same drug did not produce any of these effects [290, 291]. Thus, converging evidence suggests that the pharmacokinetics of D_2 receptor blockade during chronic treatment with haloperidol is an important determinant for the induction of D_2 receptor upregulation and for the development of VCMs, and potentially TD. The reason

why transiently high D_2 receptor blockade resulted in no receptor alteration is not clear. However, it can be postulated that blocking receptors only transiently during the day would preserve some access of endogenous DA to the receptors and that this pulsatory agonist stimulation could be sufficient to prevent a compensatory D_2 receptor upregulation.

16.5.5 Preferential Limbic D_2 Receptor Blockade

Another theory to account for atypicality is the preferential action of antipsychotics with low EPS liability on the limbic DA system. Several lines of evidence indicate that, in contrast to typical antipsychotics, atypical antipsychotics selectively target the mesolimbic DA system, while leaving the nigrostriatal system relatively unaffected. For instance, while the acute administration of haloperidol increases the number of spontaneously active DA neurons in both the SN and VTA, clozapine and olanzapine selectively increase the number of firing cells in the VTA only [207, 292, 293]. Consistent with these electrophysiological data, atypical drugs have been shown to preferentially increase DA output in the NAcc as compared to the striatum, whereas the opposite was observed after acute administration of haloperidol [294]. This regional specificity (often called limbic selectivity) of atypical agents is also observed on chronic treatment as drugs such as clozapine, quetiapine, olanzapine, and sertindole produce depolarization inactivation of VTA DA neurons, sparing those in the SN, whereas haloperidol inactivates DA neurons in both the SN and the VTA [206, 207, 292, 293, 295, 296]. Consequently, in those animal models, atypical antipsychotics can be differentiated from typical drugs on the basis of their preferential effect on DA transmission in limbic regions. Some evidence indicates that such a limbic selectivity may also exist in clinical settings. Indeed, in vivo receptor imaging studies have shown that, at clinically useful doses, several atypical antipsychotics including clozapine, olanzapine, quetiapine, risperidone, and sertindole demonstrate preferential blockade of D_2 receptors in limbic cortical regions than in striatum [297–301]. These studies suggest that preferential blockade of cortical and/or limbic D_2 receptors may be sufficient to mediate the therapeutic efficacy and low extrapyramidal symptom profile of atypical antipsychotic drugs. However, other studies report similar levels of striatal and cortical D_2 receptor blockade with atypical drugs [302–305]. The reason for this discrepancy between groups reporting similar striatal–extrastriatal occupancy and those showing much higher extrastriatal occupancy is not entirely clear and may be related to methodological differences between studies. However, the concept of limbic selectivity as a possible mechanism of atypical action is called into question. This is further emphasized by recent data showing that striatal rather than extrastriatal D_2 receptor blockade is predictive of improvement in positive symptoms as well as occurrence of motor side effects associated with antipsychotic treatment [305]. Clearly, more clinical investigations are needed to determine the exact role of limbic D2 receptors in the treatment of schizophrenia.

16.6 Other Receptors Involved in Antipsychotic Drug Action

16.6.1 Role of 5HT$_{2A}$ Receptor Blockade and 5HT$_{1A}$ Receptor Activation

The therapeutic efficacy of atypical drugs such as clozapine prompted researchers to examine how their actions on various neurotransmitter receptors differ from those of typical antipsychotics. In this respect, serotonin (5-HT) receptors have received particular attention in the treatment of schizophrenia as several antipsychotic drugs exhibit high affinities for these receptors, especially for the 5-HT$_{2A}$, 5-HT$_{2C}$, and 5-HT$_{1A}$ receptor subtypes (Table 16.1).

The finding that clozapine is a potent 5-HT$_{2A}$ receptor antagonist has generated great interest in the potential role of these receptors in antipsychotic drug actions. As a matter of fact, most currently approved atypical antipsychotic drugs are relatively potent 5-HT$_{2A}$ antagonists (Table 16.1). Moreover, subchronic treatment with clozapine but not haloperidol decreased 5-HT$_{2A}$ mRNA and the density of [^{3}H]ketanserin binding in frontal cortex, Nacc, and striatum [306–308], suggesting that modulation of 5-HT$_{2A}$ receptor levels may be important in atypical action. Several lines of evidence indicate that 5-HT$_{2A}$ antagonism per se does not confer antipsychotic effects. First, MDL 100,907, a compound largely lacking D$_2$ antagonism but which is a potent 5-HT$_{2A}$ antagonist, while showing promising antipsychotic properties in preclinical studies, was not found to be as effective as haloperidol in the treatment of schizophrenia [309]. Similarly, fananserin, a potent 5-HT$_{2A}$/D$_4$ antagonist lacking D$_2$ antagonism, does not show antipsychotic activity [310]. Second, amisulpride does not occupy any 5-HT$_{2A}$ receptors in humans even when used at high doses but still is a highly effective atypical antipsychotic [311]. Third, 5-HT$_{2A}$ receptor blockade is not necessary for, nor does it alter, the D$_2$ receptor occupancy required for clinical effectiveness [150]. Indeed, PET studies have consistently showed that, at clinically relevant doses, atypical agents such as risperidone and olanzapine have higher occupancy of 5-HT$_{2A}$ than D$_2$ receptors [158, 303, 312–314]. However, these drugs become effective only at doses which cross the conventional 65% level of D$_2$ occupancy. Doses below this D$_2$ occupancy threshold, although saturating 5-HT$_{2A}$ receptors, are not therapeutic [312]. Thus, a minimal level of D$_2$ receptor blockade is a necessary condition for an antipsychotic drug to be effective independent of any action on the 5-HT$_{2A}$ receptor.

Although 5-HT$_{2A}$ antagonism does not seem to constitute a primary mechanism for antipsychotic action, it has been suggested that a high affinity for 5-HT$_{2A}$ receptors combined with a relatively lower affinity at the D$_2$ receptors, producing a high 5-HT$_{2A}$/D$_2$ affinities ratio, is critical for conferring atypical activity [315]. Current hypotheses suggest that serotonin 5HT$_{2A}$ receptor mechanisms may improve cognitive function and negative symptoms and limit EPS by increasing DA release [316, 317]. Serotonin 5-HT$_{2A}$ receptors are indeed uniquely positioned to modulate central DA transmission. These receptors are expressed on midbrain DA neurons of the VTA [318] as well as on cortical and pallidal afferents in the dorsal striatum [319]. They are also abundant in the frontal cortex where they are localized

postsynaptically on pyramidal neurons [320, 321] and, albeit to a smaller extent, presynaptically on DA terminals [322]. Atypical antipsychotics have been reported to preferentially increase DA efflux in the mPFC compared with the NAcc in rats [323–325]. This action is due, in part, to blockade of cortical 5-HT_{2A} receptors combined with weaker blockade of D_2 receptors, since it can be mimicked by the combination of potent 5-HT_{2A} antagonism and weak blockade of D_2 receptors [326–328] whereas it does not occur with the selective 5-HT_{2A} antagonist MDL 100,907 [329]. The 5-HT_{2A}/D_2 model [316, 317] suggests that by blocking presynaptic 5-HT_{2A} receptors in the DA mesocortical and nigrostriatal pathways, atypical antipsychotics locally increase DA release. This effect would lessen the action of the drugs at D_2 receptors, thereby improving cognitive deficits and/or negative symptoms and decreasing the incidence of EPS. In contrast, with the sparse distribution of 5-HT_{2A} receptors in NAcc, D_2 blockade would still prevail in the DA mesolimbic pathways, thus preserving antipsychotic activity of these drugs. Thus, the differential distribution of D_2 and $5HT_{2A}$ receptors may explain why atypical antipsychotic drugs exert opposite action on DA transmission in cortical and limbic brain regions.

While an anti-$5HT_{2A}$ effect of atypical drugs may contribute to their ability to improve cognitive function and/or negative symptoms, it is unclear whether such a mechanism is necessary for their lower EPS propensity. Indeed, atypical drugs such as remoxipride [330] and amisulpiride [311, 331] have negligible affinities for 5-HT_{2A} receptors and still do not induce EPS at clinical dose. The reverse situation is encountered with some typical drugs, such as chlorpromazine and loxapine, which produce high 5-HT_{2A} occupancies [311, 332] but still induce EPS at clinical dose.

Although research on the role of serotonergic mechanisms in antipsychotic drug action has mainly focused on $5HT_{2A}$ receptors, $5HT_{2c}$, $5HT_{1A}$, $5HT_6$, and $5HT_7$ receptors may also be of some significance [316]. For instance, another mechanism that has been proposed to modulate the DA system and prevent or reduce the motor effects of D_2 blockade is $5HT_{1A}$ receptor agonism. Serotonin $5HT_{1A}$ receptor agonism, in combination with D_2 receptor antagonism, facilitates DA release in PFC and NAcc but not in striatum [333] and attenuates the EPS-like side effects of D_2 blockade [334, 335]. Recent observations indicate that the increase in DA output produced by atypical antipsychotics such as clozapine, ziprasidone, and aripiprazole in the PFC is dependent on the activation of 5-HT_{1A} receptors as it is partly or totally antagonized by the 5-HT_{1A} receptor antagonist WAY-100635 [324, 336–338] and by the genetic deletion of 5-HT_{1A} receptors [339]. Given the central role of prefrontal DA in cognitive function, the increase in PFC DA release may underlie the superior effects of clozapine and other atypical antipsychotic drugs on the negative and cognitive symptoms by normalizing a putative cortical hypodopaminergic transmission.

16.6.2 Role of Drugs Acting on the Glutamate System

The NMDA receptor hypofunction hypothesis of schizophrenia has provided a theoretical framework for investigating the effects of drugs acting on the glutamatergic

system in schizophrenia. Examples of new "glutamate-based" agents are agonists of the glycine recognition site of the NMDA receptor (e.g., glycine, D-serine, D-cycloserine), glycine reuptake inhibitors, glutamate release inhibitors (e.g., LY-354740 and lamotrigine), AMPA agonists and antagonists (e.g., LY-293558 and GYKI 52466), ampakines (e.g., CX-516), and mGlu receptor agonists (for review, see [340]). A recent meta-analysis [341] of randomized double-blind trials indicated that D-cycloserine seemed to be ineffective in treating schizophrenia whereas glycine and D-serine appeared to be effective in reducing negative symptoms of schizophrenia when added to ongoing antipsychotic treatment. The beneficial effects of glycine and D-serine on cognitive impairment were less robust and null on the positive symptoms. In the case of glutamate release inhibitors, evidence is not robust regarding the efficacy of lamotrigine for reducing the symptoms of schizophrenia, with some studies showing a beneficial effect and others being equivocal [342–344]. The use of the ampakine allosteric modulator CX-516 was also disappointing as it showed no clear beneficial effects as monotherapy [345], although it was found to be effective when added to clozapine, olanzapine, or risperidone [346]. Until recently, the usefulness of glutamatergic agents thus appeared somewhat limited for the treatment of schizophrenia. However, in a recent randomized, double-blind, parallel fixed-dose study, Patil et al. [347] reported a significant improvement of the positive and negative symptoms of schizophrenia following a 4-week treatment with the mGlu2/3 agonist LY2140023. If replicated, this finding is potentially important as LY2140023, and mGlu2/3 agonists in general, might be the first class of antipsychotic not acting through the D_2 receptor. However, given the functional interactions between the glutamatergic and the DA systems, their effects might still be ultimately mediated through the DA system.

16.6.3 Role of CB_1 Receptor Blockade

Another approach to the development of antipsychotic drugs has been to evaluate compounds acting through the brain cannabinoid-1 (CB1) receptor as a dysregulation of the endogenous cannabinoid system has been proposed to underlie some of the symptoms of schizophrenia [348]. Support for this hypothesis comes from studies showing that cannabinoids can lead to acute psychotic episodes in some individuals [349] and can produce short-term exacerbation or recurrences of pre-existing psychotic symptoms [350, 351]. Moreover, an overactivity of the endocannabinoid system has been revealed in schizophrenia by studies showing increased levels of endogenous cannabinoids in the cerebrospinal fluid [352] as well as increased brain densities of CB1 receptors in schizophrenia patients [353, 354]. Finally, there is substantial evidence showing that agonist stimulation of CB1 receptors increases DA neuron firing in the VTA and SN [355, 356] and, as a consequence, increases extracellular DA levels in their projection areas [357–361]. These observations have suggested that CB1

receptor antagonists may have antipsychotic properties. However, a clinical trial with the selective CB1 receptor antagonist, SR141716 (rimonabant), showed no antipsychotic efficacy [362].

16.6.4 Role of α_1 and α_2 Adrenergic Receptor Blockade

The α_1 and α_2 adrenergic receptors received much attention when it was found that clozapine binds with higher affinity to these receptors than to the D_2 receptor [363]. In fact, many antipsychotics, whether typical or atypical, are relatively strong antagonists at the α_1 and/or α_2 adrenergic receptors. Although α_1 adrenergic antagonism per se does not confer antipsychotic activity [364], it may still be of some importance in the mechanism of action of antipsychotic drugs. Svesson [365] has suggested that α_1 adrenergic blockade by antipsychotics may contribute to suppress positive symptoms of schizophrenia by inhibiting striatal hyperdopaminergia, whereas α_2 adrenergic blockade may be involved in relief of negative and cognitive symptoms by enhancing DA function in cortex. This hypothesis is consistent with studies showing that blockade of α_1 adrenergic receptors with prazosin blocks the hyperactivity and midbrain DA release induced by the NMDA antagonists MK-801 and PCP [366, 367]. Similarly, it blocks the acute locomotor effects of amphetamine and reduces the behavioral sensitization induced by repeated injections of the drug [368, 369]. Moreover, raclopride-induced activation of VTA DA neurons has been found to be suppressed by prazosin [370], suggesting a modulatory effect of α_1 adrenergic receptors on mesolimbic DA transmission. A number of studies also indicate that α_2 adrenergic antagonism may be relevant to atypicality. $Alpha_2$ blockers have been shown to increase DA levels in the rat PFC [371, 372] and to improve cognitive functioning in aged rats [373] and in patients with frontal dementias [374]. Co-medication with the α_2 adrenergic antagonist idazoxan has been found to enhance the antipsychotic efficacy of the atypical drug fluphenazine in treatment-resistant schizophrenic patients, comparing favorably with clozapine [375]. Recent experimental findings also indicate that α_2 blockade enhances the efficacy of both typical and atypical antipsychotic drugs as it enhances the suppression of CAR seen with haloperidol and olanzapine in rodents and reverses haloperidol-induced catalepsy [372]. All together, α_1 and α_2 antagonist properties of antipsychotic drugs may improve their efficacy and contribute to the reduction of their motor side effects.

16.6.5 Role of NK_3 Receptor Blockade

Neurokinin 3 (NK_3) receptors have recently become of interest with regard to the treatment of schizophrenia as those receptors play a key role in the regulation of midbrain DA function [376, 377]. Indeed, agonist-induced stimulation of NK_3 receptors has been shown to activate DA neurons in the ventral mesencephalon and to enhance DA release in NAcc, dorsal striatal, and PFC. These effects, which can be

prevented by selective NK_3 receptor blockade, have motivated the development of NK_3 receptor antagonists as potential new antipsychotic drugs. The NK_3 antagonist SR142801 (onasetant) has been evaluated in schizophrenia but showed a relatively modest antipsychotic efficacy that was intermediate between placebo and haloperidol [362] and since the future development of this line of compounds has been stopped, there may be limited information on this issue in the near future.

16.7 Conclusion and Future directions

Thus, over half a century after the discovery of the compound that revolutionized the treatment of schizophrenia, we are still tied to the same basic principle – blockade of the dopamine D_2 receptors. The intervening 50 years of science have added remarkably to our understanding of how the drugs work – the differentiation and localization of D_2 receptors, the visualization in humans, the identification of an occupancy–outcome relationship, and the introduction of compounds with more complex pharmacologies. Yet, the fundamental principle has remained unchanged – D_2 blockade remains necessary and sufficient for good antipsychotic activity.

Yet, there remain several unsolved questions and several future directions. Clozapine still remains uniquely effective in patients in whom standard typical and atypical antipsychotics do not work. The mechanism of this superior efficacy is not known. Deciphering it may provide a lead to a potentially very useful new mechanism in schizophrenia. Most of the current drugs are dopamine D_2 blockers; yet, no pathology in the dopamine D_2 receptors has been conclusively identified even after nearly a dozen such studies. The definitive abnormality of the DA system in schizophrenia lies presynaptically. This opens up a new therapeutic option. Rather than just blocking the aftereffects of inappropriately released DA, the field could focus on factors that could modulate the presynaptic release of DA. Finally, the focus on dopamine D_2 receptors draws away from the critical role of D_1 receptors in the cortex and their potential relationship with cognition and negative symptoms. A plausible hope remains that enhancing D_1 transmission in the prefrontal cortex may lead to superior symptomatic improvement in domains untouched by current drugs. The next decade will tell whether the DA system has delivered all it can for the treatment of schizophrenia or whether there are further opportunities to harness it for the benefit of our patients.

References

1. Saha S, Chant D, Welham J, McGrath J. A systematic review of the prevalence of schizophrenia. PLoS Med 2005;2:e141.
2. Kapur S, Mamo D. Half a century of antipsychotics and still a central role for dopamine D2 receptors. Prog Neuropsychopharmacol Biol Psychiatry 2003;27:1081–90.
3. Lieberman JA, Stroup TS, McEvoy JP, et al. Effectiveness of antipsychotic drugs in patients with chronic schizophrenia. N Engl J Med 2005;353:1209–23.

4. Arndt S, Andreasen NC, Flaum M, Miller D, Nopoulos P. A longitudinal study of symptom dimensions in schizophrenia. Prediction and patterns of change. Arch Gen Psychiatry 1995;52:352–60.
5. Eaton WW, Thara R, Federman B, Melton B, Liang KY. Structure and course of positive and negative symptoms in schizophrenia. Arch Gen Psychiatry 1995;52:127–34.
6. Crow TJ. The two-syndrome concept: origins and current status. Schizophr Bull 1985; 11:471–86.
7. Kirkpatrick B, Buchanan RW, Ross DE, Carpenter WT, Jr. A separate disease within the syndrome of schizophrenia. Arch Gen Psychiatry 2001;58:165–71.
8. van Rossum JM. The significance of dopamine-receptor blockade for the mechanism of action of neuroleptic drugs. Arch Int Pharmacodyn Ther 1966;160:492–4.
9. Connell PH. Amphetamine psychosis. London: Oxford University Press, 1958.
10. Carlsson A, Lindqvist M. Effect of chlorpromazine or haloperidol on formation of 3-methoxytyramine and normetanephrine in mouse brain. Acta Pharmacologica et Toxicologica 1963;20:140–4.
11. Seeman P, Lee T. Antipsychotic drugs: direct correlation between clinical potency and presynaptic action on dopamine neurons. Science 1975;188:1217–9.
12. Lee T, Seeman P, Tourtellotte WW, Farley IJ, Hornykeiwicz O. Binding of 3H-neuroleptics and 3H-apomorphine in schizophrenic brains. Nature 1978;274:897–900.
13. Seeman P, Ulpian C, Bergeron C, et al. Bimodal distribution of dopamine receptor densities in brains of schizophrenics. Science 1984;225:728–31.
14. Burt DR, Creese I, Snyder SH. Antischizophrenic drugs: chronic treatment elevates dopamine receptor binding in brain. Science 1977;196:326–8.
15. Owen F, Cross AJ, Waddington JL, Poulter M, Gamble SJ, Crow TJ. Dopamine-mediated behaviour and 3H-spiperone binding to striatal membranes in rats after nine months haloperidol administration. Life Sci 1980;26:55–9.
16. Reynolds GP, Riederer P, Jellinger K, Gabriel E. Dopamine receptors and schizophrenia: the neuroleptic drug problem. Neuropharmacology 1981;20:1319–20.
17. Mackay AV, Bird ED, Spokes EG, et al. Dopamine receptors and schizophrenia: drug effect or illness? Lancet 1980;2:915–6.
18. Mackay AV, Iversen LL, Rossor M, et al. Increased brain dopamine and dopamine receptors in schizophrenia. Arch Gen Psychiatry 1982;39:991–7.
19. Owen F, Cross AJ, Crow TJ, Longden A, Poulter M, Riley GJ. Increased dopamine-receptor sensitivity in schizophrenia. Lancet 1978;2:223–6.
20. Cross AJ, Crow TJ, Owen F. 3H-Flupenthixol binding in post-mortem brains of schizophrenics: evidence for a selective increase in dopamine D2 receptors. Psychopharmacology (Berl) 1981;74:122–4.
21. Wong DF, Wagner HN, Jr., Tune LE, et al. Positron emission tomography reveals elevated D2 dopamine receptors in drug-naive schizophrenics. Science 1986;234:1558–63.
22. Tune LE, Wong DF, Pearlson G, et al. Dopamine D2 receptor density estimates in schizophrenia: a positron emission tomography study with 11C-N-methylspiperone. Psychiatry Res 1993;49:219–37.
23. Farde L, Wiesel FA, Stone-Elander S, et al. D2 dopamine receptors in neuroleptic-naive schizophrenic patients. A positron emission tomography study with [11C]raclopride. Arch Gen Psychiatry 1990;47:213–9.
24. Hietala J, Syvalahti E, Vuorio K, et al. Striatal D2 dopamine receptor characteristics in neuroleptic-naive schizophrenic patients studied with positron emission tomography. Arch Gen Psychiatry 1994;51:116–23.
25. Seeman P, Kapur S. Schizophrenia: more dopamine, more D2 receptors. Proc Natl Acad Sci USA 2000;97:7673–5.
26. Nordstrom AL, Farde L, Eriksson L, Halldin C. No elevated D2 dopamine receptors in neuroleptic-naive schizophrenic patients revealed by positron emission tomography and [11C]N-methylspiperone. Psychiatry Res 1995;61:67–83.

27. Lomena F, Catafau AM, Parellada E, et al. Striatal dopamine D2 receptor density in neuroleptic-naive and in neuroleptic-free schizophrenic patients: an 123I-IBZM-SPECT study. Psychopharmacology (Berl) 2004;172:165–9.
28. YangYK, Yu L, Yeh TL, Chiu NT, Chen PS, Lee IH. Associated alterations of striatal dopamine D2/D3 receptor and transporter binding in drug-naive patients with schizophrenia: a dual-isotope SPECT study. Am J Psychiatry 2004;161:1496–8.
29. Glenthoj BY, Mackeprang T, Svarer C, et al. Frontal dopamine D(2/3) receptor binding in drug-naive first-episode schizophrenic patients correlates with positive psychotic symptoms and gender. Biol Psychiatry 2006;60:621–9.
30. Buchsbaum MS, Christian BT, Lehrer DS, et al. D2/D3 dopamine receptor binding with [F-18]fallypride in thalamus and cortex of patients with schizophrenia. Schizophr Res 2006;85:232–44.
31. Talvik M, Nordstrom AL, Okubo Y, et al. Dopamine D2 receptor binding in drug-naive patients with schizophrenia examined with raclopride-C11 and positron emission tomography. Psychiatry Res 2006;148:165–73.
32. Seeman P, Weinshenker D, Quirion R, et al. Dopamine supersensitivity correlates with D2High states, implying many paths to psychosis. Proc Natl Acad Sci USA 2005;102: 3513–8.
33. Graff A, Agid O, Mizrahi R, et al. The D2/3 high affinity state in drug-free schizophrenic patients. Dopamine 50 Years' Congress, May 30-June 2, Gothenburg, Sweden 2007.
34. Karlsson P, Farde L, Halldin C, Sedvall G. PET study of D(1) dopamine receptor binding in neuroleptic-naive patients with schizophrenia. Am J Psychiatry 2002;159:761–7.
35. Abi-Dargham A, Mawlawi O, Lombardo I, et al. Prefrontal dopamine D1 receptors and working memory in schizophrenia. J Neurosci 2002;22:3708–19.
36. Okubo Y, Suhara T, Suzuki K, et al. Decreased prefrontal dopamine D1 receptors in schizophrenia revealed by PET. Nature 1997;385:634–6.
37. Gurevich EV, Bordelon Y, Shapiro RM, Arnold SE, Gur RE, Joyce JN. Mesolimbic dopamine D3 receptors and use of antipsychotics in patients with schizophrenia. A postmortem study. Arch Gen Psychiatry 1997;54:225–32.
38. Seeman P, Guan H, Van Tol HHM. Dopamine D4 receptors elevated in schizophrenia. Nature 1995;365:441–5.
39. Murray AM, Hyde TM, Knable MB, et al. Distribution of putative D4 dopamine receptors in postmortem striatum from patients with schizophrenia. J Neurosci 1995;15: 2186–91.
40. Sumiyoshi T, Stockmeier CA, Overholser JC, Thompson PA, Meltzer HY. Dopamine D4 receptors and effects of guanine nucleotides on [3H]raclopride binding in postmortem caudate nucleus of subjects with schizophrenia or major depression. Brain Res 1995;681:109–16.
41. Reynolds GP, Mason SL. Are striatal dopamine D4 receptors increased in schizophrenia? J Neurochem 1994;63:1576–7.
42. Reynolds GP, Mason SL. Absence of detectable striatal dopamine D4 receptors in drug-treated schizophrenia. Eur J Pharmacol 1995;281:R5–6.
43. Lahti RA, Roberts RC, Conley RR, Cochrane EV, Mutin A, Tamminga CA. D2-type dopamine receptors in postmortem human brain sections from normal and schizophrenic subjects. Neuroreport 1996;7:1945–8.
44. Lahti RA, Roberts RC, Cochrane EV, et al. Direct determination of dopamine D4 receptors in normal and schizophrenic postmortem brain tissue: a [3H]NGD-94-1 study. Mol Psychiatry 1998;3:528–33.
45. Hietala J, Syvalahti E, Vilkman H, et al. Depressive symptoms and presynaptic dopamine function in neuroleptic-naive schizophrenia. Schizophr Res 1999;35:41–50.
46. Hietala J, Syvalahti E, Vuorio K, et al. Presynaptic dopamine function in striatum of neuroleptic-naive schizophrenic patients. Lancet 1995;346:1130–1.

47. McGowan S, Lawrence AD, Sales T, Quested D, Grasby P. Presynaptic dopaminergic dysfunction in schizophrenia: a positron emission tomographic [18F]fluorodopa study. Arch Gen Psychiatry 2004;61:134–42.
48. Kumakura Y, Cumming P, Vernaleken I, et al. Elevated [18F]fluorodopamine turnover in brain of patients with schizophrenia: an [18F]fluorodopa/positron emission tomography study. J Neurosci 2007;27:8080–7.
49. Abi-Dargham A, Rodenhiser J, Printz D, et al. Increased baseline occupancy of D2 receptors by dopamine in schizophrenia. Proc Natl Acad Sci USA 2000;97:8104–9.
50. Abi-Dargham A, Gil R, Krystal J, et al. Increased striatal dopamine transmission in schizophrenia: confirmation in a second cohort. Am J Psychiatry 1998;155:761–7.
51. Bertolino A, Breier A, Callicott JH, et al. The relationship between dorsolateral prefrontal neuronal N-acetylaspartate and evoked release of striatal dopamine in schizophrenia. Neuropsychopharmacology 2000;22:125–32.
52. Breier A, Su TP, Saunders R, et al. Schizophrenia is associated with elevated amphetamine-induced synaptic dopamine concentrations: evidence from a novel positron emission tomography method. Proc Natl Acad Sci USA 1997;94:2569–74.
53. Laruelle M, Abi-Dargham A, van Dyck CH, et al. Single photon emission computerized tomography imaging of amphetamine-induced dopamine release in drug-free schizophrenic subjects. Proc Natl Acad Sci USA 1996;93:9235–40.
54. Huttunen J, Heinimaa M, Svirskis T, et al. Striatal dopamine synthesis in first-degree relatives of patients with schizophrenia. Biol Psychiatry 2008;63:114–7.
55. Davis KL, Kahn RS, Ko G, Davidson M. Dopamine in schizophrenia: a review and reconceptualization. Am J Psychiatry 1991;148:1474–86.
56. Angrist B, Peselow E, Rubinstein M, Corwin J, Rotrosen J. Partial improvement in negative schizophrenic symptoms after amphetamine. Psychopharmacology (Berl) 1982;78:128–30.
57. Goldman-Rakic PS, Castner SA, Svensson TH, Siever LJ, Williams GV. Targeting the dopamine D1 receptor in schizophrenia: insights for cognitive dysfunction. Psychopharmacology (Berl) 2004;174:3–16.
58. Meyer-Lindenberg A, Miletich RS, Kohn PD, et al. Reduced prefrontal activity predicts exaggerated striatal dopaminergic function in schizophrenia. Nat Neurosci 2002;5:267–71.
59. Homayoun H, Moghaddam B. Bursting of prefrontal cortex neurons in awake rats is regulated by metabotropic glutamate 5 (mGlu5) receptors: rate-dependent influence and interaction with NMDA receptors. Cereb Cortex 2006;16:93–105.
60. Kim JS, Kornhuber HH, Schmid-Burgk W, Holzmuller B. Low cerebrospinal fluid glutamate in schizophrenic patients and a new hypothesis on schizophrenia. Neurosci Lett 1980;20:379–82.
61. Krystal JH, Karper LP, Seibyl JP, et al. Subanesthetic effects of the noncompetitive NMDA antagonist, ketamine, in humans. Psychotomimetic, perceptual, cognitive, and neuroendocrine responses. Arch Gen Psychiatry 1994;51:199–214.
62. Malhotra AK, Pinals DA, Weingartner H, et al. NMDA receptor function and human cognition: the effects of ketamine in healthy volunteers. Neuropsychopharmacology 1996;14:301–7.
63. Lahti AC, Koffel B, LaPorte D, Tamminga CA. Subanesthetic doses of ketamine stimulate psychosis in schizophrenia. Neuropsychopharmacology 1995;13:9–19.
64. Malhotra AK, Pinals DA, Adler CM, et al. Ketamine-induced exacerbation of psychotic symptoms and cognitive impairment in neuroleptic-free schizophrenics. Neuropsycho\-pharmacology 1997;17:141–50.
65. Kristiansen LV, Huerta I, Beneyto M, Meador-Woodruff JH. NMDA receptors and schizophrenia. Curr Opin Pharmacol 2007;7:48–55.
66. Pilowsky LS, Bressan RA, Stone JM, et al. First in vivo evidence of an NMDA receptor deficit in medication-free schizophrenic patients. Mol Psychiatry 2006;11:118–9.

67. Bressan RA, Erlandsson K, Mulligan RS, et al. Evaluation of NMDA receptors in vivo in schizophrenic patients with [123I]CNS 1261 and SPET: preliminary findings. Ann N Y Acad Sci 2003;1003:364–7.
68. Olbrich HM, Valerius G, Rusch N, et al. Frontolimbic glutamate alterations in first episode schizophrenia: Evidence from a magnetic resonance spectroscopy study. World J Biol Psychiatry 2008;9:59–63.
69. van Elst LT, Valerius G, Buchert M, et al. Increased prefrontal and hippocampal glutamate concentration in schizophrenia: evidence from a magnetic resonance spectroscopy study. Biol Psychiatry 2005;58:724–30.
70. Theberge J, Jensen JE, Rowland LM. Regarding "Increased prefrontal and hippocampal glutamate concentration in schizophrenia: evidence from a magnetic resonance spectroscopy study". Biol Psychiatry 2007;61:1218–9; author reply 9–20.
71. Javitt DC, Zukin SR. Recent advances in the phencyclidine model of schizophrenia. Am J Psychiatry 1991;148:1301–8.
72. Olney JW, Farber NB. Glutamate receptor dysfunction and schizophrenia. Arch Gen Psychiatry 1995;52:998–1007.
73. French ED. Effects of phencyclidine on ventral tegmental A10 dopamine neurons in the rat. Neuropharmacology 1986;25:241–8.
74. French ED, Ceci A. Non-competitive N-methyl-D-aspartate antagonists are potent activators of ventral tegmental A10 dopamine neurons. Neurosci Lett 1990;119:159–62.
75. Murase S, Mathe JM, Grenhoff J, Svensson TH. Effects of dizocilpine (MK-801) on rat midbrain dopamine cell activity: differential actions on firing pattern related to anatomical localization. J Neural Transm Gen Sect 1993;91:13–25.
76. Schmidt CJ, Fadayel GM. Regional effects of MK-801 on dopamine release: effects of competitive NMDA or 5-HT2A receptor blockade. J Pharmacol Exp Ther 1996;277: 1541–9.
77. Takahata R, Moghaddam B. Activation of glutamate neurotransmission in the prefrontal cortex sustains the motoric and dopaminergic effects of phencyclidine. Neuropsychopharmacology 2003;28:1117–24.
78. Lorrain DS, Baccei CS, Bristow LJ, Anderson JJ, Varney MA. Effects of ketamine and N-methyl-D-aspartate on glutamate and dopamine release in the rat prefrontal cortex: modulation by a group II selective metabotropic glutamate receptor agonist LY379268. Neuroscience 2003;117:697–706.
79. Bristow LJ, Hutson PH, Thorn L, Tricklebank MD. The glycine/NMDA receptor antagonist, R-(+)-HA-966, blocks activation of the mesolimbic dopaminergic system induced by phencyclidine and dizocilpine (MK-801) in rodents. Br J Pharmacol 1993;108:1156–63.
80. Svensson TH. Dysfunctional brain dopamine systems induced by psychotomimetic NMDA-receptor antagonists and the effects of antipsychotic drugs. Brain Res Brain Res Rev 2000;31:320–9.
81. Jentsch JD, Roth RH. The neuropsychopharmacology of phencyclidine: from NMDA receptor hypofunction to the dopamine hypothesis of schizophrenia. Neuropsychopharmacology 1999;20:201–25.
82. Carlsson A, Hansson LO, Waters N, Carlsson ML. A glutamatergic deficiency model of schizophrenia. Br J Psychiatry Suppl 1999:2–6.
83. Delay J, Deniker P, Harl JM. Utilisation en thérapeutique psychiatrique d'une phénothiazine d'action centrale élective (4560RP). Ann Méd Psychol 1952;110:112–7.
84. Hellewell JS. Treatment-resistant schizophrenia: reviewing the options and identifying the way forward. J Clin Psychiatry 1999;60(Suppl 23):14–9.
85. Kane J, Honigfeld G, Singer J, Meltzer H. Clozapine for the treatment-resistant schizophrenic. A double-blind comparison with chlorpromazine. Arch Gen Psychiatry 1988;45:789–96.
86. Levinson DF, Simpson GM, Singh H, et al. Fluphenazine dose, clinical response, and extrapyramidal symptoms during acute treatment. Arch Gen Psychiatry 1990;47:761–8.

87. Naber D, Karow A. Good tolerability equals good results: the patient's perspective. Eur Neuropsychopharmacol 2001;11(Suppl 4):S391–6.
88. Casey DE. Implications of the CATIE trial on treatment: extrapyramidal symptoms. CNS Spectr 2006;11:25–31.
89. Dossenbach M, Arango-Davila C, Silva Ibarra H, et al. Response and relapse in patients with schizophrenia treated with olanzapine, risperidone, quetiapine, or haloperidol: 12-month follow-up of the Intercontinental Schizophrenia Outpatient Health Outcomes (IC-SOHO) study. J Clin Psychiatry 2005;66:1021–30.
90. Kennedy E, Song F, Hunter R, Clark A, Gilbody S. Risperidone versus typical antipsychotic medication for schizophrenia (Cochrane Review). In: The Cochrane Library, Issue 3. Oxford: Update Software, 2000.
91. Geddes J, Freemantle N, Harrison P, Bebbington P. Atypical antipsychotics in the treatment of schizophrenia: systematic overview and meta-regression analysis. BMJ 2000;321: 1371–6.
92. Dixon LB, Lehman AF, Levine J. Conventional antipsychotic medications for schizophrenia. Schizophr Bull 1995;21:567–77.
93. Oosthuizen P, Emsley RA, Turner J, Keyter N. Determining the optimal dose of haloperidol in first-episode psychosis. J Psychopharmacol 2001;15:251–5.
94. Hippius H. A historical perspective of clozapine. J Clin Psychiatry 1999;60(Suppl 12):22–3.
95. Buchanan RW. Clozapine: efficacy and safety. Schizophr Bull 1995;21:579–91.
96. Miller DD. Review and management of clozapine side effects. J Clin Psychiatry 2000;61(Suppl 8):14–7; discussion 8–9.
97. Rosenheck R, Perlick D, Bingham S, et al. Effectiveness and cost of olanzapine and haloperidol in the treatment of schizophrenia: a randomized controlled trial. Jama 2003;290: 2693–702.
98. Conley RR, Mahmoud R. A randomized double-blind study of risperidone and olanzapine in the treatment of schizophrenia or schizoaffective disorder. Am J Psychiatry 2001;158: 765–74.
99. Chrzanowski WK, Marcus RN, Torbeyns A, Nyilas M, McQuade RD. Effectiveness of long-term aripiprazole therapy in patients with acutely relapsing or chronic, stable schizophrenia: a 52-week, open-label comparison with olanzapine. Psychopharmacology (Berl) 2006;189:259–66.
100. Glick ID, Marder SR. Long-term maintenance therapy with quetiapine versus haloperidol decanoate in patients with schizophrenia or schizoaffective disorder. J Clin Psychiatry 2005;66:638–41.
101. Correll CU, Leucht S, Kane JM. Lower risk for tardive dyskinesia associated with second-generation antipsychotics: a systematic review of 1-year studies. Am J Psychiatry 2004;161:414–25.
102. Haro JM, Salvador-Carulla L. The SOHO (Schizophrenia Outpatient Health Outcome) study: implications for the treatment of schizophrenia. CNS Drugs 2006;20:293–301.
103. Beasley CM, Jr., Tollefson G, Tran P, Satterlee W, Sanger T, Hamilton S. Olanzapine versus placebo and haloperidol: acute phase results of the North American double-blind olanzapine trial. Neuropsychopharmacology 1996;14:111–23.
104. Zimbroff DL, Kane JM, Tamminga CA, et al. Controlled, dose-response study of sertindole and haloperidol in the treatment of schizophrenia. Sertindole Study Group. Am J Psychiatry 1997;154:782–91.
105. Tran PV, Dellva MA, Tollefson GD, Wentley AL, Beasley CM, Jr. Oral olanzapine versus oral haloperidol in the maintenance treatment of schizophrenia and related psychoses. Br J Psychiatry 1998;172:499–505.
106. Csernansky JG, Mahmoud R, Brenner R. A comparison of risperidone and haloperidol for the prevention of relapse in patients with schizophrenia. N Engl J Med 2002;346: 16–22.

107. Newcomer JW. Second-generation (atypical) antipsychotics and metabolic effects: a comprehensive literature review. CNS Drugs 2005;19(Suppl 1):1–93.
108. Luft B, Taylor D. A review of atypical antipsychotic drugs versus conventional medication in schizophrenia. Expert Opin Pharmacother 2006;7:1739–48.
109. Kapur S, Zipursky RB, Remington G. Clinical and theoretical implications of 5-HT2 and D2 receptor occupancy of clozapine, risperidone, and olanzapine in schizophrenia. Am J Psychiatry 1999;156:286–93.
110. Kane JM, Eerdekens M, Lindenmayer JP, Keith SJ, Lesem M, Karcher K. Long-acting injectable risperidone: efficacy and safety of the first long-acting atypical antipsychotic. Am J Psychiatry 2003;160:1125–32.
111. Apiquian R, Fresan A, Herrera K, et al. Minimum effective doses of haloperidol for the treatment of first psychotic episode: a comparative study with risperidone and olanzapine. Int J Neuropsychopharmacol 2003;6:403–8.
112. Jones PB, Barnes TR, Davies L, et al. Randomized controlled trial of the effect on Quality of Life of second- vs first-generation antipsychotic drugs in schizophrenia: Cost Utility of the Latest Antipsychotic Drugs in Schizophrenia Study (CUtLASS 1). Arch Gen Psychiatry 2006;63:1079–87.
113. Lieberman JA, Phillips M, Gu H, et al. Atypical and conventional antipsychotic drugs in treatment-naive first-episode schizophrenia: a 52-week randomized trial of clozapine vs chlorpromazine. Neuropsychopharmacology 2003;28:995–1003.
114. Schooler N, Rabinowitz J, Davidson M, et al. Risperidone and haloperidol in first-episode psychosis: a long-term randomized trial. Am J Psychiatry 2005;162:947–53.
115. Remington G, Kapur S. Atypical antipsychotics: are some more atypical than others? Psychopharmacology (Berl) 2000;148:3–15.
116. Palacios JM, Camps M, Cortes R, Probst A. Mapping dopamine receptors in the human brain. J Neural Transm Suppl 1988;27:227–35.
117. Khan ZU, Gutierrez A, Martin R, Penafiel A, Rivera A, de la Calle A. Dopamine D5 receptors of rat and human brain. Neuroscience 2000;100:689–99.
118. Gurevich EV, Joyce JN. Distribution of dopamine D3 receptor expressing neurons in the human forebrain: comparison with D2 receptor expressing neurons. Neuropsychopharmacology 1999;20:60–80.
119. Lahti RA, Primus RJ, Gallager DW, Roberts R, Tamminga CA. Distribution of dopamine D4 receptor in human postmortem brain sections: autoradiographic studies with [3H]NGD-94-I. Schiz Res 1996;18:173.
120. Seeman P, Chau-Wong M, Tedesco J, Wong K. Brain receptors for antipsychotic drugs and dopamine: direct binding assays. Proc Natl Acad Sci USA 1975;72:4376–80.
121. Seeman P, Lee T, Chau-Wong M, Wong K. Antipsychotic drug doses and neuroleptic/dopamine receptors. Nature 1976;261:717–9.
122. Seeman P. Dopamine receptors and the dopamine hypothesis of schizophrenia. Synapse 1987;1:133–52.
123. Cools AR. Mesolimbic dopamine and its control of locomotor activity in rats: differences in pharmacology and light/dark periodicity between the olfactory tubercle and the nucleus accumbens. Psychopharmacology (Berl) 1986;88:451–9.
124. Kelly PH, Seviour PW, Iversen SD. Amphetamine and apomorphine responses in the rat following 6-OHDA lesions of the nucleus accumbens septi and corpus striatum. Brain Res 1975;94:507–22.
125. Ellenbroek BA. Treatment of schizophrenia: a clinical and preclinical evaluation of neuroleptic drugs. Pharmacol Ther 1993;57:1–78.
126. Bardin L, Auclair A, Kleven MS, et al. Pharmacological profiles in rats of novel antipsychotics with combined dopamine D2/serotonin 5-HT1A activity: comparison with typical and atypical conventional antipsychotics. Behav Pharmacol 2007;18: 103–18.

127. Protais P, Costentin J, Schwartz JC. Climbing behavior induced by apomorphine in mice: a simple test for the study of dopamine receptors in striatum. Psychopharmacology (Berl) 1976;50:1–6.
128. Gerhardt S, Gerber R, Liebman JM. SCH 23390 dissociated from conventional neuroleptics in apomorphine climbing and primate acute dyskinesia models. Life Sci 1985;37: 2355–63.
129. Ogren SO. The behavioural pharmacology of typical and atypical antipsychotic drugs. In: Csernansky JG, ed. Antipsychotics. Berlin: Springer, 1996: pp. 225–66.
130. Bardin L, Kleven MS, Barret-Grevoz C, Depoortere R, Newman-Tancredi A. Antipsychotic-like vs cataleptogenic actions in mice of novel antipsychotics having D2 antagonist and 5-HT1A agonist properties. Neuropsychopharmacology 2006;31:1869–79.
131. Newman-Tancredi A, Assie MB, Leduc N, Ormiere AM, Danty N, Cosi C. Novel antipsychotics activate recombinant human and native rat serotonin 5-HT1A receptors: affinity, efficacy and potential implications for treatment of schizophrenia. Int J Neuropsychopharmacol 2005;8:341–56.
132. Wadenberg ML, Hicks PB. The conditioned avoidance response test re-evaluated: is it a sensitive test for the detection of potentially atypical antipsychotics? Neurosci Biobehav Rev 1999;23:851–62.
133. Natesan S, Reckless GE, Nobrega JN, Fletcher PJ, Kapur S. Dissociation between in vivo occupancy and functional antagonism of dopamine D2 receptors: comparing aripiprazole to other antipsychotics in animal models. Neuropsychopharmacology 2006;31:1854–63.
134. Janssen PA, Niemegeers CJ, Schellekens KH. Is It Possible to Predict the Clinical Effects of Neuroleptic Drugs (Major Tranquillizers) from Animal Data?I. "Neuroleptic Activity Spectra" for Rats. Arzneimittelforschung 1965;15:104–17.
135. Arnt J. Pharmacological specificity of conditioned avoidance response inhibition in rats: inhibition by neuroleptics and correlation to dopamine receptor blockade. Acta Pharmacol Toxicol (Copenh) 1982;51:321–9.
136. Wadenberg ML, Ericson E, Magnusson O, Ahlenius S. Suppression of conditioned avoidance behavior by the local application of (-)sulpiride into the ventral, but not the dorsal, striatum of the rat. Biol Psychiatry 1990;28:297–307.
137. Wadenberg ML, Kapur S, Soliman A, Jones C, Vaccarino F. Dopamine D2 receptor occupancy predicts catalepsy and the suppression of conditioned avoidance response behavior in rats. Psychopharmacology (Berl) 2000;150:422–9.
138. Wadenberg ML, Soliman A, VanderSpek SC, Kapur S. Dopamine D(2) receptor occupancy is a common mechanism underlying animal models of antipsychotics and their clinical effects. Neuropsychopharmacology 2001;25:633–41.
139. Wadenberg ML. Serotonergic mechanisms in neuroleptic-induced catalepsy in the rat. Neurosci Biobehav Rev 1996;20:325–39.
140. Tang AH, Franklin SR, Himes CS, Smith MW, Tenbrink RE. PNU-96415E, a potential antipsychotic agent with clozapine-like pharmacological properties. J Pharmacol Exp Ther 1997;281:440–7.
141. Ogren SO, Archer T. Effects of typical and atypical antipsychotic drugs on two-way active avoidance. Relationship to DA receptor blocking profile. Psychopharmacology (Berl) 1994;114:383–91.
142. Deutch AY, Lee MC, Iadarola MJ. Regionally specific effects of atypical antipsychotic drugs on striatal Fos expression: the nucleus accumbens shell as a locus of antipsychotic action. Mol Cell Neuroscience 1992;3:332–41.
143. MacGibbon GA, Lawlor PA, Bravo R, Dragunow M. Clozapine and haloperidol produce a differential pattern of immediate early gene expression in rat caudate-putamen, nucleus accumbens, lateral septum and islands of Calleja. Brain Res Mol Brain Res 1994;23:21–32.
144. Robertson GS, Fibiger HC. Neuroleptics increase c-fos expression in the forebrain: contrasting effects of haloperidol and clozapine. Neuroscience 1992;46:315–28.

145. Robertson GS, Matsumura H, Fibiger HC. Induction patterns of Fos-like immunoreactivity in the forebrain as predictors of atypical antipsychotic activity. J Pharmacol Exp Ther 1994;271:1058–66.
146. Wan W, Ennulat DJ, Cohen BM. Acute administration of typical and atypical antipsychotic drugs induces distinctive patterns of Fos expression in the rat forebrain. Brain Res 1995;688:95–104.
147. Miller JC. Induction of c-fos mRNA expression in rat striatum by neuroleptic drugs. J Neurochem 1990;54:1453–5.
148. Graybiel AM, Moratalla R, Robertson HA. Amphetamine and cocaine induce drug-specific activation of the c-fos gene in striosome-matrix compartments and limbic subdivisions of the striatum. Proc Natl Acad Sci USA 1990;87:6912–6.
149. Robertson GS, Vincent SR, Fibiger HC. D1 and D2 dopamine receptors differentially regulate c-fos expression in striatonigral and striatopallidal neurons. Neuroscience 1992;49: 285–96.
150. Kapur S, Seeman P. Does fast dissociation from the dopamine d(2) receptor explain the action of atypical antipsychotics?: A new hypothesis. Am J Psychiatry 2001;158: 360–9.
151. Kapur S, Zipursky R, Jones C, Remington G, Houle S. Relationship between dopamine D(2) occupancy, clinical response, and side effects: a double-blind PET study of first-episode schizophrenia. Am J Psychiatry 2000;157:514–20.
152. Farde L, Nordstrom AL, Wiesel FA, Pauli S, Halldin C, Sedvall G. Positron emission tomographic analysis of central D1 and D2 dopamine receptor occupancy in patients treated with classical neuroleptics and clozapine. Relation to extrapyramidal side effects. Arch Gen Psychiatry 1992;49:538–44.
153. Farde L, Wiesel FA, Nordstrom AL, Sedvall G. D1- and D2-dopamine receptor occupancy during treatment with conventional and atypical neuroleptics. Psychopharmacology (Berl) 1989;99:S28–31.
154. Farde L, Mack RJ, Nyberg S, Halldin C. D2 occupancy, extrapyramidal side effects and antipsychotic drug treatment: a pilot study with sertindole in healthy subjects. Int Clin Psychopharmacol 1997;12(Suppl 1):S3–7.
155. Nordstrom AL, Farde L, Nyberg S, Karlsson P, Halldin C, Sedvall G. D1, D2, and 5-HT2 receptor occupancy in relation to clozapine serum concentration: a PET study of schizophrenic patients. Am J Psychiatry 1995;152:1444–9.
156. Kapur S, Zipursky R, Jones C, Shammi CS, Remington G, Seeman P. A positron emission tomography study of quetiapine in schizophrenia: a preliminary finding of an antipsychotic effect with only transiently high dopamine D2 receptor occupancy. Arch Gen Psychiatry 2000;57:553–9.
157. Farde L, Wiesel FA, Halldin C, Sedvall G. Central D2-dopamine receptor occupancy in schizophrenic patients treated with antipsychotic drugs. Arch Gen Psychiatry 1988;45: 71–6.
158. Kapur S, Zipursky RB, Remington G, et al. 5-HT2 and D2 receptor occupancy of olanzapine in schizophrenia: a PET investigation. Am J Psychiatry 1998;155:921–8.
159. Busatto GF, Pilowsky LS, Costa DC, Ell PJ, Verhoeff NP, Kerwin RW. Dopamine D2 receptor blockade in vivo with the novel antipsychotics risperidone and remoxipride – an 123I-IBZM single photon emission tomography (SPET) study. Psychopharmacology (Berl) 1995;117:55–61.
160. Nordstrom AL, Nyberg S, Olsson H, Farde L. Positron emission tomography finding of a high striatal D2 receptor occupancy in olanzapine-treated patients. Arch Gen Psychiatry 1998;55:283–4.
161. Tauscher J, Kufferle B, Asenbaum S, et al. In vivo 123I IBZM SPECT imaging of striatal dopamine-2 receptor occupancy in schizophrenic patients treated with olanzapine in comparison to clozapine and haloperidol. Psychopharmacology (Berl) 1999;141: 175–81.

162. Knable MB, Heinz A, Raedler T, Weinberger DR. Extrapyramidal side effects with risperidone and haloperidol at comparable D2 receptor occupancy levels. Psychiatry Res 1997;75:91–101.
163. Jauss M, Schroder J, Pantel J, Bachmann S, Gerdsen I, Mundt C. Severe akathisia during olanzapine treatment of acute schizophrenia. Pharmacopsychiatry 1998;31:146–8.
164. Tamminga CA, Gotts MD, Thaker GK, Alphs LD, Foster NL. Dopamine agonist treatment of schizophrenia with N-propylnorapomorphine. Arch Gen Psychiatry 1986;43:398–402.
165. Tamminga CA. Partial dopamine agonists in the treatment of psychosis. J Neural Transm 2002;109:411–20.
166. Clark D, Hjorth S, Carlsson A. Dopamine-receptor agonists: mechanisms underlying autoreceptor selectivity. I. Review of the evidence. J Neural Transm 1985;62:1–52.
167. Lahti RA, Mutin A, Cochrane EV, et al. Affinities and intrinsic activities of dopamine receptor agonists for the hD21 and hD4.4 receptors. Eur J Pharmacol 1996;301:R11–3.
168. Lahti AC, Weiler MA, Corey PK, Lahti RA, Carlsson A, Tamminga CA. Antipsychotic properties of the partial dopamine agonist (-)-3-(3-hydroxyphenyl)-N-n-propylpiperidine(preclamol) in schizophrenia. Biol Psychiatry 1998;43:2–11.
169. Wetzel H, Hillert A, Grunder G, Benkert O. Roxindole, a dopamine autoreceptor agonist, in the treatment of positive and negative schizophrenic symptoms. Am J Psychiatry 1994;151:1499–502.
170. Ohmori T, Koyama T, Inoue T, Matsubara S, Yamashita I. B-HT 920, a dopamine D2 agonist, in the treatment of negative symptoms of chronic schizophrenia. Biol Psychiatry 1993;33:687–93.
171. Olbrich R, Schanz H. An evaluation of the partial dopamine agonist terguride regarding positive symptoms reduction in schizophrenics. J Neural Transm Gen Sect 1991;84: 233–6.
172. Naber D, Gaussares C, Moeglen JM, Tremmel L, Bailey PE, Group tSHCS. Efficacy and tolerability of SDZ HDC 912, a partial dopamine D-2 agonist, in the treatment of schizophrenia. In: Meltzer HY, ed. Novel Antipsychotic Drugs. New York: Raven Press, 1992: pp. 99–107.
173. DeLeon A, Patel NC, Crismon ML. Aripiprazole: a comprehensive review of its pharmacology, clinical efficacy, and tolerability. Clin Ther 2004;26:649–66.
174. Lieberman JA. Aripiprazole. In: Schatzberg AF, Nemeroff CB, eds. Texbook of Psychopharmacology, 3rd edition. Washington: The American Psychiatric Press, Inc, 2004:pp. 487–94.
175. Kikuchi T, Tottori K, Uwahodo Y, et al. 7-(4-[4-(2,3-Dichlorophenyl)-1-piperazinyl]butyloxy)-3,4-dihydro-2(1H)-qui nolinone (OPC-14597), a new putative antipsychotic drug with both presynaptic dopamine autoreceptor agonistic activity and postsynaptic D2 receptor antagonistic activity. J Pharmacol Exp Ther 1995;274:329–36.
176. Natesan S, Reckless GE, Barlow KB, Nobrega JN, Kapur S. Evaluation of N-desmethylclozapine as a potential antipsychotic – preclinical studies. Neuropsycho\-pharmacology 2007;32:1540–9.
177. Kane JM, Carson WH, Saha AR, et al. Efficacy and safety of aripiprazole and haloperidol versus placebo in patients with schizophrenia and schizoaffective disorder. J Clin Psychiatry 2002;63:763–71.
178. Burris KD, Molski TF, Xu C, et al. Aripiprazole, a novel antipsychotic, is a high-affinity partial agonist at human dopamine D2 receptors. J Pharmacol Exp Ther 2002;302: 381–9.
179. Grunder G, Fellows C, Janouschek H, et al. Brain and Plasma Pharmacokinetics of Aripiprazole in Patients With Schizophrenia: An [18F]Fallypride PET Study. Am J Psychiatry 2008;165:988–995.
180. Mamo D, Graff A, Mizrahi R, Shammi CM, Romeyer F, Kapur S. Differential effects of aripiprazole on D(2), 5-HT(2), and 5-HT(1A) receptor occupancy in patients with schizophrenia: a triple tracer PET study. Am J Psychiatry 2007;164:1411–7.

181. Burstein ES, Ma J, Wong S, et al. Intrinsic efficacy of antipsychotics at human D2, D3, and D4 dopamine receptors: identification of the clozapine metabolite N-desmethylclozapine as a D2/D3 partial agonist. J Pharmacol Exp Ther 2005;315:1278–87.
182. Weiner DM, Meltzer HY, Veinbergs I, et al. The role of M1 muscarinic receptor agonism of N-desmethylclozapine in the unique clinical effects of clozapine. Psychopharmacology (Berl) 2004;177:207–16.
183. Chipkin RE, Iorio LC, Coffin VL, McQuade RD, Berger JG, Barnett A. Pharmacological profile of SCH39166: a dopamine D1 selective benzonaphthazepine with potential antipsychotic activity. J Pharmacol Exp Ther 1988;247:1093–102.
184. Tauscher J, Hussain T, Agid O, et al. Equivalent occupancy of dopamine D1 and D2 receptors with clozapine: differentiation from other atypical antipsychotics. Am J Psychiatry 2004;161:1620–5.
185. Nordstrom AL, Farde L, Nyberg S, Karlsson P, Halldin C, Sedvall G. D1, D2, and 5-HT2 receptor occupancy in relation to clozapine serum concentration: a PET study of schizophrenic patients. Am J Psychiatry 1995;152:1444–9.
186. Karle J, Clemmesen L, Hansen L, et al. NNC 01-0687, a selective dopamine D1 receptor antagonist, in the treatment of schizophrenia. Psychopharmacology (Berl) 1995;121: 328–9.
187. Karlsson P, Smith L, Farde L, Harnryd C, Sedvall G, Wiesel FA. Lack of apparent antipsychotic effect of the D1-dopamine receptor antagonist SCH39166 in acutely ill schizophrenic patients. Psychopharmacology (Berl) 1995;121:309–16.
188. de Beaurepaire R, Labelle A, Naber D, Jones BD, Barnes TR. An open trial of the D1 antagonist SCH 39166 in six cases of acute psychotic states. Psychopharmacology (Berl) 1995;121:323–7.
189. Farde L, Nordstrom AL. PET analysis indicates atypical central dopamine receptor occupancy in clozapine-treated patients. Br J Psychiatry Suppl 1992:30–3.
190. Clinton SM, Sucharski IL, Finlay JM. Desipramine attenuates working memory impairments induced by partial loss of catecholamines in the rat medial prefrontal cortex. Psychopharmacology (Berl) 2006;183:404–12.
191. Sawaguchi T. The effects of dopamine and its antagonists on directional delay-period activity of prefrontal neurons in monkeys during an oculomotor delayed-response task. Neurosci Res 2001;41:115–28.
192. Sawaguchi T, Matsumura M, Kubota K. Dopamine enhances the neuronal activity of spatial short-term memory task in the primate prefrontal cortex. Neurosci Res 1988;5:465–73.
193. Brozoski TJ, Brown RM, Rosvold HE, Goldman PS. Cognitive deficit caused by regional depletion of dopamine in prefrontal cortex of rhesus monkey. Science 1979;205:929–32.
194. Sawaguchi T, Goldman-Rakic PS. D1 dopamine receptors in prefrontal cortex: involvement in working memory. Science 1991;251:947–50.
195. Sawaguchi T, Goldman-Rakic PS. The role of D1-dopamine receptor in working memory: local injections of dopamine antagonists into the prefrontal cortex of rhesus monkeys performing an oculomotor delayed-response task. J Neurophysiol 1994;71:515–28.
196. Williams GV, Goldman-Rakic PS. Modulation of memory fields by dopamine D1 receptors in prefrontal cortex. Nature 1995;376:572–5.
197. Castner SA, Williams GV, Goldman-Rakic PS. Reversal of antipsychotic-induced working memory deficits by short-term dopamine D1 receptor stimulation. Science 2000;287: 2020–2.
198. Floresco SB, Phillips AG. Delay-dependent modulation of memory retrieval by infusion of a dopamine D1 agonist into the rat medial prefrontal cortex. Behav Neurosci 2001;115: 934–9.
199. Seamans JK, Yang CR. The principal features and mechanisms of dopamine modulation in the prefrontal cortex. Prog Neurobiol 2004;74:1–58.
200. Goldman-Rakic PS, Muly EC, 3rd, Williams GV. D(1) receptors in prefrontal cells and circuits. Brain Res Brain Res Rev 2000;31:295–301.

201. Oerther S, Ahlenius S. Atypical antipsychotics and dopamine D(1) receptor agonism: an in vivo experimental study using core temperature measurements in the rat. J Pharmacol Exp Ther 2000;292:731–6.
202. Salmi P, Karlsson T, Ahlenius S. Antagonism by SCH 23390 of clozapine-induced hypothermia in the rat. Eur J Pharmacol 1994;253:67–73.
203. Salmi P, Malmgren K, Svensson TH, Ahlenius S. Stimulation of forward locomotion by SCH-23390 and raclopride in d-amphetamine-treated rats. Naunyn Schmiedebergs Arch Pharmacol 1998;357:593–9.
204. Reavill C, Taylor SG, Wood MD, et al. Pharmacological actions of a novel, high-affinity, and selective human dopamine D(3) receptor antagonist, SB-277011-A. J Pharmacol Exp Ther 2000;294:1154–65.
205. Ashby CR, Jr., MinabeY, Stemp G, Hagan JJ, Middlemiss DN. Acute and chronic administration of the selective D(3) receptor antagonist SB-277011-A alters activity of midbrain dopamine neurons in rats: an in vivo electrophysiological study. J Pharmacol Exp Ther 2000;294:1166–74.
206. Chiodo LA, Bunney BS. Typical and atypical neuroleptics: differential effects of chronic administration on the activity of A9 and A10 midbrain dopaminergic neurons. J Neurosci 1983;3:1607–19.
207. White FJ, Wang RY. Differential effects of classical and atypical antipsychotic drugs on A9 and A10 dopamine neurons. Science 1983;221:1054–7.
208. Van Tol HH, Bunzow JR, Guan HC, et al. Cloning of the gene for a human dopamine D4 receptor with high affinity for the antipsychotic clozapine. Nature 1991;350:610–4.
209. Roth BL, Tandra S, Burgess LH, Sibley DR, Meltzer HY. D4 dopamine receptor binding affinity does not distinguish between typical and atypical antipsychotic drugs. Psychopharmacology (Berl) 1995;120:365–8.
210. Kramer MS, Last B, Getson A, Reines SA. The effects of a selective D4 dopamine receptor antagonist (L-745,870) in acutely psychotic inpatients with schizophrenia. D4 Dopamine Antagonist Group. Arch Gen Psychiatry 1997;54:567–72.
211. Bristow LJ, Collinson N, Cook GP, et al. L-745,870, a subtype selective dopamine D4 receptor antagonist, does not exhibit a neuroleptic-like profile in rodent behavioral tests. J Pharmacol Exp Ther 1997;283:1256–63.
212. Corrigan MH, Gallen CC, Bonura ML, Merchant KM. Effectiveness of the selective D4 antagonist sonepiprazole in schizophrenia: a placebo-controlled trial. Biol Psychiatry 2004;55:445–51.
213. Grace AA, Bunney BS, Moore H, Todd CL. Dopamine-cell depolarization block as a model for the therapeutic actions of antipsychotic drugs. Trends Neurosci 1997;20:31–7.
214. Kuhar MJ, Joyce AR. Slow onset of CNS drugs: can changes in protein concentration account for the delay? Trends Pharmacol Sci 2001;22:450–6.
215. Nordstrom AL, Farde L, Halldin C. Time course of D2-dopamine receptor occupancy examined by PET after single oral doses of haloperidol. Psychopharmacology (Berl) 1992;106:433–8.
216. Tauscher J, Jones C, Remington G, Zipursky RB, Kapur S. Significant dissociation of brain and plasma kinetics with antipsychotics. Mol Psychiatry 2002;7:317–21.
217. Agid O, Kapur S, Arenovich T, Zipursky RB. Delayed-onset hypothesis of antipsychotic action: a hypothesis tested and rejected. Arch Gen Psychiatry 2003;60:1228–35.
218. Agid O, Seeman P, Kapur S. The "delayed onset" of antipsychotic action – an idea whose time has come and gone. J Psychiatry Neurosci 2006;31:93–100.
219. Pae CU, Kim JJ, Lee CU, et al. Rapid versus conventional initiation of quetiapine in the treatment of schizophrenia: a randomized, parallel-group trial. J Clin Psychiatry 2007;68:399–405.
220. Small JG, Kolar MC, Kellams JJ. Quetiapine in schizophrenia: onset of action within the first week of treatment. Curr Med Res Opin 2004;20:1017–23.

221. Leucht S, Busch R, Hamann J, Kissling W, Kane JM. Early-onset hypothesis of antipsychotic drug action: a hypothesis tested, confirmed and extended. Biol Psychiatry 2005;57:1543–9.
222. Daniel DG, Zimbroff DL, Potkin SG, Reeves KR, Harrigan EP, Lakshminarayanan M. Ziprasidone 80 mg/day and 160 mg/day in the acute exacerbation of schizophrenia and schizoaffective disorder: a 6-week placebo-controlled trial. Ziprasidone Study Group. Neuropsychopharmacology 1999;20:491–505.
223. Potkin SG, Saha AR, Kujawa MJ, et al. Aripiprazole, an antipsychotic with a novel mechanism of action, and risperidone vs placebo in patients with schizophrenia and schizoaffective disorder. Arch Gen Psychiatry 2003;60:681–90.
224. Kapur S, Mizrahi R, Li M. From dopamine to salience to psychosis – linking biology, pharmacology and phenomenology of psychosis. Schizophr Res 2005;79:59–68.
225. Margolese HC, Chouinard G, Beauclair L, Belanger MC. Therapeutic tolerance and rebound psychosis during quetiapine maintenance monotherapy in patients with schizophrenia and schizoaffective disorder. J Clin Psychopharmacol 2002;22:347–52.
226. Meltzer HY, Lee MA, Ranjan R, Mason EA, Cola PA. Relapse following clozapine withdrawal: effect of neuroleptic drugs and cyproheptadine. Psychopharmacology (Berl) 1996;124:176–87.
227. Ekblom B, Eriksson K, Lindstrom LH. Supersensitivity psychosis in schizophrenic patients after sudden clozapine withdrawal. Psychopharmacology (Berl) 1984;83:293–4.
228. Llorca PM, Vaiva G, Lancon C. Supersensitivity psychosis in patients with schizophrenia after sudden olanzapine withdrawal. Can J Psychiatry 2001;46:87–8.
229. Kahne GJ. Rebound psychoses following the discontinuation of a high potency neuroleptic. Can J Psychiatry 1989;34:227–9.
230. Prien RF, Cole JO, Belkin NF. Relapse in chronic schizophrenics following abrupt withdrawal of tranquillizing medication. Br J Psychiatry 1969;115:679–86.
231. Chouinard G, Jones BD, Annable L. Neuroleptic-induced supersensitivity psychosis. Am J Psychiatry 1978;135:1409–10.
232. Chouinard G, Jones BD. Neuroleptic-induced supersensitivity psychosis: clinical and pharmacologic characteristics. Am J Psychiatry 1980;137:16–21.
233. Geurts M, Hermans E, Maloteaux JM. Enhanced striatal dopamine D(2) receptor-induced [35S]GTPgammaS binding after haloperidol treatment. Eur J Pharmacol 1999;382:119–27.
234. Gianutsos G, Drawbaugh RB, Hynes MD, Lal H. Behavioral evidence for dopaminergic supersensitivity after chronic haloperidol. Life Sci 1974;14:887–98.
235. Montanaro N, Dall'Olio R, Gandolfi O, Vaccheri A. Differential enhancement of behavioral sensitivity to apomorphine following chronic treatment of rats with (-)-sulpiride and haloperidol. Eur J Pharmacol 1982;81:1–9.
236. Sayers AC, Burki HR, Ruch W, Asper H. Neuroleptic-induced hypersensitivity of striatal dopamine receptors in the rat as a model of tardive dyskinesias. Effects of clozapine, haloperidol, loxapine and chlorpromazine. Psychopharmacologia 1975;41:97–104.
237. Smith RC, Davis JM. Behavioral evidence for supersensitivity after chronic administration of haloperidol, clozapine, and thioridazine. Life Sci 1976;19:725–31.
238. Seeger TF, Thal L, Gardner EL. Behavioral and biochemical aspects of neuroleptic-induced dopaminergic supersensitivity: studies with chronic clozapine and haloperidol. Psychopharmacology (Berl) 1982;76:182–7.
239. Smith RC, Davis JM. Behavioral supersensitivity to apomorphine and amphetamine after chronic high dose haloperidol treatment. Psychopharmacol Commun 1975;1:285–93.
240. Meng ZH, Feldpaush DL, Merchant KM. Clozapine and haloperidol block the induction of behavioral sensitization to amphetamine and associated genomic responses in rats. Brain Res Mol Brain Res 1998;61:39–50.
241. Rebec GV, Peirson EE, McPherson FA, Brugge K. Differential sensitivity to amphetamine following long-term treatment with clozapine or haloperidol. Psychopharmacology (Berl) 1982;77:360–6.

242. Halperin R, Guerin JJ, Jr., Davis KL. Chronic administration of three neuroleptics: effects of behavioral supersensitivity mediated by two different brain regions in the rat. Life Sci 1983;33:585–92.
243. Liskowsky DR, Potter LT. Dopamine D2 receptors in the striatum and frontal cortex following chronic administration of haloperidol. Neuropharmacology 1987;26:481–3.
244. Srivastava LK, Morency MA, Bajwa SB, Mishra RK. Effect of haloperidol on expression of dopamine D2 receptor mRNAs in rat brain. J Mol Neurosci 1990;2:155–61.
245. Young KA, Zavodny R, Hicks PB. Subchronic buspirone, mesulergine, and ICS 205-930 lack effects on D1 and D2 dopamine binding in the rat striatum during chronic haloperidol treatment. J Neural Transm Gen Sect 1991;86:223–8.
246. Sakai K, Gao XM, Hashimoto T, Tamminga CA. Traditional and new antipsychotic drugs differentially alter neurotransmission markers in basal ganglia-thalamocortical neural pathways. Synapse 2001;39:152–60.
247. Silvestri S, Seeman MV, Negrete JC, et al. Increased dopamine D2 receptor binding after long-term treatment with antipsychotics in humans: a clinical PET study. Psychopharmacology (Berl) 2000;152:174–80.
248. Dean B, Hussain T, Scarr E, Pavey G, Copolov DL. Extended treatment with typical and atypical antipsychotic drugs differential effects on the densities of dopamine D2-like and GABAA receptors in rat striatum. Life Sci 2001;69:1257–68.
249. Tarazi FI, Zhang K, Baldessarini RJ. Long-term effects of olanzapine, risperidone, and quetiapine on dopamine receptor types in regions of rat brain: implications for antipsychotic drug treatment. J Pharmacol Exp Ther 2001;297:711–7.
250. Klawans HL, Jr., Rubovits R. An experimental model of tardive dyskinesia. J Neural Transm 1972;33:235–46.
251. Tarsy D, Baldessarini RJ. The pathophysiologic basis of tardive dyskinesia. Biol Psychiatry 1977;12:431–50.
252. Creese I, Snyder S. Chronic neuroleptic treatment and dopamine receptor regulation. In: Cattebeni F, Racagani G, Spano P, Coata E, eds. Long-Term Effects of Neuroleptics. Adv Biochem Psychopharmacol. New York: Raven Press, 1980:89–94.
253. Davis KL, Rosenberg GS. Is there a limbic system equivalent of tardive dyskinesia? Biol Psychiatry 1979;14:699–703.
254. Kolbe H, Clow A, Jenner P, Marsden CD. Neuroleptic-induced acute dystonic reactions may be due to enhanced dopamine release on to supersensitive postsynaptic receptors. Neurology 1981;31:434–9.
255. Tsai G, Goff DC, Chang RW, Flood J, Baer L, Coyle JT. Markers of glutamatergic neurotransmission and oxidative stress associated with tardive dyskinesia. Am J Psychiatry 1998;155:1207–13.
256. Casey DE. Tardive dyskinesia: pathophysiology and animal models. J Clin Psychiatry 2000;61(Suppl 4):5–9.
257. Lohr JB, Kuczenski R, Niculescu AB. Oxidative mechanisms and tardive dyskinesia. CNS Drugs 2003;17:47–62.
258. Fernandez HH, Friedman JH. Classification and treatment of tardive syndromes. Neurologist 2003;9:16–27.
259. Kenney C, Hunter C, Jankovic J. Long-term tolerability of tetrabenazine in the treatment of hyperkinetic movement disorders. Mov Disord 2007;22:193–7.
260. Rauchverger B, Isakov V, Jabarin M. Olanzapine-induced tardive dystonia successfully treated by tetrabenazine. J Neuropsychiatry Clin Neurosci 2007;19:484–5.
261. Halperin R, Guerin JJ, Jr., Davis KL. Regional differences in the induction of behavioral supersensitivity by prolonged treatment with atypical neuroleptics. Psychopharmacology (Berl) 1989;98:386–91.
262. Rupniak NM, Hall MD, Mann S, et al. Chronic treatment with clozapine, unlike haloperidol, does not induce changes in striatal D-2 receptor function in the rat. Biochem Pharmacol 1985;34:2755–63.

263. Florijn WJ, Tarazi FI, Creese I. Dopamine receptor subtypes: differential regulation after 8 months treatment with antipsychotic drugs. J Pharmacol Exp Ther 1997;280:561–9.
264. Baldessarini RJ, Frankenburg FR. Clozapine. A novel antipsychotic agent. N Engl J Med 1991;324:746–54.
265. Butkerait P, Wang HY, Friedman E. Increases in guanine nucleotide binding to striatal G proteins is associated with dopamine receptor supersensitivity. J Pharmacol Exp Ther 1994;271:422–8.
266. Schettini G, Ventra C, Florio T, Grimaldi M, Meucci O, Marino A. Modulation by GTP of basal and agonist-stimulated striatal adenylate cyclase activity following chronic blockade of D1 and D2 dopamine receptors: involvement of G proteins in the development of receptor supersensitivity. J Neurochem 1992;59:1667–74.
267. Marcotte ER, Sullivan RM, Mishra RK. Striatal G-proteins: effects of unilateral 6-hydroxydopamine lesions. Neurosci Lett 1994;169:195–8.
268. Hall H, Sallemark M. Effects of chronic neuroleptic treatment on agonist affinity states of the dopamine-D2 receptor in the rat brain. Pharmacol Toxicol 1987;60:359–63.
269. Olianas MC, Onali P. Supersensitivity of striatal D2 dopamine receptors mediating inhibition of adenylate cyclase and stimulation of guanosine triphosphatase following chronic administration of haloperidol in mice. Neurosci Lett 1987;78:349–54.
270. Treisman GJ, Muirhead N, Gnegy ME. Increased sensitivity of adenylate cyclase activity in the striatum of the rat to calmodulin and GppNHp after chronic treatment with haloperidol. Neuropharmacology 1986;25:587–95.
271. Memo M, Pizzi M, Missale C, Carruba MO, Spano PF. Modification of the function of D1 and D2 dopamine receptors in striatum and nucleus accumbens of rats chronically treated with haloperidol. Neuropharmacology 1987;26:477–80.
272. Dewey KJ, Fibiger HC. The effects of dose and duration of chronic pimozide administration on dopamine receptor supersensitivity. Naunyn Schmiedebergs Arch Pharmacol 1983;322:261–70.
273. Samaha AN, Seeman P, Stewart J, Rajabi H, Kapur S. "Breakthrough" dopamine supersensitivity during ongoing antipsychotic treatment leads to treatment failure over time. J Neurosci 2007;27:2979–86.
274. Nilsson CL, Eriksson E. Haloperidol increases prolactin release and cyclic AMP formation in vitro: inverse agonism at dopamine D2 receptors? J Neural Transm Gen Sect 1993;92:213–20.
275. Hall DA, Strange PG. Evidence that antipsychotic drugs are inverse agonists at D2 dopamine receptors. Br J Pharmacol 1997;121:731–6.
276. Wilson J, Lin H, Fu D, Javitch JA, Strange PG. Mechanisms of inverse agonism of antipsychotic drugs at the D(2) dopamine receptor: use of a mutant D(2) dopamine receptor that adopts the activated conformation. J Neurochem 2001;77:493–504.
277. Kozell LB, Neve KA. Constitutive activity of a chimeric D2/D1 dopamine receptor. Mol Pharmacol 1997;52:1137–49.
278. Akam E, Strange PG. Inverse agonist properties of atypical antipsychotic drugs. Biochem Pharmacol 2004;67:2039–45.
279. Lefkowitz RJ, Cotecchia S, Samama P, Costa T. Constitutive activity of receptors coupled to guanine nucleotide regulatory proteins. Trends Pharmacol Sci 1993;14:303–7.
280. Strange PG. Inverse agonism of the antipsychotic drugs at the D2 dopamine receptor. International Congress Series 2003;1249:153–62.
281. Roberts DJ, Strange PG. Mechanisms of inverse agonist action at D2 dopamine receptors. Br J Pharmacol 2005;145:34–42.
282. Kapur S, Remington G. Dopamine D(2) receptors and their role in atypical antipsychotic action: still necessary and may even be sufficient. Biol Psychiatry 2001;50:873–83.
283. Kapur S, Seeman P. Antipsychotic agents differ in how fast they come off the dopamine D2 receptors. Implications for atypical antipsychotic action. J Psychiatry Neurosci 2000;25:161–6.

284. Seeman P. Atypical antipsychotics: mechanism of action. Focus 2004;2:48–58.
285. Gefvert O, Bergstrom M, Langstrom B, Lundberg T, Lindstrom L, Yates R. Time course of central nervous dopamine-D2 and 5-HT2 receptor blockade and plasma drug concentrations after discontinuation of quetiapine (Seroquel) in patients with schizophrenia. Psychopharmacology (Berl) 1998;135:119–26.
286. See RE, Toga AW, Ellison G. Autoradiographic analysis of regional alterations in brain receptors following chronic administration and withdrawal of typical and atypical neuroleptics in rats. J Neural Transm Gen Sect 1990;82:93–109.
287. Saller CF, Salama AI. Seroquel: biochemical profile of a potential atypical antipsychotic. Psychopharmacology 1993;112:285–92.
288. Parsons B, Togasaki DM, Kassir S, Przedborski S. Neuroleptics up-regulate adenosine A2a receptors in rat striatum: implications for the mechanism and the treatment of tardive dyskinesia. J Neurochem 1995;65:2057–64.
289. Lidow MS, Goldman-Rakic PS. Differential regulation of D2 and D4 dopamine receptor mRNAs in the primate cerebral cortex vs. neostriatum: effects of chronic treatment with typical and atypical antipsychotic drugs. J Pharmacol Exp Ther 1997;283:939–46.
290. Ginovart N, Wilson A, Hussey D, Houle S, Kapur S. D2-receptor upregulation is dependent upon temporal course of D2-occupancy: a longitudinal [11C]-raclopride PET study in cats. Neuropsychopharmacology 2009;34:662–671.
291. Turrone P, Remington G, Kapur S, Nobrega JN. Differential effects of within-day continuous vs. transient dopamine D2 receptor occupancy in the development of vacuous chewing movements (VCMs) in rats. Neuropsychopharmacology 2003;28:1433–9.
292. Goldstein JM, Litwin LC, Sutton EB, Malick JB. Seroquel: electrophysiological profile of a potential atypical antipsychotic. Psychopharmacology (Berl) 1993;112:293–8.
293. Stockton ME, Rasmussen K. Electrophysiological effects of olanzapine, a novel atypical antipsychotic, on A9 and A10 dopamine neurons. Neuropsychopharmacology 1996;14: 97–105.
294. Hertel P. Comparing sertindole to other new generation antipsychotics on preferential dopamine output in limbic versus striatal projection regions: mechanism of action. Synapse 2006;60:543–52.
295. Skarsfeldt T. Differential effects after repeated treatment with haloperidol, clozapine, thioridazine and tefludazine on SNC and VTA dopamine neurones in rats. Life Sci 1988;42:1037–44.
296. Skarsfeldt T. Electrophysiological profile of the new atypical neuroleptic, sertindole, on midbrain dopamine neurones in rats: acute and repeated treatment. Synapse 1992;10: 25–33.
297. Pilowsky LS, Mulligan RS, Acton PD, Ell PJ, Costa DC, Kerwin RW. Limbic selectivity of clozapine. Lancet 1997;350:490–1.
298. Kessler RM, Ansari MS, Riccardi P, et al. Occupancy of striatal and extrastriatal dopamine D2 receptors by clozapine and quetiapine. Neuropsychopharmacology 2006;31: 1991–2001.
299. Bressan RA, Erlandsson K, Jones HM, Mulligan RS, Ell PJ, Pilowsky LS. Optimizing limbic selective D2/D3 receptor occupancy by risperidone: a [123I]-epidepride SPET study. J Clin Psychopharmacol 2003;23:5–14.
300. Bigliani V, Mulligan RS, Acton PD, et al. Striatal and temporal cortical D2/D3 receptor occupancy by olanzapine and sertindole in vivo: a [123I]epidepride single photon emission tomography (SPET) study. Psychopharmacology (Berl) 2000;150:132–40.
301. Stephenson CM, Bigliani V, Jones HM, et al. Striatal and extra-striatal D(2)/D(3) dopamine receptor occupancy by quetiapine in vivo. [(123)I]-epidepride single photon emission tomography(SPET) study. Br J Psychiatry 2000;177:408–15.
302. Nyberg S, Olsson H, Nilsson U, Maehlum E, Halldin C, Farde L. Low striatal and extrastriatal D2 receptor occupancy during treatment with the atypical antipsychotic sertindole. Psychopharmacology (Berl) 2002;162:37–41.

303. Kessler RM, Ansari MS, Riccardi P, et al. Occupancy of striatal and extrastriatal dopamine D2/D3 receptors by olanzapine and haloperidol. Neuropsychopharmacology 2005;30:2283–9.
304. Talvik M, Nordstrom AL, Nyberg S, Olsson H, Halldin C, Farde L. No support for regional selectivity in clozapine-treated patients: a PET study with [(11)C]raclopride and [(11)C]FLB 457. Am J Psychiatry 2001;158:926–30.
305. Agid O, Mamo D, Ginovart N, et al. Striatal vs extrastriatal dopamine D2 receptors in antipsychotic response–a double-blind PET study in schizophrenia. Neuropsychopharmacology 2007;32:1209–15.
306. Burnet PW, Chen CP, McGowan S, Franklin M, Harrison PJ. The effects of clozapine and haloperidol on serotonin-1A, -2A and -2C receptor gene expression and serotonin metabolism in the rat forebrain. Neuroscience 1996;73:531–40.
307. Huang XF, Tan YY, Huang X, Wang Q. Effect of chronic treatment with clozapine and haloperidol on 5-HT(2A and 2C) receptor mRNA expression in the rat brain. Neurosci Res 2007;59:314–21.
308. Steward LJ, Kennedy MD, Morris BJ, Pratt JA. The atypical antipsychotic drug clozapine enhances chronic PCP-induced regulation of prefrontal cortex 5-HT2A receptors. Neuropharmacology 2004;47:527–37.
309. de Paulis T. M-100907 (Aventis). Curr Opin Investig Drugs 2001;2:123–32.
310. Truffinet P, Tamminga CA, Fabre LF, Meltzer HY, Riviere ME, Papillon-Downey C. Placebo-controlled study of the D4/5-HT2A antagonist fananserin in the treatment of schizophrenia. Am J Psychiatry 1999;156:419–25.
311. Trichard C, Paillere-Martinot ML, Attar-Levy D, Recassens C, Monnet F, Martinot JL. Binding of antipsychotic drugs to cortical 5-HT2A receptors: a PET study of chlorpromazine, clozapine, and amisulpride in schizophrenic patients. Am J Psychiatry 1998;155:505–8.
312. Kapur S, Zipursky RB, Remington G. Clinical and theoretical implications of 5-HT2 and D2 receptor occupancy of clozapine, risperidone, and olanzapine in schizophrenia. Am J Psychiatry 1999;156:286–93.
313. Gefvert O, Lundberg T, Wieselgren IM, et al. D(2) and 5HT(2A) receptor occupancy of different doses of quetiapine in schizophrenia: a PET study. Eur Neuropsychopharmacol 2001;11:105–10.
314. Nyberg S, Eriksson B, Oxenstierna G, Halldin C, Farde L. Suggested minimal effective dose of risperidone based on PET-measured D2 and 5-HT2A receptor occupancy in schizophrenic patients. Am J Psychiatry 1999;156:869–75.
315. Meltzer HY, Matsubara S, Lee JC. The ratios of serotonin2 and dopamine2 affinities differentiate atypical and typical antipsychotic drugs. Psychopharmacol Bull 1989;25:390–2.
316. Meltzer HY, Li Z, Kaneda Y, Ichikawa J. Serotonin receptors: their key role in drugs to treat schizophrenia. Prog Neuropsychopharmacol Biol Psychiatry 2003;27:1159–72.
317. Meltzer HY. What's atypical about atypical antipsychotic drugs? Curr Opin Pharmacol 2004;4:53–7.
318. Nocjar C, Roth BL, Pehek EA. Localization of 5-HT(2A) receptors on dopamine cells in subnuclei of the midbrain A10 cell group. Neuroscience 2002;111:163–76.
319. Bubser M, Backstrom JR, Sanders-Bush E, Roth BL, Deutch AY. Distribution of serotonin 5-HT(2A) receptors in afferents of the rat striatum. Synapse 2001;39:297–304.
320. Hoyer D, Pazos A, Probst A, Palacios JM. Serotonin receptors in the human brain. II. Characterization and autoradiographic localization of 5-HT1C and 5-HT2 recognition sites. Brain Res 1986;376:97–107.
321. Jakab RL, Goldman-Rakic PS. 5-Hydroxytryptamine2A serotonin receptors in the primate cerebral cortex: possible site of action of hallucinogenic and antipsychotic drugs in pyramidal cell apical dendrites. Proc Natl Acad Sci USA 1998;95:735–40.

322. Miner LA, Backstrom JR, Sanders-Bush E, Sesack SR. Ultrastructural localization of serotonin2A receptors in the middle layers of the rat prelimbic prefrontal cortex. Neuroscience 2003;116:107–17.
323. Kuroki T, Meltzer HY, Ichikawa J. Effects of antipsychotic drugs on extracellular dopamine levels in rat medial prefrontal cortex and nucleus accumbens. J Pharmacol Exp Ther 1999;288:774–81.
324. Ichikawa J, Ishii H, Bonaccorso S, Fowler WL, O'Laughlin IA, Meltzer HY. 5-HT(2A) and D(2) receptor blockade increases cortical DA release via 5-HT(1A) receptor activation: a possible mechanism of atypical antipsychotic-induced cortical dopamine release. J Neurochem 2001;76:1521–31.
325. Chung YC, Li Z, Dai J, Meltzer HY, Ichikawa J. Clozapine increases both acetylcholine and dopamine release in rat ventral hippocampus: role of 5-HT1A receptor agonism. Brain Res 2004;1023:54–63.
326. Bonaccorso S, Meltzer HY, Li Z, Dai J, Alboszta AR, Ichikawa J. SR46349-B, a 5-HT(2A/2C) receptor antagonist, potentiates haloperidol-induced dopamine release in rat medial prefrontal cortex and nucleus accumbens. Neuropsychopharmacology 2002;27:430–41.
327. Li Z, Ichikawa J, Huang M, Prus AJ, Dai J, Meltzer HY. ACP-103, a 5-HT2A/2C inverse agonist, potentiates haloperidol-induced dopamine release in rat medial prefrontal cortex and nucleus accumbens. Psychopharmacology (Berl) 2005;183:144–53.
328. Liegeois JF, Ichikawa J, Meltzer HY. 5-HT(2A) receptor antagonism potentiates haloperidol-induced dopamine release in rat medial prefrontal cortex and inhibits that in the nucleus accumbens in a dose-dependent manner. Brain Res 2002;947:157–65.
329. Gobert A, Millan MJ. Serotonin (5-HT)2A receptor activation enhances dialysate levels of dopamine and noradrenaline, but not 5-HT, in the frontal cortex of freely-moving rats. Neuropharmacology 1999;38:315–7.
330. Seeman P. Antipsychotic drugs, dopamine receptors, and schizophrenia. Clinical Neuroscience Research 2001;1:53–60.
331. Schoemaker H, Claustre Y, Fage D, et al. Neurochemical characteristics of amisulpride, an atypical dopamine D2/D3 receptor antagonist with both presynaptic and limbic selectivity. J Pharmacol Exp Ther 1997;280:83–97.
332. Kapur S, Zipursky R, Remington G, Jones C, McKay G, Houle S. PET evidence that loxapine is an equipotent blocker of 5-HT2 and D2 receptors: implications for the therapeutics of schizophrenia. Am J Psychiatry 1997;154:1525–9.
333. Ichikawa J, Meltzer HY. R(+)-8-OH-DPAT, a serotonin(1A) receptor agonist, potentiated S(-)-sulpiride-induced dopamine release in rat medial prefrontal cortex and nucleus accumbens but not striatum. J Pharmacol Exp Ther 1999;291:1227–32.
334. Neal-Beliveau BS, Joyce JN, Lucki I. Serotonergic involvement in haloperidol-induced catalepsy. J Pharmacol Exp Ther 1993;265:207–17.
335. Prinssen EP, Colpaert FC, Koek W. 5-HT1A receptor activation and anti-cataleptic effects: high-efficacy agonists maximally inhibit haloperidol-induced catalepsy. Eur J Pharmacol 2002;453:217–21.
336. Rollema H, Lu Y, Schmidt AW, Sprouse JS, Zorn SH. 5-HT(1A) receptor activation contributes to ziprasidone-induced dopamine release in the rat prefrontal cortex. Biol Psychiatry 2000;48:229–37.
337. Rollema H, Lu Y, Schmidt AW, Zorn SH. Clozapine increases dopamine release in prefrontal cortex by 5-HT1A receptor activation. Eur J Pharmacol 1997;338:R3–5.
338. Bortolozzi A, Diaz-Mataix L, Toth M, Celada P, Artigas F. In vivo actions of aripiprazole on serotonergic and dopaminergic systems in rodent brain. Psychopharmacology (Berl) 2007;191:745–58.
339. Diaz-Mataix L, Scorza MC, Bortolozzi A, Toth M, Celada P, Artigas F. Involvement of 5-HT1A receptors in prefrontal cortex in the modulation of dopaminergic activity: role in atypical antipsychotic action. J Neurosci 2005;25:10831–43.

340. Abi-Saab WM, D'Souza DC, Madonick SH, Krystal JH. Targeting the glutamate system. In: Breier A, Tran PV, Herrera JM, ollefson GD, Bymaster FP, eds. Current Issues in the Psychopharmacology of Schizophrenia. Philadelphia: Lippincott, Williams & Wilkins Healthcare, 2001:pp. 304–32.
341. Tuominen HJ, Tiihonen J, Wahlbeck K. Glutamatergic drugs for schizophrenia. Cochrane Database Syst Rev 2006:CD003730.
342. Goff DC, Keefe R, Citrome L, et al. Lamotrigine as add-on therapy in schizophrenia: results of 2 placebo-controlled trials. J Clin Psychopharmacol 2007;27:582–9.
343. Zoccali R, Muscatello MR, Bruno A, et al. The effect of lamotrigine augmentation of clozapine in a sample of treatment-resistant schizophrenic patients: a double-blind, placebo-controlled study. Schizophr Res 2007;93:109–16.
344. Premkumar TS, Pick J. Lamotrigine for schizophrenia. Cochrane Database Syst Rev 2006:CD005962.
345. Marenco S, Egan MF, Goldberg TE, et al. Preliminary experience with an ampakine (CX516) as a single agent for the treatment of schizophrenia: a case series. Schizophr Res 2002;57:221–6.
346. Goff DC, Lamberti JS, Leon AC, et al. A placebo-controlled add-on trial of the Ampakine, CX516, for cognitive deficits in schizophrenia. Neuropsychopharmacology 2008;33:465–72.
347. Patil ST, Zhang L, Martenyi F, et al. Activation of mGlu2/3 receptors as a new approach to treat schizophrenia: a randomized Phase 2 clinical trial. Nat Med 2007;13:1102–7.
348. Emrich HM, Leweke FM, Schneider U. Towards a cannabinoid hypothesis of schizophrenia: cognitive impairments due to dysregulation of the endogenous cannabinoid system. Pharmacol Biochem Behav 1997;56:803–7.
349. D'Souza DC, Perry E, MacDougall L, et al. The psychotomimetic effects of intravenous delta-9-tetrahydrocannabinol in healthy individuals: implications for psychosis. Neuropsychopharmacology 2004;29:1558–72.
350. Negrete JC, Knapp WP. The effects of cannabis use on the clinical condition of schizophrenics. NIDA Res Monogr 1986;67:321–7.
351. Treffert DA. Marijuana use in schizophrenia: a clear hazard. Am J Psychiatry 1978;135:1213–5.
352. Giuffrida A, Leweke FM, Gerth CW, et al. Cerebrospinal anandamide levels are elevated in acute schizophrenia and are inversely correlated with psychotic symptoms. Neuropsychopharmacology 2004;29:2108–14.
353. Zavitsanou K, Garrick T, Huang XF. Selective antagonist [3H]SR141716A binding to cannabinoid CB1 receptors is increased in the anterior cingulate cortex in schizophrenia. Prog Neuropsychopharmacol Biol Psychiatry 2004;28:355–60.
354. Newell KA, Deng C, Huang XF. Increased cannabinoid receptor density in the posterior cingulate cortex in schizophrenia. Exp Brain Res 2006;172:556–60.
355. French ED, Dillon K, Wu X. Cannabinoids excite dopamine neurons in the ventral tegmentum and substantia nigra. Neuroreport 1997;8:649–52.
356. Gessa GL, Melis M, Muntoni AL, Diana M. Cannabinoids activate mesolimbic dopamine neurons by an action on cannabinoid CB1 receptors. Eur J Pharmacol 1998;341:39–44.
357. Chen J, Paredes W, Lowinson JH, Gardner EL. Delta 9-tetrahydrocannabinol enhances presynaptic dopamine efflux in medial prefrontal cortex. Eur J Pharmacol 1990;190: 259–62.
358. ChenJP, Paredes W, Li J, Smith D, Lowinson J, Gardner EL. Delta 9-tetrahydrocannabinol produces naloxone-blockable enhancement of presynaptic basal dopamine efflux in nucleus accumbens of conscious, freely-moving rats as measured by intracerebral microdialysis. Psychopharmacology (Berl) 1990;102:156–62.
359. Tanda G, Pontieri FE, Di Chiara G. Cannabinoid and heroin activation of mesolimbic dopamine transmission by a common mu1 opioid receptor mechanism. Science 1997;276:2048–50.

360. Solinas M, Justinova Z, Goldberg SR, Tanda G. Anandamide administration alone and after inhibition of fatty acid amide hydrolase (FAAH) increases dopamine levels in the nucleus accumbens shell in rats. J Neurochem 2006;98:408–19.
361. Malone DT, Taylor DA. Modulation by fluoxetine of striatal dopamine release following Delta9-tetrahydrocannabinol: a microdialysis study in conscious rats. Br J Pharmacol 1999;128:21–6.
362. Meltzer HY, Arvanitis L, Bauer D, Rein W. Placebo-controlled evaluation of four novel compounds for the treatment of schizophrenia and schizoaffective disorder. Am J Psychiatry 2004;161:975–84.
363. Baldessarini RJ, Huston-Lyons D, Campbell A, Marsh E, Cohen BM. Do central antiadrenergic actions contribute to the atypical properties of clozapine? Br J Psychiatry Suppl 1992:12–6.
364. Hommer DW, Zahn TP, Pickar D, van Kammen DP. Prazosin, a specific alpha 1-noradrenergic receptor antagonist, has no effect on symptoms but increases autonomic arousal in schizophrenic patients. Psychiatry Res 1984;11:193–204.
365. Svensson TH. Alpha-adrenoceptor modulation hypothesis of antipsychotic atypicality. Prog Neuropsychopharmacol Biol Psychiatry 2003;27:1145–58.
366. Jentsch JD, Wise A, Katz Z, Roth RH. Alpha-noradrenergic receptor modulation of the phencyclidine- and delta9-tetrahydrocannabinol-induced increases in dopamine utilization in rat prefrontal cortex. Synapse 1998;28:21–6.
367. Mathe JM, Nomikos GG, Hildebrand BE, Hertel P, Svensson TH. Prazosin inhibits MK-801-induced hyperlocomotion and dopamine release in the nucleus accumbens. Eur J Pharmacol 1996;309:1–11.
368. Drouin C, Blanc G, Villegier AS, Glowinski J, Tassin JP. Critical role of alpha1-adrenergic receptors in acute and sensitized locomotor effects of D-amphetamine, cocaine, and GBR 12783: influence of preexposure conditions and pharmacological characteristics. Synapse 2002;43:51–61.
369. Auclair A, Drouin C, Cotecchia S, Glowinski J, Tassin JP. 5-HT2A and alpha1b-adrenergic receptors entirely mediate dopamine release, locomotor response and behavioural sensitization to opiates and psychostimulants. Eur J Neurosci 2004;20:3073–84.
370. Andersson JL, Marcus M, Nomikos GG, Svensson TH. Prazosin modulates the changes in firing pattern and transmitter release induced by raclopride in the mesolimbic, but not in the nigrostriatal dopaminergic system. Naunyn Schmiedebergs Arch Pharmacol 1994;349: 236–43.
371. Hertel P, Nomikos GG, Svensson TH. Idazoxan preferentially increases dopamine output in the rat medial prefrontal cortex at the nerve terminal level. Eur J Pharmacol 1999;371: 153–8.
372. Wadenberg ML, Wiker C, Svensson TH. Enhanced efficacy of both typical and atypical antipsychotic drugs by adjunctive alpha2 adrenoceptor blockade: experimental evidence. Int J Neuropsychopharmacol 2007;10:191–202.
373. Haapalinna A, Sirvio J, MacDonald E, Virtanen R, Heinonen E. The effects of a specific alpha(2)-adrenoceptor antagonist, atipamezole, on cognitive performance and brain neurochemistry in aged Fisher 344 rats. Eur J Pharmacol 2000;387:141–50.
374. Coull JT, Sahakian BJ, Hodges JR. The alpha(2) antagonist idazoxan remediates certain attentional and executive dysfunction in patients with dementia of frontal type. Psychopharmacology (Berl) 1996;123:239–49.
375. Litman RE, Su TP, Potter WZ, Hong WW, Pickar D. Idazoxan and response to typical neuroleptics in treatment-resistant schizophrenia. Comparison with the atypical neuroleptic, clozapine. Br J Psychiatry 1996;168:571–9.
376. Spooren W, Riemer C, Meltzer H. Opinion: NK3 receptor antagonists: the next generation of antipsychotics? Nat Rev Drug Discov 2005;4:967–75.
377. Smith PW, Dawson LA. Neurokinin 3 (NK3) receptor modulators for the treatment of psychiatric disorders. Recent Patents CNS Drug Discov 2008;3:1–15.

378. Arnt J, Skarsfeldt T. Do novel antipsychotics have similar pharmacological characteristics? A review of the evidence. Neuropsychopharmacology 1998;18:63–101.
379. Richelson E, Nelson A. Antagonism by neuroleptics of neurotransmitter receptors of normal human brain in vitro. Eur J Pharmacol 1984;103:197–204.

Chapter 17
Dopamine Receptor Subtypes in Reward and Relapse

David W. Self

Abstract With the advent of subtype-selective ligands and dopamine receptor knockout mice, the last two decades have seen an explosion in research on the role of dopamine receptor subtypes in reward and relapse to drug-seeking behavior. This chapter represents a relatively comprehensive review of this literature, beginning with the ability of D_1-like and D_2-like receptor agonists to support self-administration behavior and produce conditioned preference, and followed by the modulation of natural reward, brain stimulation reward, and conditioned reward with subtype-selective ligands and dopamine receptor knockout mice. Subsequent sections describe the modulation of drug and alcohol self-administration by dopamine receptor subtypes, and role of dopamine receptor subtypes in relapse to drug and alcohol seeking in animal models. Finally, down-regulation in dopamine receptors following chronic drug self-administration is discussed in reference to differential changes in dopamine receptor-mediated behavior, suggesting that better integration between biological and behavioral data is needed in future studies.

Keywords D_1 · D_2 · D_1-like · D_2-like · D_3 · D_4 · D_5 · Reinforcement · Reinstatement · Cocaine · Amphetamine · Heroin · Nicotine · Alcohol

17.1 Introduction

Dopamine involvement in natural and drug reward is well established, but the role of specific dopamine receptor subtypes in reward processes remains a topic of intensive investigation. The most common approach to investigate dopamine receptor function in reward employs pharmacological ligands with relatively good selectivity for D_1-like and D_2-like receptor classes, although their potential actions at other

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neurotransmitter receptors (e.g., serotonergic or noradrenergic) are often neglected. Unfortunately, there have been far fewer studies targeting specific D_1-like (D_1 and D_5) or D_2-like (D_2, D_3 and D_4) receptor subtypes due to a lack of selective ligands, and promising candidates that exhibit good selectivity under artificial conditions in vitro are notorious for their lack of selectivity in vivo with further scrutiny. The ability to differentiate between related dopamine receptor subtypes is even more difficult when they play similar roles in behavior, but enhanced when related subtypes mediate different behavioral effects. Another potential caveat is that opposing behavioral effects are often mediated by pre- and post-synaptic receptors in the mesolimbic dopamine system, leading to biphasic dose–effect curves for behavioral responses. Given these considerations, recent advances in antagonist selectivity profiles, especially in differentiating the effects of D_2 and D_3 receptor blockade, have yielded compelling evidence for distinct receptor subtype actions in reward and reward-seeking behavior.

Genetic deletion generally is more definitive for assessing dopamine receptor involvement in natural and drug reward, although developmental compensation must be considered especially when negative results are found. Another important consideration is that behavioral responses in mice can differ substantially from rats and primates, a caveat highlighted by uniformly inhibitory effects of D_2-like agonists on psychomotor behavior in mice over a wide dose range [1]. Dopamine receptor knockout mice can be used to demonstrate definitive selectivity of novel and putative subtype-selective ligands in vivo, most notably when their behavioral effects are completely attenuated with the deletion of a specific dopamine receptor subtype. However, the interpretation of behavioral changes that result from brain-wide receptor deletion can be complicated by competing effects of dopamine receptors in different brain regions on neural circuits regulating natural and drug reward. The recent advent of inducible and localized genetic deletion strategies in mice is a powerful approach that circumvents problems relating to developmental compensation, and provides anatomically discrete loss of specific dopamine receptor subtypes, but this approach has yet to be applied to studies on natural and drug reward. Conversely, transgenic approaches to investigate gain of dopamine receptor function have not been widely used due to the need to limit expression to cells that naturally express the dopamine receptor subtypes. Recent advances in D_1 and D_2 promoter-driven transgenics will be pivotal to modulate dopamine receptor function in the appropriate cell types in future studies.

Using anatomically discrete microinfusion of dopaminergic ligands, studies have found that dopamine receptors play different roles in mediating or modulating reward processes in different brain regions. For example, dopamine receptors in ventral striatal regions mediate primary rewarding effects. Thus, transient dopamine receptor activation in these regions is sufficient to support self-administration behavior when receptor activation is a consequence of the behavior. In contrast, dopamine receptors in neocortical or amygdala regions can modulate reward evaluation, choice, or the formation of conditioned environmental (Pavlovian) associations that acquire their own rewarding properties secondary to the role of dopamine receptors in primary reward. These regional effects, in turn, are determined by dopamine

receptor localization on specific neuronal subpopulations such as interneurons or projection neurons, and differential coupling to G protein signaling pathways, further illustrating the complexities of delineating the mechanism of dopamine receptor subtypes in reward.

In addition to reward, dopamine receptors play a prominent role in eliciting appetitive or approach behavior, a phenomenon distinguishable from reward itself by the fact that behavioral responses follow rather than precede dopamine receptor stimulation. The seminal work of Schultz and colleagues [2, 3] found that environmental predictors of reward availability activate midbrain dopamine neurons, and other studies have shown that dopamine release in forebrain regions is sufficient to elicit appetitive behavior directed at obtaining reward. This behavior often is referred to as reward-seeking behavior, and is widely studied in animal models of drug and alcohol addiction to simulate craving and relapse in humans. Thus, dopamine receptors mediate two crucial aspects of addictive behavior: (1) they mediate primary reward when stimulated consequential to self-administration behavior and (2) they elicit reward-seeking behavior during periods of forced abstinence.

This chapter reviews the role of dopamine receptor subtypes in reward and reward-seeking behavior. Initial sections review earlier work establishing that dopamine receptor agonists serve as primary rewards in self-administration studies. Other studies that use dopamine receptor agonists to induce a conditioned place preference are described; a more commonly used but indirect measure of drug reward. Subsequent sections discuss the role of dopamine receptor subtypes in natural reward, brain stimulation reward, conditioned reward, and self-administration of abused drugs and alcohol. In each section, intracranial studies that elucidate regional sites of dopamine receptor action and genetic deletion of specific receptor subtypes are reviewed where available with an emphasis on differential roles for D_1-like and D_2-like receptors. In later sections, the role of dopamine receptor subtypes in relapse to drug- and alcohol-seeking behavior is discussed. In a final section, alterations in dopamine receptors produced by chronic drug self-administration are highlighted together with discrepant changes in dopamine receptor-mediated behavior, suggesting the need for better integrative models of biological and behavioral change in drug addiction. Clearly, dopamine receptors play a role in several other aspects of reward-related behavior that are beyond the scope of this chapter.

17.2 Dopamine Receptor Subtypes that Mediate Primary Reward

The fact that selective and directly acting dopamine receptor agonists will support self-administration behavior indicates that dopamine receptors mediate a primary rewarding stimulus. The earliest study supporting this notion found that drug-naïve female rats will learn to perform a novel lever-press response to receive intravenous injections of the direct dopamine receptor agonist apomorphine, and will

maintain stable dose-dependent responding for several weeks after acquisition [4]. Apomorphine self-administration is abolished by pretreatment with the dopamine receptor antagonist pimozide. A subsequent study found that depletion of endogenous brain catecholamines with alpha-methylparatyrosine treatment has no effect on apomorphine self-administration, but attenuates self-administration of the indirect agonist amphetamine [5]. These early studies clearly established a role for dopamine receptor stimulation in mediating primary reward and are often overlooked in more modern theoretical formulations of dopamine receptor function in reward-related behavior. Thus, in addition to serving as a primary reward, various other functions ascribed to dopamine include signaling reward prediction error [6], increasing incentive salience or wanting [7], enhancing the impact of conditioned environmental stimuli on instrumental responding [8], and reducing psychological effort requirements to obtain rewards [9]. These and other attributes suggest a complex multi-functional role for dopamine in reward processing and execution of instrumental behavior, but they do not belie the fact that dopamine receptor stimulation is in itself sufficient to mediate primary rewarding events.

17.2.1 Self-Administration of D_1-Like and D_2-Like Receptor Agonists

In addition to apomorphine, animals will self-administer intravenous injections of agonists selective for either D_1-like or D_2-like receptors. Early studies with the prototypical but partial D_1-like receptor agonist found that intravenous infusions of SKF 38393 fail to support self-administration behavior [10]. Subsequent studies found that higher efficacy D_1-like agonists such as SKF 82958 and SKF 81297 will support self-administration in rats, mice, and monkeys, and even in drug-naïve animals (Fig. 17.1a) [11–16]. Similarly, several studies found that D_2-like

Fig. 17.1 (continued) of the D_1- and D_2-like agonists to a lower dose of cocaine (267 μg/kg/injection). *Significantly different from 50%, $P < 0.05$. (**c**) Intranucleus accumbens (NAc) shell self-administration of D_1- and D_2-like agonists. Groups of rats self-administered 100 nl infusions containing 0.5 mM of the D_1-like agonist SKF 38393, the D_2-like agonist quinpirole, or a mixture during the first three sessions. The SKF and quinpirole groups were switched to the mixture for sessions 4 and 5, while the group initially trained on the mixture self-administered vehicle in session 4. During sessions 1–3, rats receiving SKF + quinpirole obtained more infusions than did rats receiving either drug alone ($P < 0.001$). The SKF and quinpirole groups exhibited higher levels of self-administration in session 5, when SKF + quinpirole was given in place of SKF or quinpirole alone, than in session 3 ($P = 0.01$). The replacement of SKF + quinpirole with vehicle in session 4 diminished self-administration ($P = 0.02$). (**d**) The D_1-like agonist SKF 82958 induces dose-dependent conditioned place preferences with minimal pairings (2 drug, 2 saline). Data are expressed as mean ± S.E.M. difference in preference for the drug- minus saline-paired side. Symbols indicate post-test scores differ from saline/saline pairing (SAL; $^{*}P < 0.05$, $^{**}P < 0.01$), or from pretest scores ($^{\dagger\dagger}P < 0.01$). Reproduced with permission from (**a**) Self and Stein [11], (**b**) Manzardo et al. [21], (**c**) Ikemoto et al. [25], and (**d**) Graham et al. [43]

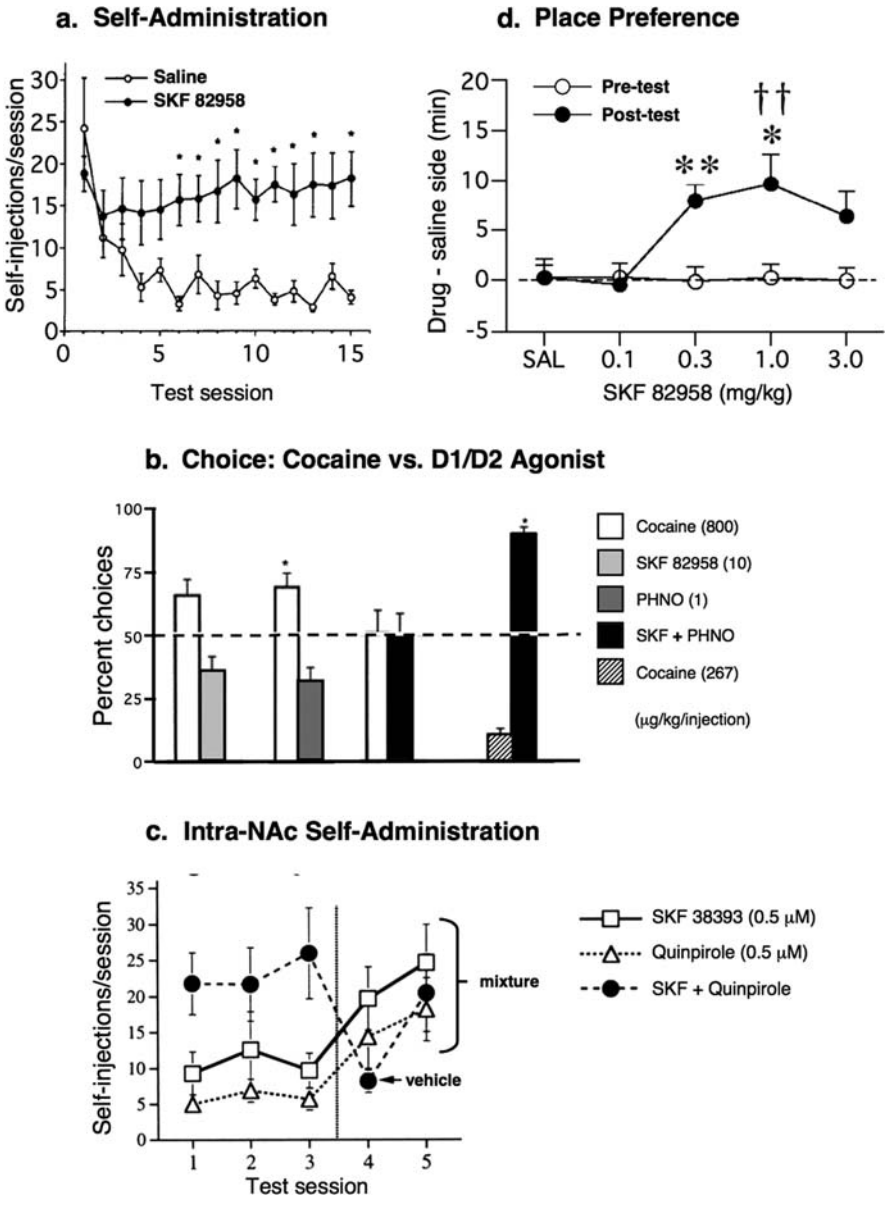


Fig. 17.1 (**a**) Self-administration of the D_1-like agonist SKF 82958 in drug-naive rats. Data points show the mean daily number of self-injections (± S.E.M.) of groups self-administering SKF 82958 at a dose of 10 μg/kg/injection or saline over 15 consecutive test sessions (*SKF 82958 differs from saline, $P < 0\ 05$). High response rates in the initial test sessions are due to lever-press training for food pellets prior to self-administration testing. (**b**) In choice tests, rats prefer to self-administer a moderate dose of cocaine (800 μg/kg/injection) to either the D_1-like agonist SKF 82958 (10 μg/kg/injection) or the D_2-like agonist (+)-PHNO (1 μg/kg/injection), but exhibit a similar preference for cocaine and a mixture of the D_1- and D_2-like agonists. Rats prefer the mixture

receptor agonists are self-administered intravenously by rats, mice and monkeys (reviewed by [17]), including highly D_2-like selective agonists such as quinpirole, 7-OH-DPAT, and (+)-PHNO [10, 18–22]. In contrast to the self-administration of D_1-like agonists in drug-naïve animals, D_2-like agonists are self-administered only when substituted for drugs such as cocaine in animals with extensive prior self-administration training. Whether drug-naïve animals will learn to self-administer D_2-like agonists has not been systematically studied, and there are no reports of experimentally or drug-naive animals acquiring D_2-like agonist self-administration, but studies have found that quinpirole and 7-OH-DPAT generally fail to support self-administration in monkeys without prior cocaine self-administration training [23, 24]. These findings indicate that D_1-like receptor stimulation is sufficient to mediate primary rewarding effects, but it is not clear whether selective D_2-like receptor stimulation is capable of mediating primary reward. One possible explanation is that D_2-like agonists maintain self-administration behavior once acquired by enhancing the conditioned rewarding properties of lever-press behavior associated with primary rewards during previous training (see Section 17.3.3). Furthermore, D_2-like receptors apparently can augment the magnitude of reward mediated by D_1-like receptor stimulation, since rats prefer to self-administer co-injections of D_2-like with D_1-like receptor agonists over cocaine, but cocaine is preferred over injections of D_1-like receptor agonists alone (Fig. 17.1b) [21]. Moreover, genetic deletion of D_1 receptors abolishes the reinforcing effects of a D_2-like agonist in mice [16], further indicating that D_2-like receptors fail to independently mediate primary reward.

Intracranial self-administration studies indicate that the ventral striatum contains D_1-like and D_2-like receptors that are important for mediating primary rewarding effects. Rats will learn to self-administer a cocktail containing both D_1-like and D_2-like receptor agonists into the nucleus accumbens shell (Fig. 17.1c), but not the core subregion [25]. While neither agonist alone supports self-administration behavior, only the partial D_1-like receptor agonist SKF 38393 has been tested, and so it is possible that high efficacy and selective D_1-like receptor agonists would support self-administration behavior in the nucleus accumbens shell. Interestingly, infusion of cocaine into more ventral olfactory tubercle regions more effectively supports self-administration behavior than infusion in the nucleus accumbens shell, an effect blocked by co-infusion with either D_1-like or D_2-like antagonists [26]. Dopamine receptors in the medial prefrontal cortex also support self-administration behavior, since rats will self-administer dopamine (or cocaine) into this region, and the effects are blocked by co-infusion with a D_2-like but not a D_1-like antagonist [27–29]. Whether animals would self-administer selective D_1-like or D_2-like agonists directly into the medial prefrontal cortex or other brain regions is unknown. Other studies found that cocaine is self-administered directly into the dopamine cell body region of the posterior ventral tegmental area in mice and rats, which may be mediated by D_1-like receptor modulation of serotonergic excitation of dopamine neurons [30, 31].

Some studies suggest that the hedonic impact of rewards may be dissociable from dopamine receptor stimulation and its role in goal-directed behavior. Thus,

for example, depletion of central dopamine has no effect on hedonic responses to natural reward in animals [7]. In humans, indirect dopamine agonists such as cocaine and amphetamine induce euphoria, but the dopamine receptor agonist apomorphine produces nausea and dysphoria, an effect that may involve peripheral dopamine receptor activation [32]. The D_2-like receptor agonist bromocriptine produces little subjective effects on its own [33], and neuroleptic drugs that block D_2-like receptors fail to consistently attenuate subjective reports of euphoria with amphetamines or cocaine in humans [34, 35]. In contrast, however, acute blockade of D_1-like receptors with ecopipam (SCH 39166) attenuates cocaine-induced euphoria in human cocaine addicts [36], although chronic blockade of D_1-like receptors actually enhances cocaine euphoria potentially due to compensatory changes [37]. These findings suggest that the primary rewarding effects of direct dopamine receptor stimulation may be dissociable from hedonic responses in animals and that D_1-like, but not D_2-like, receptors may play a role in drug-induced euphoria in humans.

17.2.2 Conditioned Place Preference with D_1-Like and D_2-Like Receptor Agonists

Conditioned place preference has been widely used as an indirect measure of the rewarding properties of dopamine receptor agonists. In conditioned place preference, animals will prefer an environment associated with primary rewarding stimuli over those paired with neutral stimuli. The environmental context acquires conditioned rewarding properties that reflect the primary rewarding properties of the dopamine receptor agonists. Several decades of research has shown differential abilities of D_1-like and D_2-like receptor agonists to produce conditioned place preference [17, 38]. Early studies found that systemic administration of high doses of the partial D_1-like agonist SKF 38393 actually produces a place aversion [39, 40], reflecting dysphoric effects, and this effect is blocked by a D_1-like but not a D_2-like antagonist [39, 41]. However, subsequent studies found that the higher efficacy D_1-like agonists SKF 82958 (Fig. 17.1d), SKF 81297, and the non-benzazepine agonist ABT-431 will produce a conditioned place preference [42, 43]. Interestingly, the long-acting full D_1-like agonist A-77636 produces a place aversion potentially due to profound receptor internalization [43, 44]. In this sense, place aversions with A-77636 and the partial D_1-like agonist SKF 38393 resemble strong place aversions that are found with D_1-like receptor blockade [45], suggesting that a loss of D_1-like receptor tone produces dysphoria in animals. Together, these studies generally agree with self-administration studies indicating that selective D_1-like receptor stimulation is sufficient to mediate primary rewarding effects.

Systemic treatment with D_2-like agonists such as quinpirole, 7-OH-DPAT, or bromocriptine can produce a conditioned place preference, a place aversion, or have no effect depending on rat strain, dose, and other experimental conditions. Systemic administration of quinpirole at doses thought to activate post-synaptic receptors produces a weak conditioned place preference in Wistar [39, 41], Lister-hooded [46],

and Long–Evans rats [40], while it is ineffective in Sprague Dawley rats unless they have received repeated cocaine injections prior to conditioning [43]. Similarly, 7-OH-DPAT has been shown to produce a conditioned place preference in Wistar [47] and Sprague Dawley rats [48, 49] in some studies, but no effect in Swiss Webster mice or Wistar rats in other studies [50, 51]. Place conditioning with low doses that are thought to selectively activate D_2-like autoreceptors actually produce conditioned place aversions [52, 53]. The less selective D_2-like agonist bromocriptine has been shown to produce place preference in both rats and mice [54, 55]. Similarly, certain full efficacy and putative D_3-selective ligands produce either a place preference [56] or an aversion [52], while a novel D_4 agonist is without effect [57]. Together these discrepancies highlight the impact of strain differences, intralaboratory variability in place conditioning procedures, or the potential differential influences of D_2 and D_3 receptors on reward substrates.

When infused into the nucleus accumbens, both D_1-like and D_2-like agonists produce conditioned place preferences [40], whereas negative findings have been found in the hippocampus and amygdala. However, dopamine receptors in the hippocampus and amygdala can modulate place conditioning with other drugs of abuse [58, 59], suggesting that dopamine receptors in these regions can alter associative learning between rewarding stimuli and conditioned environments. Results with place conditioning differ from self-administration studies by suggesting that either D_1-like or D_2-like receptors in the nucleus accumbens can produce rewarding effects in drug-naïve animals.

17.3 Modulation of Natural and Endogenous Reward by Dopamine Receptor Subtypes

Given the fact that natural rewards, including highly palatable foods, water, and sexual interaction, all increase dopamine levels in terminal regions, it is not surprising that dopamine receptors play a role in natural reward. Moreover, the rewarding effect of electrical brain stimulation, or brain stimulation reward, also is mediated by dopamine receptors to a major extent. While both D_1-like and D_2-like receptors are implicated in natural reward, D_1-like receptors in particular play an important role in reward-related learning during initial establishment of stimulus–response associations, whereas D_2-like receptors play a prominent role in augmenting the motivational salience of conditioned rewards after learning has occurred. These differences suggest that D_1- and D_2-like receptors are involved in different phases of acquisition and expression of rewarded behavior.

17.3.1 Modulation of Food, Water, and Sexual Reward by Dopamine Receptor Subtypes

Pretreatment with either dopamine agonists or antagonists can reduce instrumental responding for food rewards (e.g. [60]), but these effects usually reflect performance or other rate-altering effects unrelated to reward impact. In sham-fed rats,

where sucrose is ingested without instrumental requirements but prevented from remaining in the stomach, both D_1- and D_2-like antagonists reduce preference for rewarding sucrose [61–63]. Pretreatment with either D_1- and D_2-like agonists also reduces food intake, but only D_1-like agonists reduce sucrose-sham feeding, consistent with a role for D_1-like receptor stimulation in satiety mechanisms for highly palatable reward [64]. In contrast, instrumental responding for high sucrose content food pellets is increased by the D_2-like antagonist raclopride, consistent with an antagonist-like effect on reward processes surmountable by increased self-administration behavior [65].

Water intake in thirsty mice is not affected by D_1-like antagonists [66], while it is reduced by both D_1-like and D_2-like antagonists in rats [67]. However, the conditioned place preference to water availability in deprived rats is blocked by either the D_1-like antagonist SCH 23390 or the D_2-like antagonist raclopride at doses that have no effect on water intake during conditioning [67]. These findings suggest that both D_1- and D_2-like receptors could play a role in primary water reward, although blockade of Pavlovian learning processes independent of alterations in primary reward strength also could account for these results.

In addition, dopamine receptors are implicated in the rewarding effects of sexual interaction. Dopamine D_2-like agonists promote while antagonists reduce motivational indices of copulatory behavior in male rats [68]. In contrast, the partial D_1-like agonist SKF 38393 does not influence copulation [69]. When male rats perform an instrumental response to gain access to a receptive female, non-selective blockade of D_1- and D_2-like receptors impairs this response [70], and the pursuit of receptive females is reduced by systemic pretreatment with either D_1- or D_2-like antagonists [71]. In female rats, high doses of the D_2-like agonist quinpirole elicit sexual behavior (lordosis) in non-receptive rats, while lower (presumably autoreceptor doses) are effective in receptive rats [72], suggesting that receptivity disrupts the sensitivity to post-synaptic D_2-like stimulation. D_1-like ligands are ineffective at modulating this behavior. Many sexual responses in female rats may reflect actions in brain regions unrelated to brain reward and motivational systems. However, a recent study found that the preference for male pheromones in female mice is not blocked by D_1-like or D_2-like antagonists, but is blocked by the D_1-like agonist SKF 38393 [73], potentially reflecting a masking of the pheromone reward stimulus by tonic D_1-like receptor stimulation.

D_1-receptor knockout mice are capable of acquiring instrumental responding for sucrose or sweetened food reward, but acquire self-administration more slowly despite no difference in sucrose intake when freely available [16, 74]. Thus, these decrements may reflect performance rather than motivational deficits. In female D_5 knockout mice, the ability of apomorphine to facilitate sexual receptivity is blocked, whereas male D_5 knockout mice show an impaired conditioned place preference to environments paired with intromission, but not ejaculation [75]. D_2 receptor knockout mice show impaired acquisition of instrumental response for sweetened milk most likely due to Parkinson-like effects [76], but another study found no effect on water self-administration [77]. D_3 receptor knockout mice show no changes in responding for food or water rewards [78]. The inability to regulate the degree of

dopamine receptor inactivation, or to localize effects to specific brain regions, probably accounts for behavioral impairment and makes it difficult to assign a role for specific dopamine receptor subtypes in natural reward in these knockout studies.

Dopamine levels in the nucleus accumbens are elevated by unfettered sucrose consumption, but reverse microdialysis of either D_1- or D_2-like antagonists into the nucleus accumbens fails to alter this behavior [79]. In contrast, microinfusions of both D_1- or D_2-like antagonists into the nucleus accumbens core, but not shell, reduce instrumental responding for normal chow pellets under more demanding progressive ratio schedules, which could reflect performance rather than motivational effects [80]. Putative D_3 and D_4 antagonists are ineffective. When regular chow is available under free-feeding conditions in food-deprived rats, neither D_1- nor D_2-like receptor blockade in the nucleus accumbens impairs food intake, but the number of feeding bouts is reduced [81], consistent with a role for nucleus accumbens dopamine receptors in incentive motivational responses, but not the regulation of food intake. Kelley and colleagues found that a low dose of a D_1-like antagonist that does not impair instrumental performance will attenuate acquisition of responding rewarded by sucrose pellets when infused into the nucleus accumbens, but only when co-infused with a similar low dose of an NMDA glutamate receptor antagonist [82]. These results suggest that coincident activation of D_1-like and NMDA receptor signals are needed for reward-related learning. Post-trial infusions of the D_1-like and NMDA antagonists fail to alter acquisition of sucrose self-administration, but post-trial infusion of a protein kinase A inhibitor (PKA) does impair acquisition, suggesting that prior D_1-like receptor activation of cAMP is important for consolidating these learned stimulus–response associations [83]. A similar relationship for coincident D_1-like and NMDA receptor activation and dependence on protein kinase A is found in the medial prefrontal cortex [84]. Blockade of intra-amygdala D_1-like receptors also impairs acquisition of instrumental responses for sucrose reward without affecting performance, suggesting that amygdala D_1-like activity is important in reward-related learning [85].

17.3.2 Modulation of Brain Stimulation Reward by Dopamine Receptor Subtypes

Electrical stimulation of several brain regions will support self-stimulation behavior, including the ventral tegmental area where dopaminergic cell bodies are located, and the lateral hypothalamus where descending fibers of the medial forebrain bundle activate dopamine neurons through direct and indirect pathways [86]. Forebrain regions including the medial prefrontal cortex also support self-stimulation behavior potentially through activation of dopamine neurons in the ventral tegmental area [87]. The magnitude of brain stimulation reward can be determined in a response rate-independent manner by measuring stimulus frequency (or intensity) thresholds, or by parallel shifts in stimulus–response curves, valuable procedures given that dopaminergic ligands can produce substantial effects on performance. Systemic administration of either D_1- or D_2-like antagonists attenuate the rewarding impact

of electrical brain stimulation (e.g.[88, 89]), whereas D_3 antagonists are ineffective [48, 90]. The rate–frequency curve is shifted rightward in D_1 receptor knockout mice [91], and D_2 knockout mice require 50% more stimulus intensity to support self-stimulation [92]. Together these studies suggest that both D_1 and D_2 receptor subtypes play a necessary role in the rewarding effects of electrical brain stimulation.

In contrast to antagonists, systemic administration of D_1- and D_2-like receptor agonists produces differing effects on brain stimulation reward. Thus, the prototypical but partial D_1-agonist SKF 38393 is without effect [89] or inhibits brain stimulation reward [93], although the facilitation of ventral tegmental self-stimulation by SKF 38393 has been reported [94]. The full efficacy D_1-agonist SKF 81297 elevates brain stimulation reward thresholds in rats [95], while the less selective D_1-agonist SKF 82958 reduces thresholds in mice [96]. Pretreatment with the non-benzazepine D_1-agonist A-77636 also lowers thresholds in rats [97]. Given the differential ability of these compounds to support self-administration behavior and produce a conditioned place preference (SKF 81297 and SKF 82958), or fail self-administration tests and produce a conditioned place aversion (SKF 38393 and A-77636), the relationship between facilitation of brain stimulation reward and inherent rewarding properties of these compounds is unclear.

In contrast, pretreatment with the D_2-like agonist quinpirole lowers the threshold of brain stimulation reward [89, 98], while lower presumably autoreceptor doses increase thresholds [98]. Although the D_1-like agonist SKF 38393 is without effect, co-administration with quinpirole augments the threshold lowering effects of quinpirole alone, while the D_1-like antagonist SCH 23390 attenuates this facilitation, consistent with synergistic and enabling interactions between D_1- and D_2-like receptors [99]. Pretreatment with the D_2-like agonist 7-OH-DPAT has inconsistent or biphasic effects [48, 95, 98, 100], with low doses attenuating and high doses facilitating brain stimulation reward, consistent with biphasic activation of pre-and post-synaptic D_2-like receptors with increasing dose [101]. Similarly, 7-OH-DPAT produces biphasic effects using a progressive ratio schedule of brain stimulation reward that measures the amount of effort animals will exert to obtain brain stimulation; low doses reduce and high doses increase the amount of effort exerted [102]. The less selective D_2-like agonist bromocriptine also lowers self-stimulation thresholds [103]. These results clearly indicate that stimulation of post-synaptic D_2-like receptors enhances the rewarding effects of electrical brain stimulation.

Intracranial infusion studies have identified the nucleus accumbens as a major site for modulation of brain stimulation reward by D_1- and D_2-like receptors. Nucleus accumbens infusions of either the D_1-like antagonist SCH 23390 or the D_2-like antagonist raclopride attenuate brain stimulation reward, while the D_3 antagonist (+)-UH232 and the D_4 antagonist clozapine are ineffective [104, 105]. In contrast, agonist infusion studies have found that intra-accumbens infusions of SKF 38393 (D_1-like) enhance but infusions of quinpirole (D_2-like) attenuate brain stimulation reward, whereas medial prefrontal cortex infusions are ineffective [106]. Similarly, infusions of the D_1-like agonist A-77636 into more caudal nucleus accumbens regions lower stimulation thresholds, while quinpirole

infusions elevate thresholds [97]. Another study found that nucleus accumbens infusions of the D_2-like agonist 7-OH-DPAT have no effect on brain stimulation reward thresholds unless co-infused with AMPA glutamate receptor antagonists to remove glutamatergic tone [107]. While the effect of D_2-like agonists differs from systemic administration, it is possible that local effects mediated by D_2-like autoreceptors on dopamine terminals in the nucleus accumbens reduce dopamine release in response to brain stimulation reward. Given the reliance of D_2-like receptor-mediated reward on D_1-like receptor tone in the nucleus accumbens, local inhibition of dopamine release and D_1-like tone could explain a lack of facilitation with direct activation of post-synaptic D_2-like receptors in the nucleus accumbens.

In this regard, infusions of either D_1- or D_2-like agonists into the dopamine cell body region of the ventral tegmental area attenuate self-stimulation of the ventral tegmental area, but not self-stimulation of the lateral hypothalamus [106]. Local D_1-like receptor stimulation in the ventral tegmental area induces GABA release that inhibits dopamine neurons [108], while D_2-like autoreceptors directly inhibit dopamine neuron firing. Infusions of D_1- or D_2-like agonists into the frontal cortex or caudate-putamen are ineffective at modulating the rewarding effects of ventral tegmental area stimulation [97]. However, infusions of the D_2-like antagonists spiperone and pimozide into the medial prefrontal cortex will decrease self-stimulation of the same site [109]. Together these studies implicate both D_1 and D_2 receptors, especially in the nucleus accumbens, in mediating the rewarding effects of electrical stimulation of midbrain dopamine neurons, while a role for D_3 receptors awaits further testing in D_3 knockout mice or the availability of more selective ligands. D_4 and D_5 receptors are not highly expressed in the nucleus accumbens [110–112], but these receptors in other brain regions may play a role in modulating brain stimulation reward.

17.3.3 Modulation of Conditioned Reward by Dopamine Receptor Subtypes

The repeated temporal pairing of hedonically neutral and temporally discrete environmental stimuli (e.g., tones and lights) with response-independent delivery of primary rewards (natural or drug) leads to the formation of conditioned reward. Through Pavlovian conditioning, these stimuli acquire rewarding properties of their own, and animals subsequently will learn to perform a novel instrumental response when rewarded by presentation of the conditioned stimulus. The phenomenon has major implications for control over behavior exerted by conditioned cues in drug addiction, since these cues can trigger craving and relapse to drug use. Studies by Robbins and Taylor showed that psychostimulants greatly enhance conditioned reward by elevating dopamine levels in the nucleus accumbens and potentially other regions [113–115].

The facilitation of conditioned reward by amphetamine is blocked by systemic treatment with either D_1- or D_2-like antagonists [116]. When given alone, low doses of D_2-like antagonists can potentiate conditioned rewards, probably due to

enhanced dopamine release with autoreceptor blockade, while higher post-synaptic doses of D_1-like and D_2-like antagonists attenuate responding [117]. Systemic administration of D_1-like agonists also attenuate responding for conditioned reward, potentially due to masking of temporally discrete reward-related signals with presentation of the conditioned reward [118]. In contrast, responding for conditioned rewards is markedly enhanced by systemic pretreatment with D_2-like agonists [119]. Interestingly, the opposite effects of D_1- and D_2-like agonists on conditioned reward are remarkably similar to their effects on drug-seeking behavior as discussed in Section 17.5.1. The facilitation of conditioned reward by the D_2-like agonist bromocriptine is blocked by pretreatment with the D_1-like antagonist SCH 23390 [120], indicating the necessary role for D_1-like receptor tone on the expression of D_2-like receptor-mediated effects.

However, when directly infused into the nucleus accumbens, either the D_1-like agonist SKF 38393 or the D_2-like agonist quinpirole facilitates conditioned reward [121, 122], suggesting that the attenuation of conditioned reward by D_1-like receptor stimulation involves multiple or other brain regions. In any event, a necessary role for both D_1- and D_2-like receptors in the nucleus accumbens is indicated by the fact that antagonists for either receptor will block conditioned reward when infused in this brain region [121]. Interestingly, infusions of the D_2-like agonist 7-OH-DPAT into the amygdala during the conditioning phase prevent the subsequent expression of conditioned reward in a drug-free state [123], potentially due to D_2-like autoreceptor inhibition of reward-related dopamine release in the amygdala. Similarly, blockade of either D_1- or D_2-like receptors in the amygdala during conditioning prevents the formation of conditioned reward for cocaine-associated cues [124]. These latter results suggest that stimulation of dopamine receptors in the amygdala is important for establishing enduring learned associations between primary and conditioned rewards, whereas the former results suggest that the expression of conditioned reward requires stimulation of dopamine receptors in the nucleus accumbens.

17.4 Modulation of Drug Self-Administration by Dopamine Receptor Subtypes

Many studies have utilized dopaminergic ligands to study the role of dopamine receptors in the rewarding effects of abused drugs. Given this vast literature, this section will focus on drug self-administration studies, arguably the most pertinent animal model of human drug abuse available. The schedule requirements for drug delivery in self-administration studies are an important consideration when modeling distinct symptoms of addictive behavior. In studies where each drug injection requires a low fixed number of instrumental responses (fixed ratio), an animal's preferred level of drug intake is not encumbered by performance demands or by prolonged intervals of drug unavailability. Under these circumstances, animals titrate their preferred level of drug intake in a highly stable and dose-sensitive

manner. For example, animals will compensate for a lowering in the injection dose by increasing the rate of self-administration, and decrease self-administration rates when the injection dose is increased. This relationship is reflected by an inverted U-shaped self-administration dose–response curve, spanning subthreshold doses that are too low to support self-administration, moderate but short acting suprathreshold doses that are self-administered at increased rates, and higher doses that are self-administered at reduced rates due to prolonged effects of the drug injections. Systemic pretreatment with dopaminergic agonists such as apomorphine reduces the rate of amphetamine self-administration by prolonging the interval between successive self-injections [18]. Since this reduction resembles the effect of increasing the injection dose of amphetamine, it is thought that generalized dopamine receptor stimulation potentiates the satiating or other rate limiting effects of self-administered amphetamine in an additive manner. Conversely, blockade of post-synaptic dopamine receptors with systemic neuroleptic treatment increases the rate of cocaine intake by shortening the time interval between successive self-injections [125], similar to the effect of lowering the cocaine injection dose. Thus, dopamine receptor blockade is thought to antagonize the impact of the cocaine injections in a manner surmounted by volitional increases in cocaine intake, and a similar effect is produced when animals transit to more addicted biological states [126]. An advantage of this approach is that the performance degrading effects of dopamine receptor blockade are negated by increases in self-administration behavior.

While the stability of fixed ratio drug self-administration has its advantage, self-administration rates on these schedules are not directly related to the magnitude of reward. For assessing reward magnitude, self-administration studies often employ progressive ratio schedules, where the degree of effort (e.g., lever pressing) an animal will exert to obtain drug is used as an index of reward, rather than the actual amount of drug consumed. In progressive ratio testing, the response demands for each successive drug injection increase progressively during active self-administration, and the highest ratio of lever presses/injection achieved before animals quit responding (break point) measures a drug's rewarding efficacy. Thus, while dopamine receptor blockade can increase drug self-administration on fixed ratio schedules (Fig. 17.2a), the same treatment can decrease self-administration on progressive ratio schedules (Fig. 17.2b). The use of both schedules of drug reward is a powerful combination for clarifying the contribution of specific dopamine receptor subtypes in the regulation of drug intake (fixed ratio) and the motivation for drugs when obtaining reward is more demanding (progressive ratio).

17.4.1 Modulation of Psychostimulant Self-Administration by Dopamine Receptor Subtypes

The rewarding effects of psychostimulants such as cocaine and amphetamine involve their ability to function as indirect dopamine receptor agonists. Given that

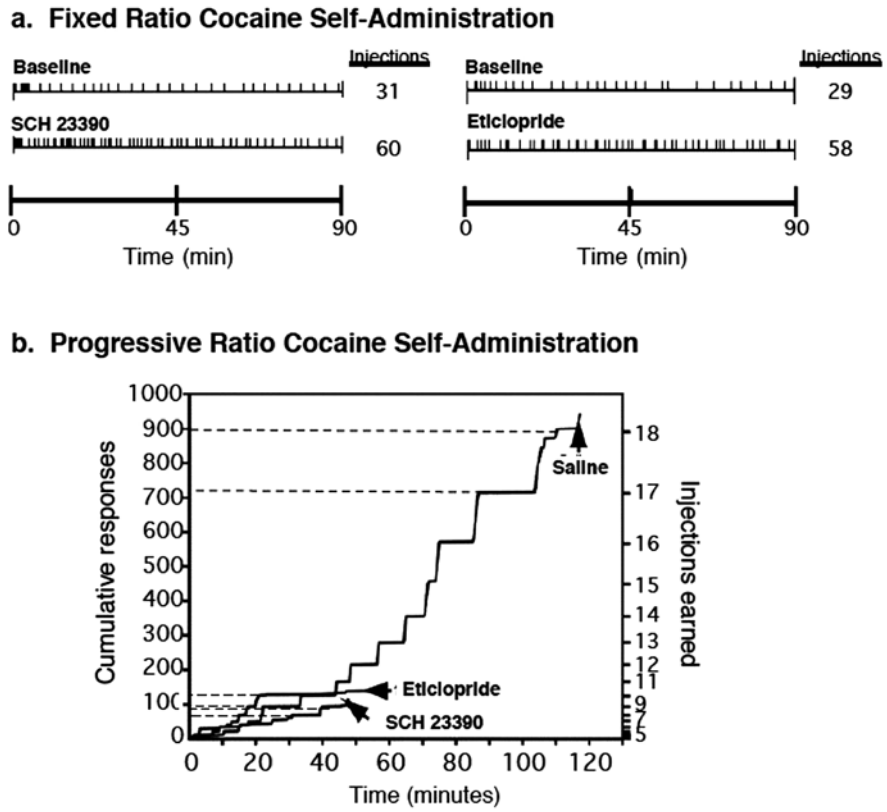


Fig. 17.2 (**a**) Intranucleus accumbens shell infusions of the D_1-like antagonist SCH 23390 (3.0 μg/side) or the D_2-like antagonist eticlopride (10.0 μg/side) increase stabilized cocaine self-administration (0.5 mg/kg/injection) on a fixed ratio 1:timeout 15 s schedule of reward. Representative self-administration records for individual animals during baseline self-administration one day prior to tests with intranucleus accumbens antagonist treatment. Upward hatchmarks denote times of self-injection. (**b**) Similar intranucleus accumbens shell infusions of SCH 23390 (1.25 μg/side) or eticlopride (10.0 μg/side) reduce cocaine self-administration on a progressive ratio self-administration schedule. Individual cumulative response records show that a saline-treated control achieves a ratio of almost 200 responses per injection (vertical distance between *dotted lines* on *left*) before quitting self-administration behavior, whereas animals treated with SCH 23390 or eticlopride achieve less than 40 responses per injection. Adapted with permission from (**a**) Bachtell et al. [157] and (**b**) Bari and Pierce [80]

both D_1- and D_2-like receptors play a role in mediating or modulating reward processes, it is not surprising that systemic pretreatment with either D_1- or D_2-like selective antagonists leads to compensatory increases in cocaine intake on fixed ratio self-administration schedules in rats [127–130], and monkeys [131]. These findings suggest that both D_1- and D_2-like receptors contribute to negative feedback regulation of cocaine intake. Conversely, both D_1- and D_2-like receptor antagonists decrease the amount of effort animals will exert to obtain cocaine or amphetamine

when self-administered on a progressive ratio schedule [130, 132–134]. Since these treatments increase self-administration rates on fixed ratio schedules, decreased self-administration on progressive ratio schedule is likely due to reduced motivational rather than performance effects. In contrast, systemic pretreatment with putative D_3 and D_4 antagonists fails to increase cocaine intake on fixed ratio schedules [135–138], but the D_3 antagonists NGB 2904 and SB-277011A attenuate cocaine reward on progressive ratio schedules [137, 138]. This differential sensitivity to D_3 antagonists with fixed and progressive ratio cocaine self-administration distinguishes these compounds from generalized D_2-like receptor antagonists. Thus, D_3 receptors may play a necessary role along with D_1- and D_2 receptors in cocaine reward, while D_1- and D_2 (but not D_3) receptors may be preferentially involved in negative feedback regulation of cocaine intake.

Genetic deletion of D_1 receptors dramatically decreases the number of mice that will acquire cocaine self-administration [16]. In contrast, D_2 receptor knockout mice acquire cocaine self-administration, and show increased cocaine intake on fixed ratio schedules similar to the effect of D_2-like receptor antagonists [135]. However, the ability of the D_2-like receptor antagonist eticlopride to further elevate cocaine intake is absent in D_2 receptor knockout mice, indicating the effect primarily reflects blockade of D_2 and not D_3 or D_4 receptors as indicated by antagonist studies in rats. These results support the notion that D_1 receptors play a major role in the primary rewarding properties of cocaine, while D_2 receptors contribute to cocaine's rate-reducing effects.

In contrast to antagonist pretreatment, pretreatment with full efficacy D_1- or D_2-like receptor agonists reduces cocaine self-administration on unrestricted fixed ratio schedules, but the qualitative aspects of this reduction differ. Thus, pretreatment with D_1-like agonists suppress the initiation of cocaine self-administration, and produce downward shifts in the inverted U-shaped dose–response function in rats and monkeys [139–142]. In contrast, pretreatment with D_2-like agonists prolongs the time interval between successive cocaine injections (post-injection pause), similar to the effect of increasing the unit cocaine dose/injection, and produces a leftward shift in the dose–response curve [20, 140, 141, 143–145]. As suggested above, these results are consistent with the idea that tonic D_1-like receptor stimulation suppresses cocaine self-administration by supplanting and occluding the primary rewarding effects of cocaine, whereas tonic stimulation of D_2-like receptors potentiates the rewarding effects of self-administered cocaine. Systemic pretreatment with the D_2-like agonist quinpirole has no effect on amphetamine self-administration on a progressive ratio schedule [134]. However, when D_1- or D_2-like agonists are combined with cocaine injections in monkeys self-administering on a progressive ratio schedule, a situation where agonists are delivered in a discrete response-contingent manner, both D_1-like and D_2-like agonists produce leftward shifts in the dose–response curve compared to cocaine alone, indicating that either receptor contributes to cocaine reward [146].

While studies with D_3-selective antagonists have been informative, studies with putative D_3-selective agonists have been complicated by the fact that the behavioral profile often resembles that of general D_2-like agonists on fixed ratio cocaine

self-administration, i.e., a prolonging of the interval between successive cocaine injections consistent with an additive interaction with cocaine [136, 147]. Previous studies suggested that the potency for this effect in vivo correlates with activity at D_3 but not D_2 receptor activation in vitro using a bioassay in cells expressing D_2 or D_3 receptors [148, 149]. However, other evidence suggests that D_3 receptors actually would oppose cocaine effects by opposing D_1 receptor function when co-localized on similar striatal neurons [150–152]. Furthermore, the ability to study the functional effects of D_3 receptors using partial D_3-preferring agonists such as BP 897 is complicated by their agonist/antagonist profiles at D_2 and D_3 receptors [153].

Intracranial infusion studies suggest that D_1- and D_2-like receptors in multiple brain regions contribute to the modulation of psychostimulant self-administration by dopamine receptor ligands. Infusions of either D_1- or D_2-like antagonists into the nucleus accumbens mimic the rate-increasing effects of systemic antagonist administration on fixed ratio cocaine and amphetamine self-administration [154–157], an effect illustrated in Fig. 17.2a. However, local stimulation of nucleus accumbens D_1- or D_2-like receptors fails to recapitulate the reduction in cocaine intake produced by systemic agonist administration [157], although infusion of dopamine itself does reduce cocaine intake on fixed ratio self-administration schedules [158]. These results may suggest that D_1- and D_2-like receptors in the nucleus accumbens are saturated with dopamine during cocaine self-administration, or that co-activation of both receptor classes and possibly in multiple brain regions is needed to produce an additive interaction with cocaine to reduce self-administration rates. However, viral-mediated over-expression of D_2 receptors in the nucleus accumbens effectively reduces the rate of fixed ratio cocaine self-administration [159], suggesting that the amount of D_2 receptors in the nucleus accumbens plays an important role in regulating preferred levels of cocaine intake.

Increases in fixed ratio cocaine self-administration produced by local blockade of nucleus accumbens D_1- or D_2-like receptors are paralleled by decreases in the motivation for cocaine (but not food) on progressive ratio schedules (Fig. 17.2b) [80, 160]. The motivational effects are selective for cocaine when infused in the shell subregion, but responding for both rewards is blocked when antagonists are infused in the core subregion potentially reflecting performance deficits [80]. Nucleus accumbens infusions of the D_3 antagonist U99194A or the D_4 antagonist L-750,667 have no effect on cocaine reward in progressive ratio testing.

Fixed ratio cocaine self-administration is increased by infusing the D_1-like antagonist SCH 23390 into several other dopamine terminal regions, including the medial prefrontal cortex [161], insular cortex [162], bed nucleus of the stria terminalis [163], and amygdala [156, 160, 162, 164]. Similar infusions of SCH 23390 in medial prefrontal cortex reduce cocaine reward on progressive ratio schedules [161], but not when infused in the amygdala [160]. These results illustrate that regulation of cocaine intake and the motivation for cocaine are distinct behavioral phenomena mediated by separate neural substrates. Interestingly, infusions of SCH 23390 in the dopamine cell body region of the ventral tegmental area also increase cocaine intake on fixed ratio schedules, and decrease the motivation for cocaine on progressive ratio schedules [165], presumably acting on D_1-like receptors located on the

axons of GABAergic, serotonergic, or glutamatergic input to dopamine neurons. Local blockade of D_2-like receptors in the arcuate nucleus of the hypothalamus increases fixed ratio cocaine intake potentially due to attenuation of cocaine-induced β-endorphin release in the nucleus accumbens [166]. Together, these findings suggest that dopamine receptors in multiple brain regions regulate cocaine reward through vastly different mechanisms.

17.4.2 Modulation of Opiate and Nicotine Self-Administration by Dopamine Receptor Subtypes

Like most drugs of abuse, opiate drugs including heroin and morphine, along with nicotine, stimulate mesolimbic dopamine release that plays a major role in their rewarding properties, although dopamine-independent mechanisms also exist for opiate reward [167, 168]. Opiate and nicotine self-administration on fixed ratio schedules can show compensatory increases in drug intake when the primary receptors (opioid and nicotinic cholinergic) are blocked with antagonists in a surmountable manner. However, the positive effects of dopamine receptor blockade on opiate and nicotine self-administration, where present, generally show reductions rather than increases in drug self-administration on fixed ratio schedules. This difference in the response may reflect an insurmountable blockade produced by blocking dopamine receptors downstream from the primary opioid and nicotinic receptors in reward pathways. Reductions in fixed ratio drug self-administration with dopamine receptor blockade also are found when the rewarding effects are relatively weak, and exhibit shallow inverted U-shaped dose–response curves as found with nicotine compared cocaine or heroin self-administration. Thus, it is important to control for possible impairments in response performance by dopamine antagonists in these studies. Given these caveats, there are far fewer reports of the modulation of opiate or nicotine self-administration with subtype-selective dopamine receptor ligands than with psychostimulant self-administration, which also could suggest that many negative effects have not been reported.

The D_1-like receptor antagonist SCH 23390 blocks the acquisition of heroin self-administration in rats when given as a systemic pretreatment prior to daily acquisition trials, but this treatment also reduces motor behavior and so performance deficits are a consideration [169]. However, similar pretreatments with SCH 23390 infused directly into the nucleus accumbens fail to impair acquisition of heroin self-administration despite decreases in motor behavior, suggesting that D_1-like receptors in the nucleus accumbens do not mediate opiate reward in self-administration paradigms (but see [170]). Interestingly, D_1 receptor knockout mice readily self-administer the opioid receptor agonist remifentanil similar to wild-type controls, despite impairments in cocaine self-administration [16]. Discrepancies between pharmacological blockade and D_1 receptor knockout could suggest a role for D_5 receptors, or involve species differences or compensatory changes with a total loss of D_1 receptors. Regarding the latter, chronic blockade of dopamine receptors causes a compensatory sensitization in dopamine-independent opiate reward in rats self-administering heroin [171].

In contrast, D_2 receptor knockout eliminates intravenous morphine self-administration on either fixed or progressive ratio schedules, without affecting acquisition of water self-administration [77]. Since dopamine-dependent opiate reward is mediated by disinhibition of dopamine neurons in the ventral tegmental area, the loss of both pre- and post-synaptic D_2 receptors complicates the interpretation of these findings. A loss of autoreceptor inhibition of dopamine neurons could occlude disinhibition by opiates. Pre- rather than post-synaptic sites of action are supported by the finding that systemic administration of the D_2-like receptor antagonist sulpiride leads to extinction of intracranial morphine self-administration directly into the ventral tegmental area, but sulpiride fails to alter morphine self-administration directly into the nucleus accumbens [172]. The acquisition of morphine self-administration infused directly into the lateral septum is prevented by systemic administration of either D_1- or D_2-like receptor antagonists using a spatial discrimination Y-maze task less sensitive to performance issues [173].

The ability of D_1-like receptor stimulation to augment heroin reward is indicated by the fact that co-injections of D_1-like agonists with heroin cause an additive leftward shift in the self-administration dose–response curve on a progressive ratio schedule in monkeys, similar to their effect on cocaine self-administration [146]. However, in contrast to cocaine self-administration, heroin co-injected with D_2-like agonists causes a rightward shift of the dose–response curves, again, potentially due to D_2-like autoreceptor stimulation counteracting heroin's ability to disinhibit midbrain dopamine neurons. Together, these findings suggest that while dopamine-dependent opiate reward may involve post-synaptic D_1-like receptors similar to psychostimulant reward, dopamine-independent opiate reward may play a major role especially when dopamine systems are chronically compromised.

Intravenous nicotine is self-administered at modest rates by animals, but inhibition of monoamine oxidase, as produced by compounds found in tobacco, dramatically enhances nicotine self-administration in rats [174]. The enhanced nicotine self-administration is blocked by systemic pretreatment with D_1-like receptor antagonist SCH 23390. When nicotine is self-administered directly into the ventral tegmental area, self-administration is reduced by systemic administration of SCH 23390 [175], or by co-infusion with the D_2-like receptor agonist quinpirole [176], the latter reflecting autoreceptors counteracting the ability of nicotine to excite dopamine neurons. A high dose of the D_3 antagonist SB-277011A reduces nicotine but not food self-administration on a more demanding progressive ratio schedule, even though this dose also impairs motor activity [177]. Another study found that SB-277011A has no effect on stable fixed ratio nicotine self-administration at doses that block nicotine-primed relapse to nicotine seeking in the absence of reward [178]. Oral nicotine self-administration is increased by clozapine but not haloperidol, potentially reflecting surmountable blockade of D_4 receptors [179]. Together, these studies suggest that both D_1-like and D_3 receptors play a role in the rewarding efficacy of nicotine, although the role of D_2, D_4, and D_5 receptors remains undetermined.

17.4.3 Modulation of Alcohol Self-Administration by Dopamine Receptor Subtypes

Studies on alcohol self-administration also support a role for dopamine receptors in modulating alcohol intake and reward. Most studies measure volitional but freely available alcohol consumption in a choice over water or sucrose, but some investigators employ instrumental responses rewarded by alcohol presentation. Previous studies have found that either D_1- or D_2-like receptor blockade with SCH 23390 or raclopride can reduce alcohol consumption, but with the caveat that similar doses also reduce water intake [180]. Another study found that SCH 23390 and spiperone (D_2-like) reduce water but not alcohol consumption [181]. Other studies using alcohol-preferring rats found that D_2-like blockade with spiperone slightly increases alcohol intake, while the D_1-like receptor antagonist SCH 23390 reduces consumption [182]. The D_1-like receptor antagonist SCH 31966, devoid of serotonergic receptor activity, also reduces intake in alcohol-preferring rats, and at doses that reduce sucrose but not water intake [183]. In contrast, the D_2-like antagonist remoxipride fails to affect alcohol intake when instrumental responses are required, even at doses that reduce appetitive responding in non-rewarded sessions [184]. Thus, studies in rats suggest that D_1- rather than D_2-like receptors may be necessary for alcohol reward.

In mice, pretreatment with either the D_1-like agonist SKF 38393 or the D_2-like agonist bromocriptine reduces alcohol consumption, but the effect of bromocriptine is reduced after mice are sensitized to alcohol exposure [185]. In alcohol-preferring rats, SKF 38393 reduces alcohol intake similar to the D_1-like antagonist SCH 23390, potentially reflecting the partial agonist properties of SKF 38393 or satiating effects with D_1-like stimulation [182]. Pretreatment with the D_2-like agonist quinpirole also reduces alcohol intake via dopamine autoreceptor effects as discussed below. However, pretreatment with the D_2-like agonist 7-OH-DPAT at very low presumably autoreceptor doses increases alcohol drinking [180]. The putative D_3 antagonist U99194A fails to alter preference for alcohol in mice [186], while a high dose of the D_3 antagonist SB-277011A reduces alcohol intake in rats [187, 188]. It is interesting that both D_1 and D_2 receptor knockout mice actually show an aversion to alcohol [189, 190]; while loss of pre-synaptic D_2 autoreceptors could occlude the rewarding effects of alcohol that otherwise disinhibit dopamine neurons, post-synaptic D_2 receptor responses also could be involved in D_2 receptor knockout mice. In any event, findings in knockout mice strongly implicate dopamine in the rewarding effect of alcohol.

When alcohol is self-administered directly into the ventral tegmental area, coinfusion with quinpirole reduces self-administration to control levels, reflecting the ability of direct autoreceptor stimulation to oppose alcohol-mediated disinhibition of dopamine neurons [191]. However, blockade of D_1-like receptors in dopamine terminal region of the nucleus accumbens with SCH 2390 also decreases alcohol intake without altering the overall rate of instrumental responding [192]. Nucleus accumbens infusions of the D_2-like receptor antagonist raclopride reduce both intake and instrumental response rates [192, 193], potentially due to motor

impairment. In the bed nucleus of the stria terminalis, D_1- but not D_2-like receptor blockade reduces instrumental responding for alcohol while producing lesser reductions in responding for sucrose [194].

Conversely, in a free choice procedure, intranucleus accumbens infusions of SCH 23390 have little effect on alcohol preference in alcohol-preferring rats, while the D_2-like antagonist sulpiride dose dependently increases consumption of alcohol without altering intake of sucrose or saccharine [195]. Microinfusion of sulpiride into the ventral pallidum also increases alcohol intake under free choice conditions, while SCH 23390 has little effect, although both antagonist treatments elevate extracellular dopamine levels [196]. These findings suggest that alcohol intake is differentially sensitive to blockade of dopamine receptors under free choice or instrumental response procedures. D_1-like receptors in the nucleus accumbens and bed nucleus of the stria terminalis may play a necessary role in regulating alcohol reward when instrumental responses are required. In contrast, D_2-like receptors in the nucleus accumbens and ventral pallidum may mediate inhibitory feedback regulation of alcohol intake under free choice conditions.

Stimulation of nucleus accumbens D_2-like receptors with quinpirole infusions increases instrumental responding for alcohol at low doses, opposite to the effect of receptor blockade, but decreases responding at high doses [193, 197]. Infusions of the D_1-like agonist SKF 38393 have no effect. The increase in self-administration with low dose quinpirole is prevented by co-infusion of either SKF 38393 or SCH 23390 [197], suggesting that D_1-like receptor tone is important for the expression of post-synaptic D_2-like-mediated increases in alcohol self-administration. In contrast to instrumental responding for alcohol, viral vector-mediated increases in nucleus accumbens D_2 receptors decrease both alcohol preference and intake in preferring and non-preferring rats [198], indicating that the D_2 receptor itself is sufficient to regulate alcohol reward. Together these findings suggest that while stimulation of D_2-like receptors in the nucleus accumbens is sufficient to facilitate instrumental responding for alcohol, increases in the amount of D_2 receptors reduce alcohol intake under free choice conditions consistent with negative feedback regulation of intake discussed above.

17.5 Dopamine Receptor Subtypes in Relapse to Drug-Seeking Behavior

In addition to playing a critical role in drug reward, the mesolimbic dopamine system is a major neural substrate for drug-seeking behavior. The mesolimbic dopamine system is activated by exposure to drug-related environmental cues, stress, and other pharmacological stimuli that trigger relapse to drug seeking during withdrawal [199–204]. Dopamine release in forebrain regions such as the nucleus accumbens is sufficient to trigger relapse to drug seeking. Moreover, in some but not

all cases, such dopamine release is necessary for environmental or pharmacological stimuli to trigger drug-seeking behavior.

Relapse to drug seeking is reflected by approach behavior aimed at performing responses that delivered drug injections on prior occasions during self-administration. Like self-administration on progressive ratio schedules, most studies measure the level of effort animals will exert to obtain drug when reward is withheld as an index of drug-seeking behavior, and this behavior is thought to reflect wanting or craving that would precipitate relapse to drug use in humans [7, 205]. An important distinction from self-administration behavior is that responding is measured in the non-rewarded or drug-free state either without or before reward delivery.

The most commonly used method to model the propensity for relapse to drug seeking is the extinction/reinstatement paradigm. The paradigm has face validity because environmental and pharmacological stimuli that reinstate drug seeking in animals also trigger drug craving in humans [206–208]. In the extinction phase of this procedure, drug-seeking behavior is elicited by environmental and contextual cues associated with drug use in the self-administration test chambers, and ultimately diminishes with repeated training in the absence of drug reward. Following extinction of drug-seeking behavior, the ability of specific experimenter-delivered stimuli to elicit or "reinstate" drug-paired lever responding is measured. The reinstatement of drug-seeking behavior can be induced by priming injections of drugs, presentation of discrete or environmental cues associated with drug injections, and by brief exposure to moderate intermittent footshock stress. There are numerous extinction/reinstatement studies in rats and monkeys. However, there are very few reports in mice, since commonly used strains do not exhibit effective reinstatement of drug seeking with non-contingent priming injections of drugs [209, 210], although alcohol-primed reinstatement of alcohol seeking has been reported in mice [188]. More recently, the reinstatement paradigm has been adapted to the Pavlovian conditioned place preference model of drug reward, but it is difficult to conceive that conditioned place preference reflects drug-seeking behavior without volitional drug self-administration on prior occasions. Furthermore, the role of dopamine receptor subtypes in the reinstatement of volitional drug seeking markedly differs from their role in the reinstatement of a drug-induced conditioned place preference [43, 139].

Another model of drug-seeking behavior involves the use of second-order schedules of drug self-administration, where initial responding is rewarded by discrete cues predictive of ultimate drug availability. The second-order schedule illustrates the powerful control over drug seeking exerted by these cues. However, since this cue-rewarded drug seeking declines without ultimate delivery of the drug reward, it has been used less extensively to model long-term drug withdrawal. Reinstatement paradigms, on the other hand, suffer from the fact that drug seeking is triggered after extinction of the behavior, a situation vastly different from human drug abuse where craving and relapse occur without extinction experience.

17.5.1 Modulation of Cocaine Seeking by Dopamine Receptor Subtypes: Systemic Administration

Most work on the role of dopamine receptors in drug-seeking behavior has been conducted in animals self-administering cocaine. As discussed above, cocaine self-administration is reduced by systemic pretreatment with either D_1- or D_2-like receptor agonists, suggesting that both receptors provide inhibitory feedback regulation of cocaine intake during self-administration. Cocaine seeking in the absence of reward also is strongly regulated by both D_1- and D_2-like dopamine receptor classes, except that they mediate opposite effects on this relapse behavior (Fig. 17.3a). Thus, selective stimulation of post-synaptic D_2-like receptors is a powerful trigger of cocaine-seeking behavior during or after responses extinguish [19, 139, 211–216]. Conversely, selective D_1-like receptor stimulation is virtually without effect, even when locomotor activation is similar to D_2-like receptor stimulation [139, 211, 214, 215]. In addition, pretreatment with full efficacy D_1-like receptor agonists will block the ability of a single cocaine priming injection, or the presentation of cocaine-associated cues, to reinstate cocaine seeking in the absence of increased stereotypy [139, 142, 216–218]. Conversely, pretreatment with D_2-like agonists facilitates cocaine-primed reinstatement [139]. A similar D_1-/D_2-like dichotomy regulates cocaine seeking in monkeys [219, 220] and also has been shown in some studies to suppress and stimulate craving responses in humans, respectively [221, 222]. The D_3-preferring agonist PD 128,907 fails to mimic the reinstating effects of D_2-like agonists in monkeys [219]. Together, these studies suggest that D_2 receptors play a major role in eliciting relapse to cocaine seeking when environmental stimuli such as cocaine-related cues or stress activate the mesolimbic dopamine system [223], while high D_1 receptor tone may provide inhibitory regulation over cocaine seeking by satiating primary reward processes.

While systemic administration of D_2-like but not D_1-like receptor agonists is sufficient to trigger cocaine-seeking behavior, both receptor types are necessary for the expression of cocaine seeking. Thus, systemic pretreatment with either D_1- or D_2-like receptor antagonists attenuates cocaine-seeking behavior elicited by priming injections of cocaine [217, 219, 224], discrete cues associated with prior cocaine injections [217, 225], cues predictive of cocaine availability [213, 226], or exposure to a cocaine-associated environmental context [227]. In monkeys, cocaine-primed reinstatement is not reduced by the D_3-preferring antagonists UH 232 and AJ-76 [219]. In rats, however, the D_3 antagonist SB-277011-A reduces cocaine seeking during initial extinction conditions [228], and reduces responding maintained by cocaine-associated but not sucrose-associated cues with a second-order self-administration schedule [229]. Similarly, the D_3 antagonists SB-277011A and/or NGB 2904 dose dependently attenuate cue-primed [230], cocaine-primed [138, 231], and footshock stress-primed [232] reinstatement of cocaine seeking following extinction, without altering reinstatement of sucrose-seeking behavior [232]. Since these compounds also reduce cocaine self-administration on progressive ratio self-administration schedules, but fail to increase cocaine intake on fixed ratio schedules

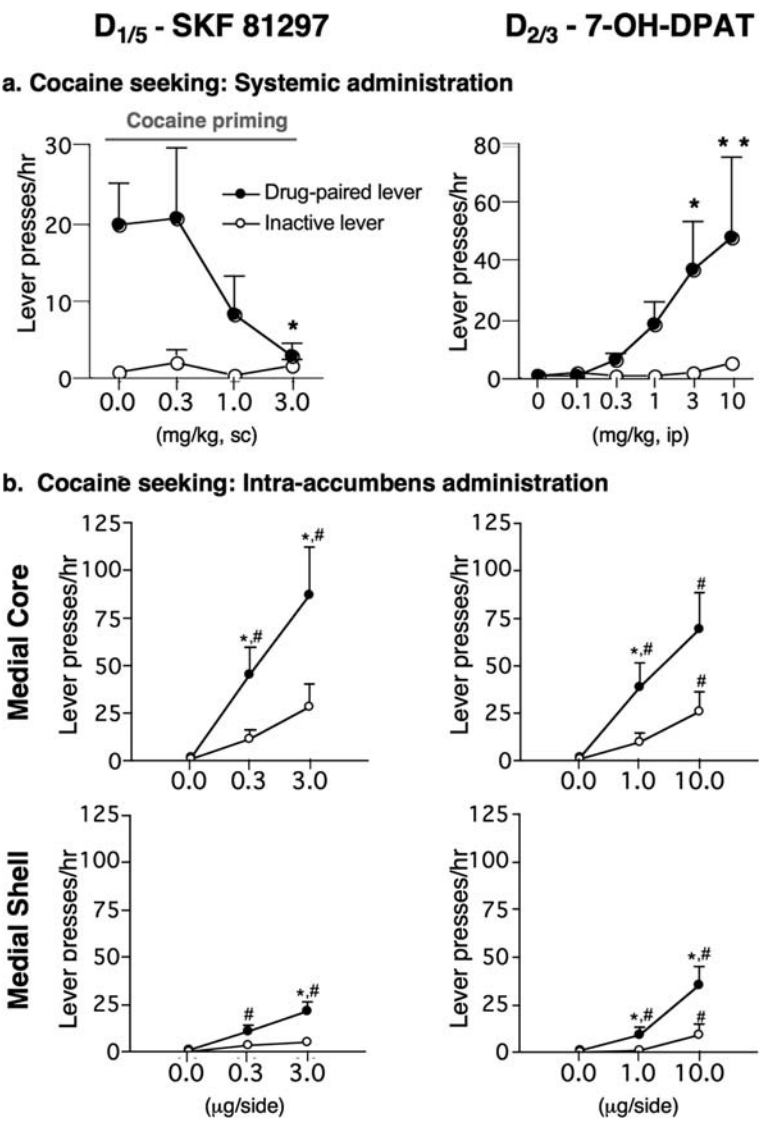


Fig. 17.3 (**a**) *Left*, systemic subcutaneous (sc) pretreatment with the highly selective D_1-like dopamine agonist SKF 81297 attenuates the ability of intravenous cocaine (2.0 mg/kg) priming to reinstate cocaine-seeking behavior (non-rewarded responding on the drug-paired lever) in rats. The priming injections of cocaine were given 30 min after pretreatment with SKF 81297 following extinction of cocaine-seeking behavior in the reinstatement paradigm. *Right*, intraperitoneal (ip) priming injections with the D_2-like agonist 7-OH-DPAT trigger cocaine-seeking behavior ($^*P < 0.05$ compared to vehicle pretreatment). (**b**) When infused directly into the nucleus accumbens, both D_1- and D_2-like agonists reinstate cocaine-seeking behavior in rats, with greater effects in the medial core than medial shell subregions of nucleus accumbens ($^*P < 0.05$ compared to inactive lever responses or vehicle-infused controls $^{\#}P < 0.05$). Reinstatement of non-rewarded lever-press responding at the drug-paired lever is thought to model relapse behavior. Adapted with permission from (**a**) Self et al. [139] and (**b**) Bachtell et al. [157]

(unlike general D_2-like antagonists), D_3 receptors may play a distinct role in the motivation for cocaine independent of regulation of cocaine intake as discussed in Section 17.4.1.

17.5.2 Modulation of Cocaine Seeking by Dopamine Receptor Subtypes: IntraCranial Administration

Although systemic D_1-like agonist administration attenuates cocaine-seeking behavior, infusion of SKF 81297 directly into the nucleus accumbens actually triggers cocaine seeking in reinstatement paradigms [157, 233]. This effect involves D_1-like modulation of calcium/calmodulin-mediated kinase II and activation of L-type calcium channels [234]. Nucleus accumbens infusions of D_2-like agonists also reinstate cocaine seeking [157, 233], an effect that may involve D_2-like receptor-mediated inhibition of cyclic AMP-protein kinase A signaling [235]. In both cases, D_1- and D_2-like agonists induce more robust cocaine seeking when infused into the medial core rather than the shell subregion as shown in Fig. 17.3b, while lateral core infusions are ineffective [157, 233]. Such enhanced sensitivity of dopamine receptor responses in the medial nucleus accumbens core may be important for cocaine seeking elicited by cocaine-associated cues, since unanticipated cues elevate dopamine levels in the core rather than shell [203, 236].

As shown in Fig. 17.4a, the reinstating effects of D_2-like agonists in the nucleus accumbens probably involve the D_2 subtype, since the D_3 agonist PD 128,907 and the D_4 agonist PD 168,077 fail to mimic reinstatement of cocaine seeking elicited by the D_2-like agonist quinpirole [233]. Synergistic interactions between D_1- and D_2-like receptors are demonstrated by the ability of subthreshold doses of SKF 81297 and quinpirole to elicit far greater cocaine-seeking behavior when co-infused in the nucleus accumbens than when infused alone (Fig. 17.4b) [237]. Similarly, cooperativity between D_1- and D_2-like receptors and a role for endogenous dopamine receptor tone is indicated by the ability of the D_1-like antagonists to block cocaine seeking induced by D_2-like agonists, while D_2-like antagonists block cocaine seeking induced by D_1-like agonists, when co-infused in the nucleus accumbens (Fig. 17.4c) [157, 237].

In contrast to direct activation of dopamine receptors, systemic priming injections of cocaine preferentially elevate dopamine levels in the shell subregion of the nucleus accumbens [238, 239]. Blockade of either D_1- or D_2-like receptors in the nucleus accumbens shell attenuates cocaine seeking elicited by systemic cocaine priming injections [157, 240, 241]. Nucleus accumbens infusions of the D_3 antagonist U99194A and the D_4 antagonist L-750667 are ineffective [241]. However, nucleus accumbens infusion of the D_3 antagonist SB-277011A is effective at blocking reinstatement of cocaine seeking induced by footshock stress [232], but SB-277011A fails to block cue-maintained cocaine seeking on a second-order self-administration schedule [242]. Since response-contingent cue presentation in second-order schedules does not increase dopamine levels in the nucleus accumbens [236], it is not clear whether local D_3 blockade would attenuate reinstatement of cocaine

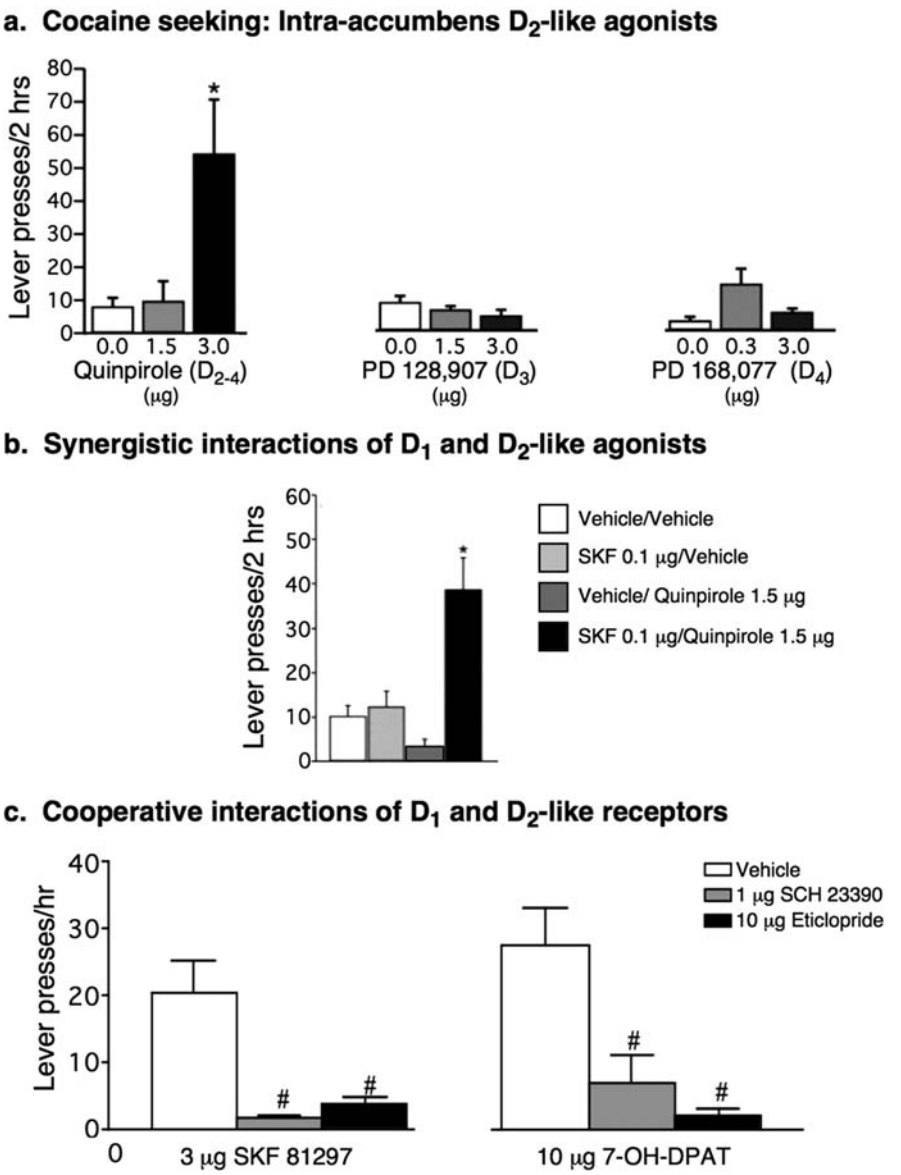


Fig. 17.4 (**a**) Intranucleus accumbens shell infusions of the D_2-like agonist quinpirole, but not the D_3 agonist PD 128,907 or the D_4 agonist PD 168,077, elicit cocaine-seeking behavior in a reinstatement paradigm in rats. Total number of responses (mean ± S.E.M.) on the drug-paired lever differs from saline ($^*P < 0.05$). (**b**) Co-infusion of subthreshold doses of SKF 81297 and quinpirole into the nucleus accumbens shell reinstates cocaine seeking. Total number of drug-paired lever responses differs from either SKF 81297 or quinpirole alone ($^*P < 0.05$). (**c**) Cross blockade of reinstatement induced by intra-accumbens D_1- and D_2-like agonists by D_1- and D_2-like antagonists. Co-infusion of SCH 23390 or eticlopride in the nucleus accumbens blocks SKF 81297- and 7-OH-DPAT-induced reinstatement of cocaine seeking. $^{\#}P < 0.05$ compared to vehicle/agonist co-infused controls. Adapted with permission from (**a**) Schmidt et al. [233], (**b**) Schmidt and Pierce [237], and (**c**) Bachtell et al. [157]

seeking induced by unanticipated cues that do elevate nucleus accumbens dopamine. In any event, it appears that D_1- and D_2-like receptor stimulations in the nucleus accumbens is both sufficient and in most cases necessary for induction of cocaine-seeking behavior. Given the ability of systemic D_3 antagonist administration to block cocaine- and cue-mediated cocaine seeking, these findings suggest that D_3 receptors in other brain regions may be involved, while D_3 receptors in the nucleus accumbens could play a role in stress-induced relapse. Conversely, the failure to block cue- and cocaine-primed cocaine seeking with nucleus accumbens infusions of D_3 antagonists indirectly implicates D_2 receptors in these behaviors, since D_4 receptors are only marginally expressed in the nucleus accumbens [110, 111]. Clearly, further investigation with multiple and selective D_3 ligands is needed.

Reinstatement of cocaine seeking by response-contingent cues is blocked by local infusion of the D_1-like receptor antagonist SCH 23390 in the amygdala, while the D_2-like antagonist raclopride is ineffective [243]. However, amygdala infusions of the D_3 antagonist SB-277011A reduce cue-maintained responding using a second-order self-administration schedule [242]. These results suggest that D_1-like and D_3 receptors in the amygdala may be critical for the expression of conditioned reward mediated by cocaine-associated cues, similar to their role in the formation of these associations during conditioning as discussed in Section 17.3.3. Cocaine-primed reinstatement of cocaine seeking also is blocked by amygdala infusions of the D_1-like antagonist SCH 23390 [162].

Modulation of neocortical dopamine receptors also regulates cocaine-seeking behavior. Local blockade of D_1- or D_2-like receptors in the medial prefrontal cortex with SCH 23390 and eticlopride, respectively, attenuates cocaine-primed relapse to cocaine seeking without affecting food self-administration [244]. Another study found no effect when specifically targeting the prelimbic subregion with SCH 23390 and raclopride [245]. However, this study found that blockade of D_1-like, but not D_2-like, receptors in the medial prefrontal cortex attenuates relapse to cocaine seeking induced by footshock stress. Cocaine- and cue-primed reinstatement of cocaine seeking also is attenuated by D_1-like blockade in the insular cortex [162]. Interestingly, stimulation of D_1-like receptors in the agranular insular area of prefrontal cortex with SKF 81297 actually reduces cocaine seeking maintained by cues on a second-order schedule of cocaine self-administration [246]. In this sense, the agranular insular subregion of cortex represents the only brain site known to date where a D_1-like agonist attenuates cocaine seeking in a manner consistent with systemic administration. However, focal D_1-like receptor stimulation in regions such as the nucleus accumbens may have different effects on limbic circuits than when the same accumbens D_1-like receptors are stimulated concomitant with brain-wide D_1-like receptor activation. For example, D_1-like receptor stimulation of prefrontal cortical afferents to nucleus accumbens alters the physiological response to coincident D_1-like receptor stimulation in nucleus accumbens neurons, producing excitatory rather than inhibitory effects [247]. If so, the behavioral responses of such isolated and localized receptor activation could be epiphenomenal to more natural situations.

17.5.3 Modulation of Heroin, Nicotine, and Alcohol Seeking by Dopamine Receptor Subtypes

Far fewer studies have been conducted on dopamine receptor involvement in relapse behavior with other drugs of abuse. As found with cocaine-trained animals, reinstatement of heroin-seeking behavior is induced by systemic administration of the D_2-like receptor agonist quinpirole, but only as long as animals remain sensitized to D_2-like receptor stimulation in withdrawal [19, 248]. Similarly, the D_1-like agonist SKF 82958 is ineffective and tends to reduce heroin seeking [211]. Systemic pretreatment with the D_1-like antagonist SCH 23390 or the D_2-like agonist raclopride blocks reinstatement induced by non-contingent heroin priming injections, but fails to block relapse elicited by footshock stress [249]. However, generalized blockade of both D_1- and D_2-like receptors with flupenthixol effectively blocks this behavior [249], suggesting a role for dopamine in stress-induced relapse. Systemic administration of SCH 23390, but not raclopride or the D_3 antagonist NGB 2904, attenuates reinstatement of heroin seeking induced by food deprivation [250], indicting a specific role for D_1-like receptors in the motivational effects of hunger on heroin-seeking behavior. However, stimulation of D_2-like receptors with 7-OH-DPAT effectively reinstates food-seeking behavior in animals self-administering food pellets, although D_2-like antagonists fail to block food seeking elicited by non-contingent priming with food pellets [251]. These results suggest that D_2-like receptor activation may underlie a fundamental mechanism for reward-seeking behavior in the absence of primary reward, whereas both D_1- and D_2-like receptors are necessary for expression of heroin seeking induced by heroin priming and footshock stress.

Blockade of D_1-like receptors in the nucleus accumbens shell reduces reinstatement of heroin seeking induced by environmental contextual cues, whereas heroin seeking elicited by discrete cues paired with prior heroin injections is reduced by blockade of D_1-like receptors in the nucleus accumbens core [252], consistent with the ability of discrete cocaine-associated cues to induce dopamine release in the core but not in the shell [236]. Cues that predict food availability will reinstate food-seeking behavior, and this effect is blocked by local D_1-like receptor blockade in the nucleus accumbens [253].

Systemic administration of a novel D_3 antagonist or SB-277011A attenuates nicotine-primed reinstatement of nicotine seeking in rats, without affecting cue-primed reinstatement or fixed ratio nicotine self-administration [178, 254]. Since D_3 antagonists also reduce nicotine self-administration on a progressive ratio schedule (see Section 17.4.2), D_3 receptors may be critical for the incentive motivational effects of nicotine. Pretreatment with SB-277011A also blocks alcohol- and cue-primed reinstatement of alcohol seeking in rats and mice [188, 255], and the withdrawal-induced binge in alcohol consumption known as the alcohol deprivation effect [255]. More generalized D_2-like receptor blockade with remoxipride reduces alcohol-seeking behavior under initial extinction conditions at doses that have no effect on instrumental responding for alcohol [184]. The effects of systemic remoxipride on alcohol seeking are recapitulated by infusions of raclopride

directly into the nucleus accumbens at doses that have no effect on alcohol consumption [256, 257]. D_1- and D_2-like antagonists also attenuate alcohol seeking induced by cues that predict alcohol availability in rats [258], and the D_1-like antagonist SCH 23390 blocks renewal of alcohol seeking elicited by an environmental context associated with alcohol self-administration [259]. These findings suggest that both D_1- and D_2-like receptors, potentially in the nucleus accumbens, are important for relapse to nicotine- and alcohol-seeking behavior, similar to cocaine relapse. A role for D_3 receptors in the motivation for nicotine and alcohol during withdrawal also is strongly indicated.

17.6 Future Directions

With the advent of subtype-selective ligands and knockout mice, a vast body of work on dopamine receptor function in natural and drug reward and in relapse to drug-seeking behavior has accumulated in the past two decades. An equally expansive literature has shown that chronic drug and alcohol use can change the amount of D_1- and D_2-like receptors in specific brain regions in animals and more recently, in humans using neuroimaging procedures. A major challenge for future work will involve integrating observed changes in the amount of dopamine receptors in specific brain regions with changes in dopamine receptor regulation of reward and relapse. The breadth of our current knowledge will facilitate this integration, but it is important for future research to track changes in the biochemical, physiological, and behavioral responses mediated by D_1- and D_2-like receptors as animals transit from initial drug self-administration to more addicted biological states. It is also important to identify premorbid alterations in D_1- and D_2-like receptors that predispose individual vulnerability to drug and alcohol addiction.

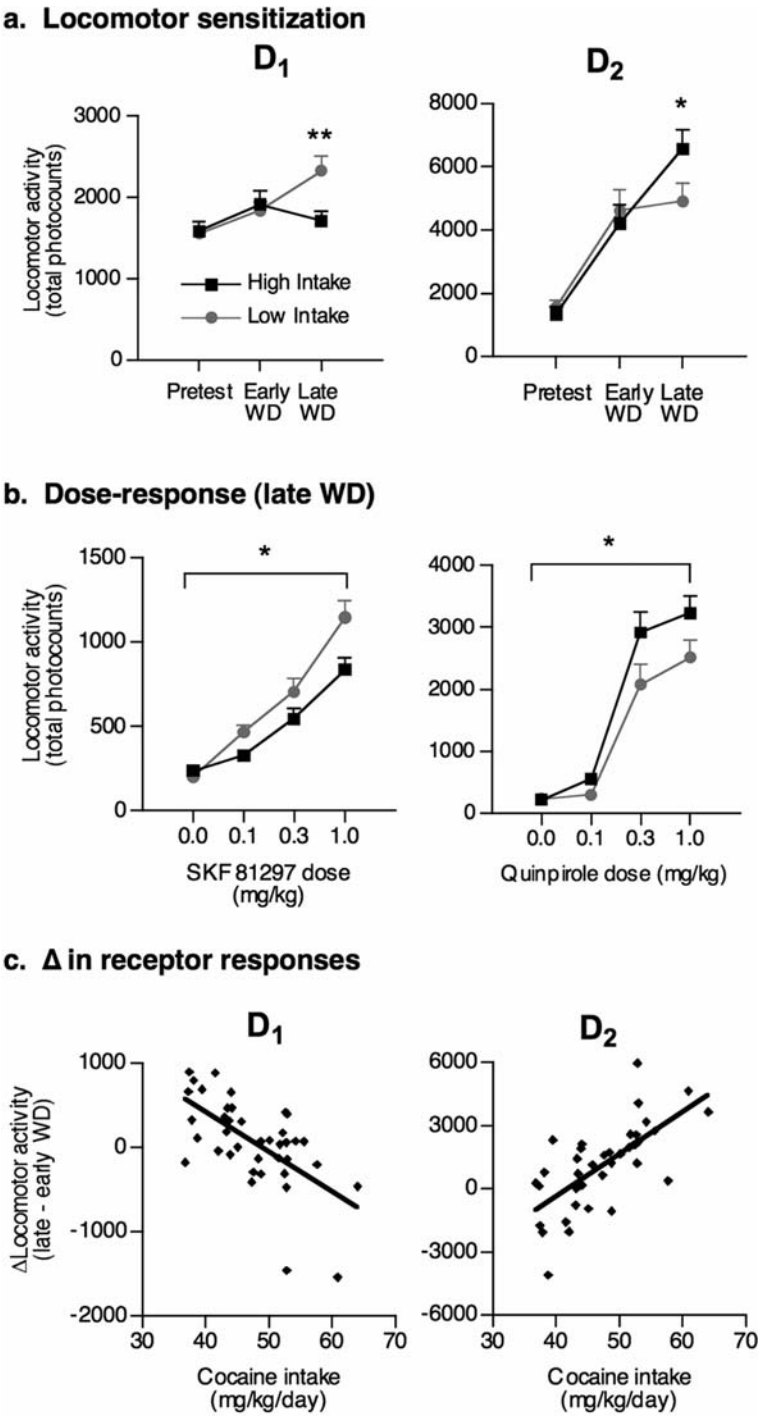
For example, decreases in the amount of D_2-like receptors in the nucleus accumbens of rats are predictive of impulsive behavior and early escalation of cocaine self-administration [260], whereas increases in D_2-like receptors that accompany social dominance in monkeys are associated with resistance to initial propensity for cocaine self-administration [261]. After self-administration is initiated, prolonged daily access to drugs leads to profound escalation in drug intake, along with a greater propensity for relapse to drug seeking [126, 262, 263]. Animals that escalate cocaine self-administration also show enhanced sensitivity to the rate-altering effects of generalized dopamine receptor blockade on a fixed ratio self-administration schedule[264] suggesting a loss of D_1- and/or D_2-like dopamine receptor function with the transition to addiction. These results are consistent with enduring reductions in cortical and striatal D_2-like receptors that are found in human cocaine, methamphetamine, heroin, and alcohol addicts [265–268].

These decreases in D_2-like receptors are intriguing given that D_2-like receptor stimulation triggers relapse to drug-seeking behavior as discussed above. Furthermore, animal data clearly show that chronic cocaine or heroin self-administration [211, 216, 248], and opiate withdrawal [269], increases sensitivity

to D_2-like receptor-mediated behaviors. Similarly, rats with higher preferred levels of cocaine intake are more sensitive to D_2-like agonist-induced relapse to cocaine seeking [216], consistent with the development of greater sensitization in D_2-like receptor responses in more addicted biological states. Conversely, rats with lower preferred levels of cocaine intake are more sensitive to the ability of a D_1-like agonist to suppress cocaine-primed relapse to cocaine-seeking behavior [216], potentially reflecting a compensatory neuroadaptation that prevents low intake animals from developing a more addicted phenotype. These opposing differences in D_1- and D_2-like receptor regulation of cocaine seeking are paralleled by changes in the unconditioned locomotor response to D_1- and D_2-like receptor challenge after 4 weeks withdrawal, where both D_1- and D_2-like receptors mediate directionally similar behavioral effects (Fig. 17.5a–b). Furthermore, higher levels of cocaine intake are positively correlated with sensitization in D_2-like receptor responsiveness after 4 weeks withdrawal from self-administration, and negatively correlated with changes in D_1-like receptor responsiveness (Fig. 17.5c).

Thus, a challenge for future work will be to relate changes in the amount of D_1- and D_2-like receptors in human drug abusers or in animal studies to seemingly discrepant changes in behavioral responses mediated by dopamine receptors in animal models of drug addiction. One possible explanation for these discrepancies could involve the finding that chronic drug administration increases the amount of high affinity (G protein-coupled) D_2-like receptors in striatal regions, despite decreases in the total amount of D_2-like receptors [270–273]. Thus, drug-induced neuroadaptations that reduce the total amount of D_2-like receptors often are associated with increases in the amount of functional D_2-like receptors. It would be interesting to determine whether the amount of functionally coupled D_2-like receptors parallels or diverges from sensitization in D_2-like receptor-mediated relapse in the same study. In this regard, decreases in the amount of total D_2-like receptor binding in striatal subregions in human cocaine abusers fail to correlate with cocaine-induced euphoria or cocaine-primed self-administration [274], which could reflect an inability to measure functionally coupled D_2-like receptors in vivo.

Fig. 17.5 Differential development of D_1- and D_2-like receptor sensitization in low and high intake rats during withdrawal from chronic cocaine self-administration. (**a**) Low intake animals develop D_1-like receptor sensitization from early to late withdrawal, whereas high intake animals develop greater D_2-like receptor sensitization. Data in *top panels* show changes in cumulative locomotor responses for dose–response tests (0.1–1.0 mg/kg) with the D_1-like agonist SKF 81297 and the D_2-like agonist quinpirole conducted before self-administration (pretest) and at early (2–3 days) and late (28–29 days) withdrawal (WD) times. (**b**) Locomotor dose–response data for low and high intake animals challenged with the D_1-like and D_2-like agonists at late withdrawal. Asterisks indicate that high differ from low intake animals $^{*}p < 0.05$, ** $p < 0.01$. (**c**) Average daily cocaine intake during the last 6 days of acquisition training is negatively correlated with the change (Δ) in D_1 responsiveness from early to late withdrawal ($r = -0.507$, $p = 0.001$), but positively correlated with the change in D_2 responsiveness ($r = 0.686$, $p < 0.001$) among individual animals based on cumulative locomotor responses in dose–response tests. Reproduced with permission from Edwards et al. [216]


a. Locomotor sensitization
D1
D2
Locomotor activity (total photocounts)
3000
2000
1000
0
**
High Intake
Low Intake
Pretest
Early WD
Late WD
8000
6000
4000
2000
0
*
b. Dose-response (late WD)
1500
1000
500
0
0.0
0.1
0.3
1.0
SKF 81297 dose (mg/kg)
4000
3000
2000
1000
Quinpirole dose (mg/kg)
c. Δ in receptor responses
ΔLocomotor activity (late - early WD)
1000
0
-1000
-2000
30
40
50
60
70
Cocaine intake (mg/kg/day)
6000
3000
-3000
-6000

Given that D_1- and D_2-like receptors play such integral roles in reward and relapse behavior, alterations in these receptors due to genetic or environmental factors will remain a major focus for addiction research. It is increasingly apparent that changes in the amount of D_1- and D_2-like receptors in drug and alcohol addiction belie a straightforward interpretation that integrates these findings with their functional roles in reward and relapse phenomena. More work is needed to compare changes in the amount of receptors with changes in D_1-like and D_2-like receptor function using biochemical and physiological measures. It is imperative that findings from multiple approaches ultimately converge with changes in behavioral responses mediated by D_1- and D_2-like receptors before an unambiguous understanding of alterations in dopamine receptor function in drug and alcohol addiction can be appreciated.

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References

1. Halberda JP, Middaugh LD, Gard BE, Jackson BP. DAD1- and DAD2-like agonist effects on motor activity of C57 mice: differences compared to rats. Synapse 1997;26:81–92.
2. Mirenowicz J, Schultz W. Preferential activation of midbrain dopamine neurons by appetitive rather than aversive stimuli. Nature 1996;379:449–51.
3. Schultz W. Predictive reward signal of dopamine neurons. J Neurophysiol 1998;80:1–27.
4. Baxter BL, Gluckman MI, Stein L, Scerni RA. Self-injection of apomorphine in the rat: positive reinforcement by a dopamine receptor stimulant. Pharmacol Biochem Behav 1974;2:387–91.
5. Baxter BL, Gluckman MI, Scerni RA. Apomorphine self-injection is not affected by alpha-methylparatyrosine treatment: support for dopaminergic reward. Pharmacol Biochem Behav 1976;4:611–2.
6. Schultz W. Multiple dopamine functions at different time courses. Annu Rev Neurosci 2007;30:259–88.
7. Berridge KC, Robinson TE. What is the role of dopamine in reward: hedonic impact, reward learning, or incentive salience? Brain Res Rev 1998;28:309–69.
8. Everitt BJ, Dickinson A, Robbins TW. The neuropsychological basis of addictive behaviour. Brain Res Rev 2001;36:129–38.
9. Salamone JD, Correa M, Farrar A, Mingote SM. Effort-related functions of nucleus accumbens dopamine and associated forebrain circuits. Psychopharmacology 2007;191:461–82.
10. Woolverton WL, Goldberg LI, Jinos JZ. Intravenous self-administration of dopamine receptor agonists by rhesus monkeys. J Pharmacol Exp Ther 1984;230:678–83.
11. Self DW, Stein L. The D_1 agonists SKF 82958 and SKF 77434 are self-administered by rats. Brain Res 1992;582:349–52.
12. Weed MR, Vanover KE, Woolverton WL. Reinforcing effect of the D_1 dopamine agonist SKF 81297 in rhesus monkeys. Psychopharmacology 1993;113:51–2.
13. Weed MR, Woolverton ML. The reinforcing effects of D1 receptor agonists in rhesus monkeys. J Pharmacol Exp Ther 1995;275:1367–74.
14. Grech DM, Spealman RD, Bergman J. Self-administration of D1 receptor agonists by squirrel monkeys. Psychopharmacology 1996;125:97–104.
15. Self DW, Belluzzi JD, Kossuth S, Stein L. Self-administration of the D1 agonist SKF 82958 is mediated by D1, and not D2, receptors. Psychopharmacology 1996;123:303–6.

16. Caine SB, Thomsen M, Gabriel KI, et al. Lack of self-administration of cocaine in dopamine D_1 receptor knock-out mice. J Neurosci 2007;27:13140–50.
17. Self DW, Stein L. Receptor subtypes in opioid and stimulant reward. Pharmacol Toxicol 1992;70:87–94.
18. Yokel RA, Wise RA. Amphetamine-type reinforcement by dopaminergic agonists in the rat. Psychopharmacology 1978;58:289–96.
19. Wise RA, Murray A, Bozarth MA. Bromocriptine self-administration and bromocriptine-reinstatement of cocaine-trained and heroin-trained lever pressing in rats. Psychopharmacology 1990;100:355–60.
20. Caine SB, Koob GF. Modulation of cocaine self-administration in the rat through D-3 dopamine receptors. Science 1993;260:1814–6.
21. Manzardo AM, Del Rio JA, Stein L, Belluzzi JD. Rats choose cocaine over dopamine agonists in a two-lever self-administration preference test. Pharmacol Biochem Behav 2001;70:257–65.
22. Woolverton WL, Ranaldi R. Comparison of the reinforcing efficacy of two dopamine D2-like receptor agonists in rhesus monkeys using a progressive-ratio schedule of reinforcement. Pharmacol Biochem Behav 2002;72:803–9.
23. Nader MA, Mach RH. Self-administration of the dopamine D_3 agonist 7-OH-DPAT in rhesus monkeys is modified by prior cocaine exposure. Psychopharmacology 1996;125:13–22.
24. Sinnott RS, Mach RH, Nader MA. Dopamine D2/D3 receptors modulate cocaine's reinforcing and discriminative stimulus effects in rhesus monkeys. Drug Alcohol Depend 1999;54:97–110.
25. Ikemoto S, Glazier BS, Murphy JM, McBride WJ. Role of dopamine D1 and D2 receptors in the nucleus accumbens in mediating reward. J Neurosci 1997;17:8580–7.
26. Ikemoto S. Involvement of the olfactory tubercle in cocaine reward: intracranial self-administration studies. J Neurosci 2003;23:9305–11.
27. Goeders NE, Smith JE. Reinforcing properties of cocaine in the medical prefrontal cortex: primary action on presynaptic dopaminergic terminals. Pharmacol Biochem Behav 1986;25:191–9.
28. Goeders NE, Dworkin SI, Smith JE. Neuropharmacological assessment of cocaine self-administration into the medial prefrontal cortex. Pharmacol Biochem Behav 1986;24: 1429–40.
29. Goeders NE, Smith JE. Cortical dopaminergic involvement in cocaine reinforcement. Science 1983;221:773–5.
30. David V, Segu L, Buhot MC, Ichaye M, Cazala P. Rewarding effects elicited by cocaine microinjections into the ventral tegmental area of C57BL/6 mice: involvement of dopamine D1 and serotonin1B receptors. Psychopharmacology 2004;174:367–75.
31. Rodd ZA, Bell RL, Kuc KA, Zhang Y, Murphy JM, McBride WJ. Intracranial self-administration of cocaine within the posterior ventral tegmental area of Wistar rats: evidence for involvement of serotonin-3 receptors and dopamine neurons. J Pharmacol Exp Ther 2005;313:134–45.
32. Corsini GU, Del Zompo M, Gessa GL, Mangoni A. Therapeutic efficacy of apomorphine combined with an extracerebral inhibitor of dopamine receptors in Parkinson's disease. Lancet 1979;1(8123):954–6.
33. Kumor K, Sherer M, Jaffe J. Effects of bromocriptine pretreatment on subjective and physiological responses to i.v. cocaine. Pharmacol Biochem Behav 1989;33: 829–37.
34. Brauer LH, Goudie AJ, de Wit H. Dopamine ligands and the stimulus effects of amphetamine: animal models versus human laboratory data. Psychopharmacology 1997; 130:2–13.
35. Wachtel SR, Ortengren A, de Wit H. The effects of acute haloperidol or risperidone on subjective responses to methamphetamine in healthy volunteers. Drug Alcohol Depend 2002;68:23–33.

36. Romach MK, Glue P, Kampman K, et al. Attenuation of the euphoric effects of cocaine by the dopamine D1/D5 antagonist ecopipam (SCH 39166). Arch Gen Psychiat 1999;56: 1101–6.
37. Haney M, Ward AS, Foltin RW, Fischman MW. Effects of ecopipam, a selective dopamine D1 antagonist, on smoked cocaine self-administration by humans. Psychopharmacology 2001;155:330–7.
38. Tzschentke TM. Measuring reward with the conditioned place preference paradigm: a comprehensive review of drug effects, recent progress and new issues. Prog Neurobiol 1998;56:613–72.
39. Hoffman DC, Beninger RJ. Selective D1 and D2 dopamine agonists produce opposing effects in place conditioning but not in conditioned taste aversion learning. Pharmacol Biochem Behav 1988;31:1–8.
40. White NM, Packard MG, Hiroi N. Place conditioning with dopamine D1 and D2 agonists injected peripherally or into nucleus accumbens. Psychopharmacology 1991;103:271–6.
41. Hoffman DC, Beninger RJ. The effects of selective dopamine D1 or D2 receptor antagonists on the establishment of agonist-induced place conditioning in rats. Pharmacol Biochem Behav 1989;33:273–9.
42. Abrahams BS, Rutherford JD, Mallet PE, Beninger RJ. Place conditioning with the dopamine D1-like receptor agonist SKF 82958 but not SKF 81297 or SKF 77434. Eur J Pharmacol 1998;343:111–8.
43. Graham DL, Hoppenot R, Hendryx A, Self DW. Differential ability of D1 and D2 dopamine receptor agonists to induce and modulate expression and reinstatement of cocaine place preference in rats. Psychopharmacology 2007;191:719–30.
44. Blanchet PJ, Grondin R, Bedard PJ, Shiosaki K, Britton DJ. Dopamine D_1 receptor desensitization profile in MPTP-lesioned primates. Eur J Pharmacol 1996;309:13–20.
45. Shippenberg TS, Herz A. Place preference reveals the involvement of D-1 dopamine receptors in the motivational properties of μ- and κ-opioid agonists. Brain Res 1987;436:169–72.
46. Papp M, Muscat R, Willner P. Subsensitivity to rewarding and locomotor stimulant effects of a dopamine agonist following chronic mild stress. Psychopharmacology 1993;110:152–8.
47. Mallet PE, Beninger RJ. 7-OH-DPAT produces place conditioning in rats. Eur J Pharmacol 1994;261:R5–6.
48. Kling-Petersen T, Ljung E, Wollter L, Svensson K. Effects of dopamine D3 preferring compounds on conditioned place preference and intracranial self-stimulation in the rat. J Neural Trans 1995;101:27–39.
49. Biondo AM, Clements RL, Hayes DJ, Eshpeter B, Greenshaw AJ. NMDA or AMPA/kainate receptor blockade prevents acquisition of conditioned place preference induced by D(2/3) dopamine receptor stimulation in rats. Psychopharmacology 2005;179:189–97.
50. Rodriguez De Fonseca F, Rubio P, Martin-Calderon JL, Caine SB, Koob GF, Navarro M. The dopamine receptor agonist 7-OH-DPAT modulates the acquisition and expression of morphine-induced place preference. Eur J Pharmacol 1995;274:47–55.
51. Frances H, Smirnova M, Leriche L, Sokoloff P. Dopamine D3 receptor ligands modulate the acquisition of morphine-conditioned place preference. Psychopharmacology 2004;175:127–33.
52. Gyertyan I, Gal K. Dopamine D3 receptor ligands show place conditioning effect but do not influence cocaine-induced place preference. Neuroreport 2003;14:93–8.
53. Khroyan TV, Baker DA, Neisewander JL. Dose-dependent effects of the D3-preferring agonist 7-OH-DPAT on motor behaviors and place conditioning. Psychopharmacology 1995;122:351–7.
54. Hoffman DC, Dickson PR, Beninger RJ. The dopamine D2 receptor agonists, quinpirole and bromocriptine produce conditioned place preferences. Prog Neuropsychopharmacol Biol Psychiatry 1988;12:315–22.
55. Kuz'min AV, Zvartau EE. The reinforcing properties of psychostimulating preparations on models of conditioned-reflex place preference in mice. Zh Vyssh Nerv Deiat Im I P Pavlova 1995;45:802–10.

56. Khroyan TV, Fuchs RA, Baker DA, Neisewander JL. Effects of D3-preferring agonists 7-OH-PIPAT and PD-128,907 on motor behaviors and place conditioning. Behav Pharmacol 1997;8:65–74.
57. Woolley ML, Waters KA, Reavill C, et al. Selective dopamine D4 receptor agonist (A-412997) improves cognitive performance and stimulates motor activity without influencing reward-related behaviour in rat. Behav Pharmacol 2008;19:765–76.
58. Rezayof A, Zarrindast MR, Sahraei H, Haeri-Rohani A. Involvement of dopamine receptors of the dorsal hippocampus on the acquisition and expression of morphine-induced place preference in rats. J Psychopharmacol 2003;17:415–23.
59. Zarrindast MR, Rezayof A, Sahraei H, Haeri-Rohani A, Rassouli Y. Involvement of dopamine D1 receptors of the central amygdala on the acquisition and expression of morphine-induced place preference in rat. Brain Res 2003;965:212–21.
60. Barrett AC, Miller JR, Dohrmann JM, Caine SB. Effects of dopamine indirect agonists and selective D1-like and D2-like agonists and antagonists on cocaine self-administration and food maintained responding in rats. Neuropharmacology 2004;47(Suppl 1): 256–73.
61. Weatherford SC, Greenberg D, Gibbs J, Smith GP. The potency of D-1 and D-2 receptor antagonists is inversely related to the reward value of sham-fed corn oil and sucrose in rats. Pharmacol Biochem Behav 1990;37:317–23.
62. Hsiao S, Smith GP. Raclopride reduces sucrose preference in rats. Pharmacol Biochem Behav 1995;50:121–5.
63. Yu WZ, Silva RM, Sclafani A, Delamater AR, Bodnar RJ. Pharmacology of flavor preference conditioning in sham-feeding rats: effects of dopamine receptor antagonists. Pharmacol Biochem Behav 2000;65:635–47.
64. Terry P, Gilbert DB, Cooper SJ. Dopamine receptor subtype agonists and feeding behavior. Obes Res 1995;3(Suppl 4):515S–23S.
65. Phillips G, Willner P, Muscat R. Suppression or facilitation of operant behaviour by raclopride dependent on concentration of sucrose reward. Psychopharmacology 1991;105:239–46.
66. Price KL, Middaugh LD. The dopamine D1 antagonist reduces ethanol reward for C57BL/6 mice. Alcohol Clin Exp Res 2004;28:1666–75.
67. Agmo A, Federman I, Navarro V, Padua M, Velazquez G. Reward and reinforcement produced by drinking water: role of opioids and dopamine receptor subtypes. Pharmacol Biochem Behav 1993;46:183–94.
68. Melis MR, Argiolas A. Dopamine and sexual behavior. Neurosci Biobehav Rev 1995;19: 19–38.
69. Beck J, Bialy M, Kostowski W. Effects of D(1) receptor agonist SKF 38393 on male rat sexual behavior and postcopulatory departure in the goal compartment-runway paradigm. Physiol Behav 2002;76:91–7.
70. Everitt BJ. Sexual motivation: a neural and behavioural analysis of the mechanisms underlying appetitive and copulatory responses of male rats. Neurosci Biobehav Rev 1990;14:217–32.
71. Pfaus JG, Philipps AG. Role of dopamine in anticipatory and consummatory aspects of sexual behavior in the male rat: I. Effects of systemic administration of dopamine antagonists. Behav Neurosci 1991;105:727–43.
72. Grierson JP, James MD, Pearson JR, Wilson CA. The effect of selective D1 and D2 dopaminergic agents on sexual receptivity in the female rat. Neuropharmacology 1988;27: 181–9.
73. Agustin-Pavon C, Martinez-Ricos J, Martinez-Garcia F, Lanuza E. Effects of dopaminergic drugs on innate pheromone-mediated reward in female mice: a new case of dopamine-independent "liking." Behav Neurosci 2007;121:920–32.
74. El-Ghundi M, O'Dowd BF, Erclik M, George SR. Attenuation of sucrose reinforcement in dopamine D1 receptor deficient mice. Eur J Neurosci 2003;17:851–62.

75. Kudwa AE, Dominguez-Salazar E, Cabrera DM, Sibley DR, Rissman EF. Dopamine D5 receptor modulates male and female sexual behavior in mice. Psychopharmacology 2005;180:206–14.
76. Fowler SC, Zarcone TJ, Vorontsova E, Chen R. Motor and associative deficits in D2 dopamine receptor knockout mice. Int J Dev Neurosci 2002;20:309–21.
77. Elmer GI, Pieper JO, Rubinstein M, Low MJ, Grandy DK, Wise RA. Failure of intravenous morphine to serve as an effective instrumental reinforcer in dopamine D2 receptor knock-out mice. J Neurosci 2002;22:RC224.
78. Boyce-Rustay JM, Risinger FO. Dopamine D3 receptor knockout mice and the motivational effects of ethanol. Pharmacol Biochem Behav 2003;75:373–9.
79. Hajnal A, Norgren R. Accumbens dopamine mechanisms in sucrose intake. Brain Res 2001;904:76–84.
80. Bari AA, Pierce RC. D1-like and D2 dopamine receptor antagonists administered into the shell subregion of the rat nucleus accumbens decrease cocaine, but not food, reinforcement. Neuroscience 2005;135:959–68.
81. Baldo BA, Sadeghian K, Basso AM, Kelley AE. Effects of selective dopamine D1 or D2 receptor blockade within nucleus accumbens subregions on ingestive behavior and associated motor activity. Behav Brain Res 2002;137:165–77.
82. Smith-Roe SL, Kelley AE. Coincident activation of NMDA and dopamine D1 receptors within the nucleus accumbens core is required for appetitive instrumental learning. J Neurosci 2000;20:7737–42.
83. Baldwin AE, Sadeghian K, Holahan MR, Kelley AE. Appetitive instrumental learning is impaired by inhibition of cAMP-dependent protein kinase within the nucleus accumbens. Neurobiol Learn Mem 2002;77:44–62.
84. Baldwin AE, Sadeghian K, Kelley AE. Appetitive instrumental learning requires coincident activation of NMDA and dopamine D1 receptors within the medial prefrontal cortex. J Neurosci 2002;22:1063–71.
85. Andrzejewski ME, Spencer RC, Kelley AE. Instrumental learning, but not performance, requires dopamine D1-receptor activation in the amygdala. Neuroscience 2005;135:335–45.
86. Wise RA. Forebrain substrates of reward and motivation. J Comp Neurol 2005;493:115–21.
87. You ZB, Tzschentke TM, Brodin E, Wise RA. Electrical stimulation of the prefrontal cortex increases cholecystokinin, glutamate, and dopamine release in the nucleus accumbens: an in vivo microdialysis study in freely moving rats. J Neurosci 1998;18:6492–500.
88. Gallistel CR, Davis AJ. Affinity for the dopamine D_2 receptor predicts neuroleptic potency in blocking the reinforcing effect of MFB stimulation. Pharmacol Biochem Behav 1983;19:867–72.
89. Nakajima S, O'Regan NB. The effects of dopaminergic agonists and antagonists on the frequency-response function for hypothalamic self-stimulation in the rat. Pharmacol Biochem Behav 1991;39:465–8.
90. Carr KD, Yamamoto N, Omura M, Cabeza de Vaca S, Krahne L. Effects of the D(3) dopamine receptor antagonist, U99194A, on brain stimulation and d-amphetamine reward, motor activity, and c-fos expression in ad libitum fed and food-restricted rats. Psychopharmacology 2002;163:76–84.
91. Tran AH, Tamura R, Uwano T, Kobayashi T, Katsuki M, Ono T. Dopamine D1 receptors involved in locomotor activity and accumbens neural responses to prediction of reward associated with place. Proc Natl Acad Sci USA 2005;102:2117–22.
92. Elmer GI, Pieper JO, Levy J, et al. Brain stimulation and morphine reward deficits in dopamine D2 receptor-deficient mice. Psychopharmacology 2005;182:33–44.
93. Hunt GE, Atrens DM, Jackson DM. Reward summation and the effects of dopamine D1 and D2 agonists and antagonists on fixed-interval responding for brain stimulation. Pharmacol Biochem Behav 1994;48:853–62.
94. Singh J, Desiraju T, Raju TR. Dose-response functions of apomorphine, SKF 38393, LY 171555, haloperidol and clonidine on the self-stimulation evoked from lateral hypothalamus and ventral tegmentum. Indian J Physiol Pharmacol 1996;40:15–22.

95. Baldo BA, Jain K, Veraldi L, Koob GF, Markou A. A dopamine D1 agonist elevates self-stimulation thresholds: comparison to other dopamine-selective drugs. Pharmacol Biochem Behav 1999;62:659–72.
96. Gilliss B, Malanga CJ, Pieper JO, Carlezon WA, Jr. Cocaine and SKF-82958 potentiate brain stimulation reward in Swiss-Webster mice. Psychopharmacology 2002;163:238–48.
97. Ranaldi R, Beninger RJ. The effects of systemic and intracerebral injections of D1 and D2 agonists on brain stimulation reward. Brain Res 1994;651:283–92.
98. Depoortere R, Perrault G, Sanger DJ. Behavioural effects in the rat of the putative dopamine D3 receptor agonist 7-OH-DPAT: comparison with quinpirole and apomorphine. Psychopharmacology 1996;124:231–40.
99. Nakajima S, Liu X, Lau CL. Synergistic interaction of D1 and D2 dopamine receptors in the modulation of the reinforcing effect of brain stimulation. Behav Neurosci 1993;107:161–5.
100. Panagis G, Spyraki C. Neuropharmacological evidence for the role of dopamine in ventral pallidum self-stimulation. Psychopharmacology 1996;123:280–8.
101. Gilbert DB, Millar J, Cooper SJ. The putative dopamine D3 agonist, 7-OH-DPAT, reduces dopamine release in the nucleus accumbens and electrical self-stimulation to the ventral tegmentum. Brain Res 1995;681:1–7.
102. Depoortere R, Perrault G, Sanger DJ. Intracranial self-stimulation under a progressive-ratio schedule in rats: effects of strength of stimulation, d-amphetamine, 7-OH-DPAT and haloperidol. Psychopharmacology 1999;142:221–9.
103. Knapp CM, Kornetsky C. Bromocriptine, a D_2 receptor agonist, lowers the threshold for rewarding brain stimulation. Pharmacol Biochem Behav 1994;49:901–4.
104. Kurumiya S, Nakajima S. Dopamine D1 receptors in the nucleus accumbens: involvement in the reinforcing effect of tegmental stimulation. Brain Res 1988;448:1–6.
105. Nakajima S, Patterson RL. The involvement of dopamine D2 receptors, but not D3 or D4 receptors, in the rewarding effect of brain stimulation in the rat. Brain Res 1997;760:74–9.
106. Singh J, Desiraju T, Raju TR. Dopamine receptor sub-types involvement in nucleus accumbens and ventral tegmentum but not in medial prefrontal cortex: on self-stimulation of lateral hypothalamus and ventral mesencephalon. Behav Brain Res 1997;86:171–9.
107. Choi KH, Clements RL, Greenshaw AJ. Simultaneous AMPA/kainate receptor blockade and dopamine D(2/3) receptor stimulation in the nucleus accumbens decreases brain stimulation reward in rats. Behav Brain Res 2005;158:79–88.
108. Cameron DL, Williams JT. Dopamine D1 receptors facilitate transmitter release. Nature 1993;366:344–7.
109. Ferrer JM, Sanguinetti AM, Vives F, Mora F. Effects of agonists and antagonists of D1 and D2 dopamine receptors on self-stimulation of the medial prefrontal cortex in the rat. Pharmacol Biochem Behav 1983;19:211–7.
110. Van Tol HH, Bunzow JR, Guan HC, et al. Cloning of the gene for a human dopamine D4 receptor with high affinity for the antipsychotic clozapine. Nature 1991;350(6319):610–4.
111. Seeman P, Van Tol HH. Dopamine receptor pharmacology. Trends Pharmacol Sci 1994;15:264–70.
112. Tiberi M, Jarvie KR, Silvia C, et al. Cloning, molecular characterization, and chromosomal assignment of a gene encoding a second D1 dopamine receptor subtype: differential expression pattern in rat brain compared with the D1A receptor. Proc Natl Acad Sci USA 1991;88:7491–5.
113. Robbins TW. The potentiation of conditioned reinforcement by psychomotor stimulant drugs: A test of Hill's hypthesis. Psychopharmacolgy 1975;45:103–14.
114. Taylor JR, Robbins TW. Enhanced behavioral control by conditioned reinforcers following microinjections of d-amphetamine into the nucleus accumbens. Psychopharmacology 1984;84:405–12.
115. Taylor JR, Robbins TW. 6-hydroxydopamine lesions of the nucleus accumbens, but not of the caudate nucleus, attenuate enhanced responding with reward-related stimuli produced by intra-accumbens d-amphetamine. Psychopharmacology 1986;90:390–7.

116. Ranaldi R, Beninger RJ. Dopamine D1 and D2 antagonists attenuate amphetamine-produced enhancement of responding for conditioned reward in rats. Psychopharmacology 1993;113:110–8.
117. Smith JK, Neill JC, Costall B. Bidirectional effects of dopamine D2 receptor antagonists on responding for a conditioned reinforcer. Pharmacol Biochem Behav 1997;57:843–9.
118. Beninger RJ, Rolfe NG. Dopamine D1-like receptor agonists impair responding for conditioned reward in rats. Behav Pharmacol 1995;6:785–93.
119. Beninger RJ, Ranaldi R. The effect of amphetamine, apomorphine, SKF 38393, quinpirole and bromocriptine on responding for conditioned reward in rats. Behav Pharmacol 1992;3:155–63.
120. Ranaldi R, Beninger RJ. Bromocriptine enhancement of responding for conditioned reward depends on intact D1 receptor function. Psychopharmacology 1995;118:437–43.
121. Wolterink G, Phillips G, Cador M, Donselaar-Wolterink I, Robbins TW, Everitt BJ. Relative roles of ventral striatal D_1 and D_2 dopamine receptors in responding with conditioned reinforcement. Psychopharmacology 1993;110:355–64.
122. Phillips GD, Robbins TW, Everitt BJ. Mesoaccumbens dopamine-opiate interactions in the control over behaviour by a conditioned reinforcer. Psychopharmacology 1994;114:345–59.
123. Hitchcott PK, Phillips GD. Effects of intra-amygdala R(+) 7-OH-DPAT on intra-accumbens d-amphetamine-associated learning. II. Instrumental conditioning. Psychopharmacology 1998;140:310–8.
124. Berglind WJ, Case JM, Parker MP, Fuchs RA, See RE. Dopamine D1 or D2 receptor antagonism within the basolateral amygdala differentially alters the acquisition of cocaine-cue associations necessary for cue-induced reinstatement of cocaine-seeking. Neuroscience 2006;137:699–706.
125. Roberts DCS, Vickers G. Atypical neuroleptics increase self-administration of cocaine: an evaluation of a behavioral screen for antipsychotic activity. Psychopharmacology 1984;82:135–9.
126. Ahmed SH, Koob GF. Transition from moderate to excessive drug intake: change in hedonic set point. Science 1998;282:298–300.
127. Koob GF, Le HT, Creese I. The D_1 dopamine receptor antagonist SCH 23390 increases cocaine self-administration in the rat. Neurosci Lett 1987;79:315–20.
128. Britton DR, Mackenzie RG, Kebabian JW, Williams JEG, Kerkmen D. Evidence for involvement of both D1 and D2 receptors in maintaining cocaine self-administration. Pharmacol Biochem Behav 1991;39:911–5.
129. Corrigall WA, Coen KM. Cocaine self-administration is increased by both D1 and D2 dopamine receptor antagonists. Pharmacol Biochem Behav 1991;39:799–802.
130. Hubner CB, Moreton JB. Effects of selective D_1 and D_2 dopamine antagonists on cocaine self-administration in the rat. Psychopharmacology 1991;105:151–6.
131. Glowa JR, Wojnicki FH. Effects of drugs on food- and cocaine-maintained responding, III: Dopaminergic antagonists. Psychopharmacology 1996;128:351–8.
132. Depoortere RY, Li DH, Lane JD, Emmett-Oglesby MW. Parameters of self-administration of cocaine in rats under a progressive-ratio schedule. Pharmacol Biochem Behav 1993;45:539–48.
133. Fletcher PJ. A comparison of the effects of risperidone, raclopride, and ritanserin on intravenous self-administration of d-amphetamine. Pharmacol Biochem Behav 1998;60:55–60.
134. Izzo E, Orsini C, Koob GF, Pulvirenti L. A dopamine partial agonist and antagonist block amphetamine self-administration in a progressive ratio schedule. Pharmacol Biochem Behav 2001;68:701–8.
135. Caine SB, Negus SS, Mello NK, et al. Role of dopamine D2-like receptors in cocaine self-administration: studies with D2 receptor mutant mice and novel D2 receptor antagonists. J Neurosci 2002;22:2977–88.
136. Gal K, Gyertyan I. Targeting the dopamine D3 receptor cannot influence continuous reinforcement cocaine self-administration in rats. Brain Res Bull 2003;61:595–601.

137. Xi ZX, Gilbert JG, Pak AC, Ashby CR, Jr., Heidbreder CA, Gardner EL. Selective dopamine D3 receptor antagonism by SB-277011A attenuates cocaine reinforcement as assessed by progressive-ratio and variable-cost-variable-payoff fixed-ratio cocaine self-administration in rats. Eur J Neurosci 2005;21:3427–38.
138. Xi ZX, Newman AH, Gilbert JG, et al. The novel dopamine D3 receptor antagonist NGB 2904 inhibits cocaine's rewarding effects and cocaine-induced reinstatement of drug-seeking behavior in rats. Neuropsychopharmacology 2006;31:1393–405.
139. Self DW, Barnhart WJ, Lehman DA, Nestler EJ. Opposite modulation of cocaine-seeking behavior by D_1- and D_2-like dopamine receptor agonists. Science 1996;271:1586–9.
140. Caine SB, Negus SS, Mello NK, Bergman J. Effects of dopamine D(1-like) and D(2-like) agonists in rats that self-administer cocaine. J Pharmacol Exp Ther 1999;291:353–60.
141. Caine SB, Negus SS, Mello NK. Effects of dopamine D(1-like) and D(2-like) agonists on cocaine self-administration in rhesus monkeys: rapid assessment of cocaine dose-effect functions. Psychopharmacology 2000;148:41–51.
142. Self DW, Karanian DA, Spencer JJ. Effects of the novel D1 agonist ABT-431on cocaine self-administration and reinstatement. Annl NY Acad Sci 2000;909:133–44.
143. Hubner CB, Koob GF. Bromocriptine produces decreases in cocaine self-administration in the rat. Neuropsychopharmacology 1990;3:101–8.
144. Pulvirenti L, Koob GF. Dopamine receptor agonists, partial agonists and psychostimulant addiction. Trends Pharmacol Sci 1994;15:374–9.
145. Caine SB, Koob GF. Pretreatment with the dopamine agonist 7-OH-DPAT shifts the cocaine self-administration dose-effect function to the left under different schedules in the rat. Behav Pharmacol 1995;6:333–47.
146. Rowlett JK, Platt DM, Yao WD, Spealman RD. Modulation of heroin and cocaine self-administration by dopamine D1- and D2-like receptor agonists in rhesus monkeys. J Pharmacol Exp Ther 2007;321:1135–43.
147. Parsons LH, Caine SB, Sokoloff P, Schwartz J-C, Koob GF, Weiss F. Neurochemical evidence that post-synaptic nucleus accumbens D_3 receptor stimulation enhances cocaine reinforcement. J Neurochem 1996;67:1078–89.
148. Caine SB, Koob GF, Parsons LH, Everitt BJ, Schwartz JC, Sokoloff P. D3 receptor test in vitro predicts decreased cocaine self-administration in rats. Neuroreport 1997;8:2373–7.
149. Le Foll B, Schwartz JC, Sokoloff P. Dopamine D3 receptor agents as potential new medications for drug addiction. Eur Psychiatry 2000;15:140–6.
150. Xu M, Koeltzow TE, Santiago GT, et al. Dopamine D3 receptor mutant mice exhibit increased behavioral sensitivity to concurrent stimulation of D1 and D2 receptors. Neuron 1997;19:837–48.
151. Karasinska JM, George SR, Cheng R, O'Dowd BF. Deletion of dopamine D1 and D3 receptors differentially affects spontaneous behaviour and cocaine-induced locomotor activity, reward and CREB phosphorylation. Eur J Neurosci 2005;22(7):1741–50.
152. Jiao H, Zhang L, Gao F, Lou D, Zhang J, Xu M. Dopamine D(1) and D(3) receptors oppositely regulate NMDA- and cocaine-induced MAPK signaling via NMDA receptor phosphorylation. J Neurochem 2007;103:840–8.
153. Pilla M, Perachon S, Sautel F, et al. Selective inhibition of cocaine-seeking behaviour by a partial dopamine D3 receptor agonist. Nature 1999;400:371–5.
154. Maldonado R, Robledo P, Chover AJ, Caine SB, Koob JF. D_1 dopamine receptors in the nucleus accumbens modulate cocaine self-administration in the rat. Pharmacol Biochem Behav 1993;45:239–42.
155. Phillips GD, Robbins TW, Everitt BJ. Bilateral intra-accumbens self-administration of d-amphetamine: antagonism with intra-accumbens SCH-23390 and sulpiride. Psychopharmacology 1994;114:477–85.
156. Caine SB, Heinrichs SC, Coffin VL, Koob GF. Effects of the dopamine D-1 antagonist SCH 23390 microinjected into the accumbens, amygdala or striatum on cocaine self-administration in the rat. Brain Res 1995;692:47–56.

157. Bachtell RK, Whisler K, Karanian DA, Self DW. Effects of intranucleus accumbens shell administration of dopamine agonists and antagonists on cocaine-taking and cocaine-seeking behaviors in the rat. Psychopharmacology 2005;183:41–53.
158. Hurd YL, Ponten M. Cocaine self-administration behavior can be reduced or potentiated by the addition of specific dopamine concentrations in the nucleus accumbens and amygdala using in vivo microdialysis. Behav Brain Res 2000;116:177–86.
159. Thanos PK, Michaelides M, Umegaki H, Volkow ND. D2R DNA transfer into the nucleus accumbens attenuates cocaine self-administration in rats. Synapse 2008;62:481–6.
160. McGregor A, Roberts DCS. Dopaminergic antagonism within the nucleus accumbens or the amygdala produces differential effects on intravenous cocaine self-administration under fixed and progressive ratio schedules of reinforcement. Brain Res 1993;624: 245–52.
161. McGregor A, Roberts DC. Effect of medial prefrontal cortex injections of SCH 23390 on intravenous cocaine self-administration under both a fixed and progressive ratio schedule of reinforcement. Behav Brain Res 1995; 67:75–80.
162. Alleweireldt AT, Hobbs RJ, Taylor AR, Neisewander JL. Effects of SCH-23390 infused into the amygdala or adjacent cortex and basal ganglia on cocaine seeking and self-administration in rats. Neuropsychopharmacology 2006;31:363–74.
163. Epping-Jordan MP, Markou A, Koob GF. The dopamine D-1 receptor antagonist SCH 23390 injected into the dorsolateral bed nucleus of the stria terminalis decreased cocaine reinforcement in the rat. Brain Res 1998;784:105–15.
164. Hurd YL, McGregor A, Ponten M. In vivo amygdala dopamine levels modulate cocaine self-administration behaviour in the rat: D1 dopamine receptor involvement. Eur J Neurosci 1997;9:2541–8.
165. Ranaldi R, Wise RA. Blockade of D1 dopamine receptors in the ventral tegmental area decreases cocaine reward: possible role for dendritically released dopamine. J Neurosci 2001;21:5841–6.
166. Doron R, Fridman L, Yadid G. Dopamine-2 receptors in the arcuate nucleus modulate cocaine-seeking behavior. Neuroreport 2006;17:1633–6.
167. Ettenberg A, Pettit HO, Bloom FE, Koob GF. Heroin and cocaine intravenous self-administration in rats: mediation by separate neural systems. Psychopharmacology 1982;78:204–9.
168. Pettit HO, Ettenberg A, Bloom FE, Koob GF. Destruction of dopamine in the nucleus accumbens selectively attenuates cocaine but not heroin self-administration in rats. Psychopharmacology 1984;84:167–73.
169. Gerrits MA, Ramsey NF, Wolterink G, van Ree JM. Lack of evidence for an involvement of nucleus accumbens dopamine D1 receptors in the initiation of heroin self-administration in the rat. Psychopharmacology 1994;114:486–94.
170. Shippenberg TS, Bals-Kubik R, Herz A. Examination of the neurochemical substrates mediating the motivational effects of opioids: role of the mesolimbic dopamine system and D-1 vs. D-2 dopamine receptors. J Pharmacol Exp Ther 1993;265:53–9.
171. Stinus L, Nadaud D, Deminiere JM, Jauregui J, Hand TT, Le Moal M. Chronic flupentixol treatment potentiates the reinforcing properties of systemic heroin administration. Biol Psychiatry 1989;26:363–71.
172. David V, Durkin TP, Cazala P. Differential effects of the dopamine D2/D3 receptor antagonist sulpiride on self-administration of morphine into the ventral tegmental area or the nucleus accumbens. Psychopharmacology 2002;160:307–17.
173. Le Merrer J, Gavello-Baudy S, Galey D, Cazala P. Morphine self-administration into the lateral septum depends on dopaminergic mechanisms: Evidence from pharmacology and Fos neuroimaging. Behav Brain Res 2007;180:203–17.
174. Villegier AS, Lotfipour S, McQuown SC, Belluzzi JD, Leslie FM. Tranylcypromine enhancement of nicotine self-administration. Neuropharmacology 2007;52:1415–25.

175. David V, Besson M, Changeux JP, Granon S, Cazala P. Reinforcing effects of nicotine microinjections into the ventral tegmental area of mice: dependence on cholinergic nicotinic and dopaminergic D1 receptors. Neuropharmacology 2006;50:1030–40.
176. Ikemoto S, Qin M, Liu ZH. Primary reinforcing effects of nicotine are triggered from multiple regions both inside and outside the ventral tegmental area. J Neurosci 2006;26:723–30.
177. Ross JT, Corrigall WA, Heidbreder CA, LeSage MG. Effects of the selective dopamine D3 receptor antagonist SB-277011A on the reinforcing effects of nicotine as measured by a progressive-ratio schedule in rats. Eur J Pharmacol 2007;559:173–9.
178. Andreoli M, Tessari M, Pilla M, Valerio E, Hagan JJ, Heidbreder CA. Selective antagonism at dopamine D3 receptors prevents nicotine-triggered relapse to nicotine-seeking behavior. Neuropsychopharmacology 2003;28:1272–80.
179. Kameda G, Dadmarz M, Vogel WH. Influence of various drugs on the voluntary intake of nicotine by rats. Neuropsychobiology 2000;41:205–9.
180. Silvestre JS, O'Neill MF, Fernandez AG, Palacios JM. Effects of a range of dopamine receptor agonists and antagonists on ethanol intake in the rat. Eur J Pharmacol 1996;318:257–65.
181. Linseman MA. Effects of dopaminergic agents on alcohol consumption by rats in a limited access paradigm. Psychopharmacology 1990;100:195–200.
182. Dyr W, McBride WJ, Lumeng L, Li TK, Murphy JM. Effects of D1 and D2 dopamine receptor agents on ethanol consumption in the high-alcohol-drinking (HAD) line of rats. Alcohol 1993;10:207–12.
183. Panocka I, Ciccocioppo R, Mosca M, Polidori C, Massi M. Effects of the dopamine D1 receptor antagonist SCH 39166 on the ingestive behaviour of alcohol-preferring rats. Psychopharmacology 1995;120:227–35.
184. Czachowski CL, Santini LA, Legg BH, Samson HH. Separate measures of ethanol seeking and drinking in the rat: effects of remoxipride. Alcohol 2002;28:39–46.
185. Ng GY, George SR. Dopamine receptor agonist reduces ethanol self-administration in the ethanol-preferring C57BL/6 J inbred mouse. Eur J Pharmacol 1994;269:365–74.
186. Boyce JM, Risinger FO. Dopamine D3 receptor antagonist effects on the motivational effects of ethanol. Alcohol 2002;28:47–55.
187. Thanos PK, Katana JM, Ashby CR, Jr., et al. The selective dopamine D3 receptor antagonist SB-277011-A attenuates ethanol consumption in ethanol preferring (P) and non-preferring (NP) rats. Pharmacol Biochem Behav 2005;81:190–7.
188. Heidbreder CA, Andreoli M, Marcon C, Hutcheson DM, Gardner EL, Ashby CR, Jr. Evidence for the role of dopamine D3 receptors in oral operant alcohol self-administration and reinstatement of alcohol-seeking behavior in mice. Addict Biol 2007;12:35–50.
189. El-Ghundi M, George SR, Drago J, et al. Disruption of dopamine D1 receptor gene expression attenuates alcohol-seeking behavior. Eur J Pharmacol 1998;353:149–58.
190. Phillips TJ, Brown KJ, Burkhart-Kasch S, et al. Alcohol preference and sensitivity are markedly reduced in mice lacking dopamine D2 receptors. Nat Neurosci 1998;1:610–5.
191. Rodd ZA, Melendez RI, Bell RL, et al. Intracranial self-administration of ethanol within the ventral tegmental area of male Wistar rats: evidence for involvement of dopamine neurons. J Neurosci 2004;24:1050–7.
192. Hodge CW, Samson HH, Chappelle AM. Alcohol self-administration: further examination of the role of dopamine receptors in the nucleus accumbens. Alcohol Clin Exp Res 1997;21:1083–91.
193. Samson HH, Chappell A. Dopaminergic involvement in medial prefrontal cortex and core of the nucleus accumbens in the regulation of ethanol self-administration: a dual-site microinjection study in the rat. Physiol Behav 2003;79:581–90.
194. Eiler WJ, 2nd, Seyoum R, Foster KL, Mailey C, June HL. D1 dopamine receptor regulates alcohol-motivated behaviors in the bed nucleus of the stria terminalis in alcohol-preferring (P) rats. Synapse 2003;48:45–56.
195. Levy AD, Murphy JM, McBride WJ, Lumeng L, Li TK. Microinjection of sulpiride into the nucleus accumbens increases ethanol drinking in alcohol-preferring (P) rats. Alcohol Alcohol Suppl 1991;1:417–20.

196. Melendez RI, Rodd ZA, McBride WJ, Murphy JM. Dopamine receptor regulation of ethanol intake and extracellular dopamine levels in the ventral pallidum of alcohol preferring (P) rats. Drug Alcohol Depend 2005;77:293–301.
197. Hodge CW, Chappelle AM, Samson HH. Dopamine receptors in the medial prefrontal cortex influence ethanol and sucrose-reinforced responding. Alcohol Clin Exp Res 1996;20: 1631–8.
198. Thanos PK, Taintor NB, Rivera SN, et al. DRD2 gene transfer into the nucleus accumbens core of the alcohol preferring and nonpreferring rats attenuates alcohol drinking. Alcohol Clin Exp Res 2004;28:720–8.
199. Self DW, Nestler EJ. Relapse to drug seeking: neural and molecular mechanisms. Drug Alcohol Depend 1998;51:49–60.
200. Spealman RD, Barrett-Larimore RL, Rowlett JK, Platt DM, Khroyan TV. Pharmacological and environmental determinants of relapse to cocaine-seeking behavior. Pharmacol Biochem Behav 1999;64:327–36.
201. Stewart J. Pathways to relapse: the neurobiology of drug- and stress-induced relapse to drug-taking. J Psychiat Neurosci 2000;25:125–36.
202. Shalev U, Grimm JW, Shaham Y. Neurobiology of relapse to heroin and cocaine seeking: a review. Pharmacol Rev 2002;54:1–42.
203. Phillips PE, Stuber GD, Heien ML, Wightman RM, Carelli RM. Subsecond dopamine release promotes cocaine seeking. Nature 2003;422:614–8.
204. Pruessner JC, Champagne F, Meaney MJ, Dagher A. Dopamine release in response to a psychological stress in humans and its relationship to early life maternal care: a positron emission tomography study using [11C] raclopride. J Neurosci 2004;24:2825–31.
205. Wise RA. The brain and reward. In: Liebman JM, Cooper SJ, eds. The Neuropharmacological Basis of Reward. New York: Oxford University Press; 1989:377–424.
206. Jaffe JH, Cascella NG, Kumor KM, Sherer MA. Cocaine-induced cocaine craving. Psychopharmacology 1989;97:59–64.
207. Robbins SJ, Ehrman RN, Childress AR, O'Brien CP. Relationships among physiological and self-report responses produced by cocaine-related cues. Addict Behav 1997;22:157–67.
208. Sinha R, Catapano D, O'Malley S. Stress induced craving and stress response in cocaine dependent individuals. Psychopharmacology 1999;142:343–51.
209. Fuchs RA, See RE, Middaugh LD. Conditioned stimulus-induced reinstatement of extinguished cocaine seeking in C57BL/6 mice: a mouse model of drug relapse. Brain Res 2003;973:99–106.
210. Yan Y, Nitta A, Mizoguchi H, Yamada K, Nabeshima T. Relapse of methamphetamine-seeking behavior in C57BL/6 J mice demonstrated by a reinstatement procedure involving intravenous self-administration. Behav Brain Res 2006;168:137–43.
211. De Vries TJ, Schoffelmeer ANM, Binnekade R, Vanderschuren LJMJ. Dopaminergic mechanisms mediating the incentive to seek cocaine and heroin following long-term withdrawal if IV drug self-administration. Psychopharmacology 1999;143:254–60.
212. Fuchs RA, Tran-Nguyen LT, Weber SM, Khroyan TV, Neisewander JL. Effects of 7-OH-DPAT on cocaine-seeking behavior and on re-establishment of cocaine self-administration. Pharmacol Biochem Behav 2002;72:623–32.
213. Cervo L, Carnovali F, Stark JA, Mennini T. Cocaine-seeking behavior in response to drug-associated stimuli in rats: involvement of D3 and D2 dopamine receptors. Neuropsychopharmacology 2003;28:1150–9.
214. Dias C, Lachize S, Boilet V, Huitelec E, Cador M. Differential effects of dopaminergic agents on locomotor sensitisation and on the reinstatement of cocaine-seeking and food-seeking behaviour. Psychopharmacology 2004;175:414–27.
215. Koeltzow TE, Vezina P. Locomotor activity and cocaine-seeking behavior during acquisition and reinstatement of operant self-administration behavior in rats. Behav Brain Res 2005;160:250–9.

216. Edwards S, Whisler KN, Fuller DC, Orsulak PJ, Self DW. Addiction-related alterations in D_1 and D_2 dopamine receptor behavioral responses following chronic cocaine self-administration. Neuropsychopharmacology 2007;32:354–66.
217. Alleweireldt AT, Weber SM, Kirschner KF, Bullock BL, Neisewander JL. Blockade or stimulation of D1 dopamine receptors attenuates cue reinstatement of extinguished cocaine-seeking behavior in rats. Psychopharmacology 2002;159:284–93.
218. Alleweireldt AT, Kirschner KF, Blake CB, Neisewander JL. D1-receptor drugs and cocaine-seeking behavior: investigation of receptor mediation and behavioral disruption in rats. Psychopharmacology 2003;168:109–17.
219. Khroyan TV, Barrett-Larimore RL, Rowlett JK, Spealman RD. Dopamine D1- and D2-like receptor mechanisms in relapse to cocaine-seeking behavior: effects of selective antagonists and agonists. J Pharmacol Exp Ther 2000;294:680–7.
220. Khroyan TV, Platt DM, Rowlett JK, Spealman RD. Attenuation of relapse to cocaine seeking by dopamine D1 receptor agonists and antagonists in non-human primates. Psychopharmacology 2003;168:124–31.
221. Haney M, Foltin RW, Fischman MW. Effects of pergolide on intravenous cocaine self-administration in men and women. Psychopharmacology 1998;137:15–24.
222. Haney M, Collins ED, Ward AS, Foltin RW, Fischman MW. Effect of a selective D_1 agonist (ABT-431) on smoked cocaine self-administration in humans. Psychopharmacology 1999;143:102–10.
223. Phillips PE, Stuber GD, Heien ML, Wightman RM, Carelli RM. Subsecond dopamine release promotes cocaine seeking. Nature 2003;422:614–8.
224. Schenk S, Gittings D. Effects of SCH 23390 and eticlopride on cocaine-seeking produced by cocaine and WIN 35,428 in rats. Psychopharmacology 2003;168:118–23.
225. Weissenborn R, Deroche V, Koob G, Weiss F. Effects of dopamine agonists and antagonists on cocaine-induced operant responding for a cocaine-associated stimulus. Psychopharmacology 1996;126:311–22.
226. Ciccocioppo R, Sanna PP, Weiss F. Cocaine-predictive stimulus induces drug-seeking behavior and neural activation in limbic brain regions after multiple months of abstinence: reversal by D(1) antagonists. Proc Natl Acad Sci USA 2001;98:1976–81.
227. Crombag HS, Grimm JW, Shaham Y. Effect of dopamine receptor antagonists on renewal of cocaine seeking by reexposure to drug-associated contextual cues. Neuropsychopharmacology 2002;27:1006–15.
228. Gal K, Gyertyan I. Dopamine D3 as well as D2 receptor ligands attenuate the cue-induced cocaine-seeking in a relapse model in rats. Drug Alcohol Depend 2006;81:63–70.
229. Di Ciano P, Underwood RJ, Hagan JJ, Everitt BJ. Attenuation of cue-controlled cocaine-seeking by a selective D3 dopamine receptor antagonist SB-277011-A. Neuropsychopharmacology 2003;28:329–38.
230. Gilbert JG, Newman AH, Gardner EL, et al. Acute administration of SB-277011A, NGB 2904, or BP 897 inhibits cocaine cue-induced reinstatement of drug-seeking behavior in rats: role of dopamine D3 receptors. Synapse 2005;57:17–28.
231. Vorel SR, Ashby CR, Jr., Paul M, et al. Dopamine D3 receptor antagonism inhibits cocaine-seeking and cocaine-enhanced brain reward in rats. J Neurosci 2002;22:9595–603.
232. Xi ZX, Gilbert J, Campos AC, et al. Blockade of mesolimbic dopamine D3 receptors inhibits stress-induced reinstatement of cocaine-seeking in rats. Psychopharmacology 2004;176: 57–65.
233. Schmidt HD, Anderson SM, Pierce RC. Stimulation of D1-like or D2 dopamine receptors in the shell, but not the core,of the nucleus accumbens reinstates cocaine-seeking behaviour in the rat. Eur J Neurosci 2006;23:219–28.
234. Anderson SM, Famous KR, Sadri-Vakili G, et al. CaMKII: a biochemical bridge linking accumbens dopamine and glutamate systems in cocaine seeking. Nat Neurosci 2008;11: 344–53.

235. Self DW, Genova LM, Hope BT, Barnhart WJ, Spencer JJ, Nestler EJ. Involvement of cAMP-dependent protein kinase in the nucleus accumbens in cocaine self-administration and relapse of cocaine-seeking behavior. J Neurosci 1998;18:1848–59.
236. Ito R, Dalley JW, Howes SR, Robbins TW, Everitt BJ. Dissociation in conditioned dopamine release in the nucleus accumbens core and shell in response to cocaine cues and during cocaine-seeking behavior in rats. J Neurosci 2000;20:7489–95.
237. Schmidt HD, Pierce RC. Cooperative activation of D1-like and D2-like dopamine receptors in the nucleus accumbens shell is required for the reinstatement of cocaine-seeking behavior in the rat. Neuroscience 2006;142:451–61.
238. Pontieri FE, Tanda G, Di Chiara G. Intravenous cocaine, morphine, and amphetamine preferentially increase extracellular dopamine in the "shell" as compared with the "core" of the rat nucleus accumbens. Proc Natl Acad Sci USA 1995;92:12304–8.
239. Hedou G, Feldon J, Heidbreder CA. Effects of cocaine on dopamine in subregions of the rat prefrontal cortex and their efferents to subterritories of the nucleus accumbens. Eur J Pharmacol 1999;372:143–55.
240. Anderson SM, Bari AA, Pierce RC. Administration of the D1-like dopamine receptor antagonist SCH-23390 into the medial nucleus accumbens shell attenuates cocaine priming-induced reinstatement of drug-seeking behavior in rats. Psychopharmacology 2003;168:132–8.
241. Anderson SM, Schmidt HD, Pierce RC. Administration of the D2 dopamine receptor antagonist sulpiride into the shell, but not the core, of the nucleus accumbens attenuates cocaine priming-induced reinstatement of drug seeking. Neuropsychopharmacology 2006;31:1452–61.
242. Di Ciano P. Drug seeking under a second-order schedule of reinforcement depends on dopamine D3 receptors in the basolateral amygdala. Behav Neurosci 2008;122(1):129–39.
243. See RE, Kruzich PJ, Grimm JW. Dopamine, but not glutamate, receptor blockade in the basolateral amygdala attenuates conditioned reward in a rat model of relapse to cocaine-seeking behavior. Psychopharmacology 2001;154:301–10.
244. Sun W, Rebec GV. The role of prefrontal cortex D1-like and D2-like receptors in cocaine-seeking behavior in rats. Psychopharmacology 2005;177:315–23.
245. Capriles N, Rodaros D, Sorge RE, Stewart J. A role for the prefrontal cortex in stress- and cocaine-induced reinstatement of cocaine seeking in rats. Psychopharmacology 2003;168:66–74.
246. Di Pietro NC, Mashhoon Y, Heaney C, Yager LM, Kantak KM. Role of dopamine D1 receptors in the prefrontal dorsal agranular insular cortex in mediating cocaine self-administration in rats. Psychopharmacology 2008;200:81–91.
247. O'Donnell P. Dopamine gating of forebrain neural ensembles. Eur J Neurosci 2003;17:429–35.
248. De Vries TJ, Schoffelmeer AN, Binnekade R, Raaso H, Vanderschuren LJ. Relapse to cocaine- and heroin-seeking behavior mediated by dopamine D2 receptors is time-dependent and associated with behavioral sensitization. Neuropsychopharmacology 2002;26:18–26.
249. Shaham Y, Stewart J. Effects of opioid and dopamine receptor antagonists on relapse induced by stress and re-exposure to heroin in rats. Psychopharmacology 1996;125:385–91.
250. Tobin S, Newman AH, Quinn T, Shalev U. A role for dopamine D1-like receptors in acute food deprivation-induced reinstatement of heroin seeking in rats. Int J Neuropsychopharmacol 2008:1–10.
251. Duarte C, Biala G, Le Bihan C, Hamon M, Thiebot MH. Respective roles of dopamine D2 and D3 receptors in food-seeking behaviour in rats. Psychopharmacology 2003;166:19–32.
252. Bossert JM, Poles GC, Wihbey KA, Koya E, Shaham Y. Differential effects of blockade of dopamine D1-family receptors in nucleus accumbens core or shell on reinstatement of heroin seeking induced by contextual and discrete cues. J Neurosci 2007;27:12655–63.

253. Yun IA, Nicola SM, Fields HL. Contrasting effects of dopamine and glutamate receptor antagonist injection in the nucleus accumbens suggest a neural mechanism underlying cue-evoked goal-directed behavior. Eur J Neurosci 2004;20:249–63.
254. Micheli F, Bonanomi G, Blaney FE, et al. 1,2,4-triazol-3-yl-thiopropyl-tetrahydrobenzazepines: a series of potent and selective dopamine D(3) receptor antagonists. J Med Chem 2007;50:5076–89.
255. Vengeliene V, Leonardi-Essmann F, Perreau-Lenz S, et al. The dopamine D3 receptor plays an essential role in alcohol-seeking and relapse. FASEB J 2006;20:2223–33.
256. Czachowski CL, Chappell AM, Samson HH. Effects of raclopride in the nucleus accumbens on ethanol seeking and consumption. Alcohol Clin Exp Res 2001;25:1431–40.
257. Samson HH, Chappell AM. Effects of raclopride in the core of the nucleus accumbens on ethanol seeking and consumption: the use of extinction trials to measure seeking. Alcohol Clin Exp Res 2004;28:544–9.
258. Liu X, Weiss F. Reversal of ethanol-seeking behavior by D1 and D2 antagonists in an animal model of relapse: differences in antagonist potency in previously ethanol-dependent versus nondependent rats. J Pharmacol Exp Ther 2002;300:882–9.
259. Hamlin AS, Newby J, McNally GP. The neural correlates and role of D1 dopamine receptors in renewal of extinguished alcohol-seeking. Neuroscience 2007;146:525–36.
260. Dalley JW, Fryer TD, Brichard L, et al. Nucleus accumbens D2/3 receptors predict trait impulsivity and cocaine reinforcement. Science 2007;315:1267–70.
261. Morgan D, Grant KA, Gage HD, et al. Social dominance in monkeys: dopamine D2 receptors and cocaine self-administration. Nat Neurosci 2002;5:169–74.
262. Ahmed SH, Walker JR, Koob GF. Persistent increase in the motivation to take heroin in rats with a history of drug escalation. Neuropsychopharmacology 2000;22:413–21.
263. Lenoir M, Ahmed SH. Heroin-induced reinstatement is specific to compulsive heroin use and dissociable from heroin reward and sensitization. Neuropsychopharmacology 2007;32:616–24.
264. Ahmed SH, Koob GF. Changes in response to a dopamine receptor antagonist in rats with escalating cocaine intake. Psychopharmacology 2004;172:450–54.
265. Volkow ND, Fowler JS, Wang GJ, et al. Decreased dopamine D2 receptor availability is associated with reduced frontal metabolism in cocaine abusers. Synapse 1993;14:169–77.
266. Volkow ND, Wang GJ, Fowler JS, et al. Decreases in dopamine receptors but not in dopamine transporters in alcoholics. Alcohol Clin Exp Res 1996;20:1594–8.
267. Wang GJ, Volkow ND, Fowler JS, et al. Dopamine D2 receptor availability in opiate-dependent subjects before and after naloxone-precipitated withdrawal. Neuropsychopharmacology 1997;16:174–82.
268. Volkow ND, Chang L, Wang GJ, et al. Low level of brain dopamine D2 receptors in methamphetamine abusers: association with metabolism in the orbitofrontal cortex. Am J Psychiat 2001;158:2015–21.
269. Druhan JP, Walters CL, Aston-Jones G. Behavioral activation induced by D(2)-like receptor stimulation during opiate withdrawal. J Pharmacol Exp Ther 2000;294:531–8.
270. Seeman P, Tallerico T, Ko F, Tenn C, Kapur S. Amphetamine-sensitized animals show a marked increase in dopamine D2 high receptors occupied by endogenous dopamine, even in the absence of acute challenges. Synapse 2002;46:235–9.
271. Seeman P, Weinshenker D, Quirion R, et al. Dopamine supersensitivity correlates with D2High states, implying many paths to psychosis. Proc Natl Acad Sci USA 2005;102:3513–8.
272. Seeman P, McCormick PN, Kapur S. Increased dopamine D2(High) receptors in amphetamine-sensitized rats, measured by the agonist [(3)H](+)PHNO. Synapse 2007;61:263–7.
273. Briand LA, Flagel SB, Seeman P, Robinson TE. Cocaine self-administration produces a persistent increase in dopamine D2 High receptors. Eur Neuropsychopharmacol 2008;18:551–6.

274. Martinez D, Broft A, Foltin RW, et al. Cocaine dependence and d2 receptor availability in the functional subdivisions of the striatum: relationship with cocaine-seeking behavior. Neuropsychopharmacology 2004;29:1190–202.

Chapter 18
Dopamine Receptors and the Treatment of Parkinson's Disease

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Abstract Parkinson's disease is a devastating disorder caused by progressive degeneration of dopaminergic neurons in the substantia nigra and the consequent loss of dopamine in the striatum. Dopamine replacement therapy with the dopamine precursor levodopa (L-DOPA), introduced in the 1960s, remains the most effective treatment. Unfortunately, L-DOPA, upon long-term administration, gradually loses its efficacy and eventually leads to severe motor complications, including dyskinesia. The data from numerous studies on Parkinson's patients and animal models of the disease show a complex pattern of changes in multiple signaling pathways in the striatum induced by dopamine depletion. These include modulation of the expression and activity of several subtypes of dopamine receptors, G proteins, effectors, multiple protein kinases, components of the machinery for desensitization and trafficking of G protein-coupled receptors, ionotropic glutamate receptors, and transcription factors. Dopamine replacement therapy reverses many of these changes. However, select signaling effects are exacerbated and/or induced de novo by chronic treatment with L-DOPA. The L-DOPA-induced dyskinesia appears closely associated with selective increases in the activity of specific D_1 receptor-dependent pathways. The contribution of D_2 and D_3 receptor-mediated signaling to dyskinesia development remains largely unexplored. The mechanisms underlying the further enhancement by L-DOPA of signaling pathways already made supersensitive by dopamine depletion need to be elucidated. The recently introduced long-lived dopamine agonists cause less dyskinesia than L-DOPA but are also less efficacious as antiparkinsonian agents. The clinically used DA agonists, which target D2-like receptors and often show preference for the D_3 over D_2 subtype, in addition to their antiparkinsonian action, may protect surviving dopaminergic neurons. Continuous delivery of L-DOPA or dopamine agonists, which mimics the physiological tonic stimulation of dopamine receptors, holds the promise of providing therapeutic benefits with minimal side effects. A much better understanding of the molecular

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processes underlying the therapeutic action of dopaminergic drugs and the development of dyskinesia is necessary in order to modify existing treatments and/or devise new approaches to maximize the beneficial effects of dopamine replacement and minimize the side effects.

Keywords Parkinson's disease · L-DOPA · Dyskinesia · Motor complications · Sensitization · Receptor supersensitivity

18.1 Dopamine Receptors in the Pathology of Parkinson's Disease

Parkinson's disease (PD) is a progressive neurodegenerative disorder characterized by bradykinesia or akinesia, resting tremor, and muscle rigidity. These symptoms are often accompanied by changes of posture, gait, and sleep; autonomous disturbances; cognitive decline; and depression as the disease progresses. The main underlying pathology in PD is the death of dopaminergic neurons in the substantia nigra pars compacta. These neurons project to the striatum, and their demise results in the loss of dopamine (DA) in the striatum. DA exerts a critical modulatory influence over the striato-thalamo-cortical circuit. DA depletion in the striatum enhances overall striatal output, bringing about excessive inhibition of the excitatory thalamo-cortical projections. As a result, the motor cortical output is reduced, leading to the paucity of voluntary movements in PD patients [1]. This model is applicable to akinesia. Another major symptom of PD, rest tremor, has a somewhat different, and poorly understood, pathophysiology. Tremor is caused by nigrostriatal deficits, but its generation involves extrastriatal areas such as the cerebellum and thalamus [2]. Due to difficulties of reliably modeling tremor in animals [3], no studies have yet been performed addressing the molecular mechanisms of parkinsonian tremor. Therefore, we will confine the discussion to the mechanisms of akinesia.

Obviously, the best treatment of PD would be prevention of the degeneration of dopaminergic neurons, or, at least, an arrest of their loss at early stages. Unfortunately, no means to achieve these goals are available at the moment. Some of the DA agonists used to alleviate parkinsonian features have shown neuroprotective properties improving survival of dopaminergic neurons in culture and animal models of PD. Currently, though, attempts to compensate for the diminishing production of endogenous dopamine are the prevailing therapeutic strategy. Although DA receptor agonists are used for this purpose, the most common and effective replacement is achieved with the dopamine precursor levodopa (L-DOPA). Two problems develop over the years as a result of L-DOPA treatment: (1) its therapeutic effectiveness gradually decreases and (2) patients develop severe motor complications. Attempts to alleviate these unwanted effects simply by varying the doses and delivery regimens of L-DOPA and/or DA agonists do not appear to solve these problems. To design effective therapies with minimum side effects, we need a detailed understanding of the molecular mechanisms of dopaminergic

signaling in the normal brain, as well as of the changes in DA-driven signaling pathways induced by DA depletion in PD and by different types of DA replacement therapy. Therefore, here we primarily focus on DA-mediated signaling and its modulation in PD, with the objective of reconstructing a coherent picture from the rather fragmentary information and identifying gaps in our knowledge that need to be filled.

18.1.1 Expression Pattern of DA Receptors in the Forebrain of Rodents and Primates

DA acts via five subtypes of G protein-coupled DA receptors. Two of the receptors belong to the D1-like subfamily (D_1 and D_5) and three to the D2-like subfamily (D_2, D_3, and D_4). D1-like receptors couple to $G\alpha_s/G\alpha_{olf}$ and induce accumulation of cyclic adenosine monophosphate (cAMP), whereas D2-like receptors couple to $G\alpha_i/G\alpha_o$ and inhibit cAMP production. The D_1 subtype is the main D1-like receptor expressed at a very high level in the striatum (Fig. 18.1). D_1 receptors are largely localized on the striatal medium spiny neurons, projecting to the internal segment of the globus pallidus and the substantia nigra pars reticulata (direct striatal output pathway), which also express the neuropeptides substance P and dynorphin [4–6]. D_1 receptors are also expressed at moderate levels throughout the cortex [7, 8]. D_2 receptors are highly abundant in the caudate nucleus, putamen, and nucleus accumbens (Fig. 18.1). In the striatum, they are located on a different population of medium spiny neurons than those expressing D_1 receptors. D_2 receptors are found on neurons expressing enkephalin and projecting to the globus pallidus external (indirect pathway) [4, 9, 10]. D_2 receptors are also localized presynaptically on nigrostriatal dopaminergic terminals and on the substantia nigra neurons [11–14]. D_2 receptors are found at low levels throughout the cortex [7]. D_2 receptors located on presynaptic corticostriatal terminals inhibit striatal glutamate release [7, 15, 16].

The experimental support for the model of segregated localization of the two major DA receptor subtypes is somewhat mixed. Double in situ hybridization labeling studies invariably demonstrate practically exclusive expression of D_1 receptors in substance P/dynorphin-positive neurons and expression of D_2 receptors in enkephalin-positive neurons [5, 9, 17, 18]. However, physiological, histochemical, and single cell PCR studies demonstrate co-expression of D_1 and D_2 subtypes [19, 20], although the estimates of the degree of co-expression vary from 10–25 to 100%. It is equally unclear whether the direct pathway neurons express only substance P and dynorphin and whether the indirect pathway neurons express exclusively enkephalin. Another source of controversy is that the segregation of the direct and indirect pathways is incomplete. Most striatal output neurons in both the rodent and the primate brain extensively collateralize, often sending collateral projections to all striatal output targets [10, 21, 22]. Thus, it is no longer possible to adhere to the simplistic model of complete segregation of D_1 and D_2 DA receptors. Nonetheless, in situ hybridization experiments convincingly demonstrate that D_1 receptor mRNA is present at a high level in approximately half of striatal medium

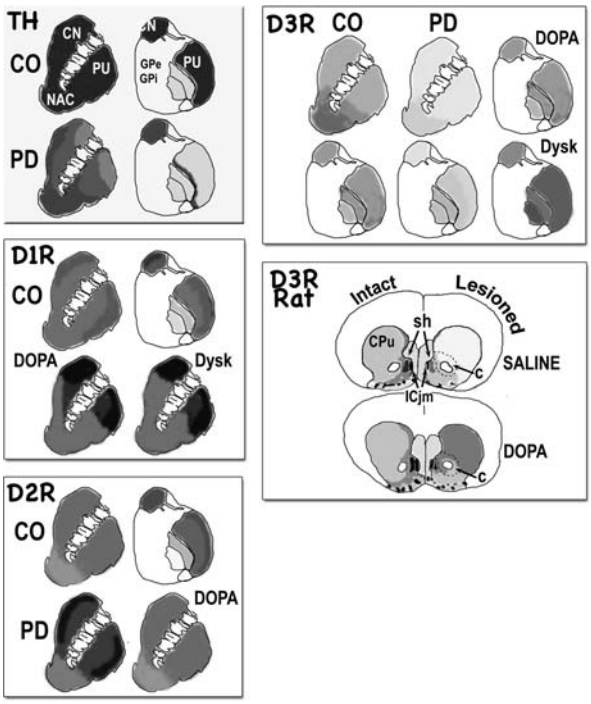

Fig. 18.1 Changes in the dopamine receptor number in Parkinson's disease. Schematic illustrations of alterations in dopamine receptors in the primate, including human, brain following dopamine depletion or in Parkinson's disease. The concentrations of the proteins are color-coded: darker colors denote higher density of the proteins. *TH box* (*top left*): The density of dopaminergic terminals in the striatum in the normal brain (CO) and loss of dopaminergic innervation in Parkinson's disease (PD) at the rostral (left) and caudal (right) levels. The expression of the D_1 (*D1R box*), D_2 (*D2R box*), and D_3 (*D3R box*) dopamine receptors in the normal primate striatum (CO) is shown at different rostro-caudal levels. The alterations in the receptor densities specific for PD, chronic L-DOPA treatment (regardless of the presence of dyskinesia), or overt dyskinesia (Dysk) are also shown. *D3R Rat box*: The expression of the D_3 receptor in the brain of the hemiparkinsonian rat is shown (upper image): Intact – the intact hemisphere; Lesioned – the hemisphere depleted of dopamine with 6-hydroxydopamine. Lower image – alterations in the D_3 receptor density induced by chronic L-DOPA treatment (in comparison with the saline treatment; upper image).

Abbreviations: c – the core subdivision of the nucleus accumbens (dashed outline); CN – caudate nucleus; CO – control subjects; CPu – caudatoputamen; DOPA – the group chronically treated with L-DOPA; Dysk – the group of subjects displaying overt dyskinesia; GPe – external segment of the globus pallidus; GPi – internal segment of the globus pallidus; ICjm – island of Calleja magna; NAC – nucleus accumbens; PD – subjects with Parkinson's disease or drug-naïve animals following dopamine depletion by toxins; PU – putamen; sh – the shell subdivision of the nucleus accumbens.

spiny neurons, whereas the remaining half shows no detectable signal. Similarly, a non-overlapping population of striatal neurons expresses high levels of D_2 mRNA. Since there is no reason to disregard these findings, an explanation consistent with all available data would be that the D_1 and D_2 receptors are expressed at a high

level by substance P/dynorphin- and enkephalin-positive neurons, respectively, but many neurons express the "wrong" receptors at lower levels. This explanation would account for single cell PCR data as well as for physiological data showing that most striatal neurons respond to both D_1 and D_2 agonists [19, 20, 23]. The D_1 and D_2 receptors co-expressed on the same striatal neurons may have differential subcellular distribution resulting in the subtype-specific role in the striatal function [23]. It is important to remember that most of these data come from studies of the rodent brain. There is evidence that a similar distribution exists in the brain of non-human primates [4, 10], but no direct evidence has been produced so far for the human brain. D_2, but probably not D_1, receptors localize to cholinergic striatal interneurons [11, 24, 25] in both the rodent and the primate brains and may play an important regulatory role via these neurons in the normal and diseased brain [26]. D_2 receptors are also found throughout the neocortex and hippocampus [7, 8, 27].

The D_3 DA receptor is another member of the D2-like subfamily. It is often said that it has a more restricted distribution than the D_2 receptor. Indeed, in the rodent brain, D_3-binding sites and mRNA are detectable in a limited number of brain regions: the nucleus accumbens, mostly rostral pole and shell subdivisions, the islands of Calleja, the olfactory tubercle, the ventral pallidum, and at low levels in the striatum [11, 28, 29]. However, the pattern of D_3 receptor expression in the primate brain is quite different from that in the rodent (Fig. 18.1). In primates, including humans, D_3 receptors are seen throughout the striatum, although they are most abundant in the nucleus accumbens [8, 11, 30–32]. D_3 receptors are also detected in the globus pallidus, particularly the internal segment (where they are undetectable in rodents), anterior thalamus, amygdala, and throughout the cortex and hippocampus [11, 32]. The concentration of D_3 receptors in the striatum in the human brain is approximately 30% of that of D_2 receptors, whereas in the rodent brain it is barely 5% [11, 31]. In the rat and human brain, D_3 receptors colocalize with both D_1 and D_2 receptors [11, 20, 33]. Functional importance of the D_3 receptor stems from its high affinity to most D_2 agonists, including the endogenous agonist DA [29]. Thus, many DA agonists used in the treatment of PD are D_3-preferring drugs targeting the D_3 receptor more than the D_2 [34].

Pharmacological properties of the D_3 receptor are peculiar. In contrast to the D_2 receptor, the D_3 receptor is not converted into the low-affinity state by GTP and there is little difference in the affinity of agonists to the D_3 receptor in the presence or absence of GTP [35]. This feature has been extensively used to achieve selective labeling of the D_3 receptor in radioligand-binding experiments [11, 12, 31, 35]. Originally, when the receptor was first cloned, this phenomenon gave rise to the idea that the D_3 receptor is not functional. Indeed, it proved difficult to define the signal transduction pathways activated by the D_3 receptor and attribute specific functions to it. However, D_3 certainly plays a functional role, which is yet to be understood. An interesting feature of the D_3 receptor is its expression in the proliferative zones during prenatal and early postnatal development [12, 36, 37] and the transient expression in the primary sensory cortical areas during postnatal development [38, 39]. This expression pattern suggests a role for the D_3 receptor in neurogenesis and brain maturation.

Much less is known about the distribution of the other two DA receptors in the brain. The D2-like D_4 receptor is largely found in extrastriatal areas, the septum, thalamus, cortex, and hippocampus [8, 27]. D4 receptors are present in both populations of the striatal output neurons at a modest level [20, 40, 41] but are not detectable in striatal interneurons [40]. The D1-like D_5 receptor has been detected in the striatum, where it has a somewhat different subcellular distribution than its closest relative the D_1 receptor, localizing more to dendritic shafts, whereas the D_1 subtype is found more often on dendritic spines [24, 42]. D_5, but not D_1, receptors are expressed by most cholinergic striatal interneurons [24, 25, 43, 44] as well as by parvalbumin-positive [43] and nitric oxide-positive [45] interneurons. The D_5 receptor is also found in the hippocampus and cortex of the human brain, as well as in the substantia nigra, thalamus, and cerebellum [24, 27, 44].

18.1.2 Changes in Dopamine Receptor Expression in Parkinson's Disease

In PD, when dopaminergic neurons degenerate and the brain is deprived of DA, DA receptors undergo multiple plastic changes (Fig. 18.1). One type of alteration that is easy to measure and that has been studied extensively is the change in receptor expression. D_2 receptors located on nigrostriatal terminals are obviously lost when the terminals degenerate, which leads to a reduction in the D_2 receptor concentration in the striatum. However, most studies found that the concentration of D_2 receptors increases in the striatum of patients with PD (detected by live imaging methods) [46–54] or at postmortem [55–58], as well as in animal models of PD [59–62]. These data suggest compensatory up-regulation of postsynaptic D_2 receptors (and, possibly, presynaptic receptors on the remaining nigrostriatal terminals). The magnitude of this increase is quite modest (20–50%). With less severe DA depletion in MPTP-treated monkeys, a down-regulation of D_2 receptors is detected, apparently reflecting the loss of presynaptic D_2 receptors, which in this case is not masked by the up-regulation of postsynaptic receptors observed after extensive DA depletion [63]. There have been conflicting reports regarding changes in the expression of striatal D_1 receptors in the parkinsonian brain, but most studies found no significant changes or a slight decrease in the D_1 receptor number [46, 52, 56, 59, 62, 64]. In the rat, D_3 receptors in the nucleus accumbens and striatum decrease following DA depletion [60, 65–67]. Most studies found a similar decrease in the striatum of monkeys depleted of DA by treatment with the neurotoxin 1-methyl 4-phenyl 1,2,3,4-tetrahydropyridine (MPTP) [30, 68] and parkinsonian patients at postmortem [57, 58]. No information is currently available about changes in the concentration of striatal D_4 or D_5 receptors induced by DA depletion.

The striatum receives the bulk of dopaminergic projections, and striatal neurons express DA receptors at high levels. In PD, there is a strict pattern of degeneration of dopaminergic cells, with neurons of the ventral tier of the substantia nigra pars compacta projecting to the dorso-lateral striatum being the most vulnerable

[69–71]. Thus, the highest degree of DA depletion is seen in the dorso-lateral putamen [72, 73], and DA receptors are most likely to change in this region (Fig. 18.1). The dopaminergic neurons of the mesolimbic and mesocortical systems are relatively spared in PD, resulting in less pronounced or minimal loss of DA in other brain regions. Among cortical regions, only the prefrontal cortex has relatively dense dopaminergic innervation, and it is unknown how much it is affected by dopaminergic degeneration in PD. Human postmortem studies found no changes in the concentration of DA receptors in extrastriatal regions of the basal ganglia [55, 58]. Imaging studies detected reduced densities of D_2 receptors in selected cortical areas [74].

18.1.3 Modifications of Dopamine Receptor Signaling in Parkinson's Disease

The most striking change in the function of DA receptors brought about by the loss of DA is their supersensitivity. This supersensitivity, first described by Ungerstedt [75], in rats unilaterally lesioned with the dopaminergic toxin 6-hydroxydopamine (6-OHDA), manifested itself as rotations induced by dopaminergic drugs at doses 10–100 times lower than those required to cause noticeable behavioral effects in intact animals. This model, the hemiparkinsonian rat, was to become the most important animal model of PD for molecular and signaling studies, since it allowed for the comparison of signaling events between the intact and the dopamine-depleted striatum in the same animal. When hemiparkinsonian animals are injected with direct DA agonists such as apomorphine or the DA precursor L-DOPA, they rotate contralaterally to the lesioned side, but the animals rotate ipsilaterally upon injection of the indirect agonist amphetamine, which induces the release of endogenous DA. The widely accepted interpretation for these observations is that dopaminergic drugs induce activation seen as rotations due to the imbalance in dopaminergic activity between the intact and the lesioned hemispheres. Thus, the animals rotate away from the side of stronger stimulation. The DA releaser amphetamine stimulates the intact side, where endogenous DA is present, whereas direct DA agonists stimulate the lesioned side, where DA receptors are supersensitive. Similarly, the locomotor response to dopaminergic stimulation is supersensitive in DA null mice (in which the gene of tyrosine hydroxylase, the rate-limiting enzyme in the DA biosynthesis, is inactivated) [76].

18.1.3.1 Changes in the Responsiveness of Signaling Pathways Caused by DA Depletion

The stimulation of DA receptors in the DA-depleted striatum produces exaggerated molecular responses, which presumably underlie the supersensitive behavioral response (Fig. 18.2). Multiple reports showed enhanced DA-induced activity of adenylyl cyclase and suggested that elevated accumulation of cAMP underlies the

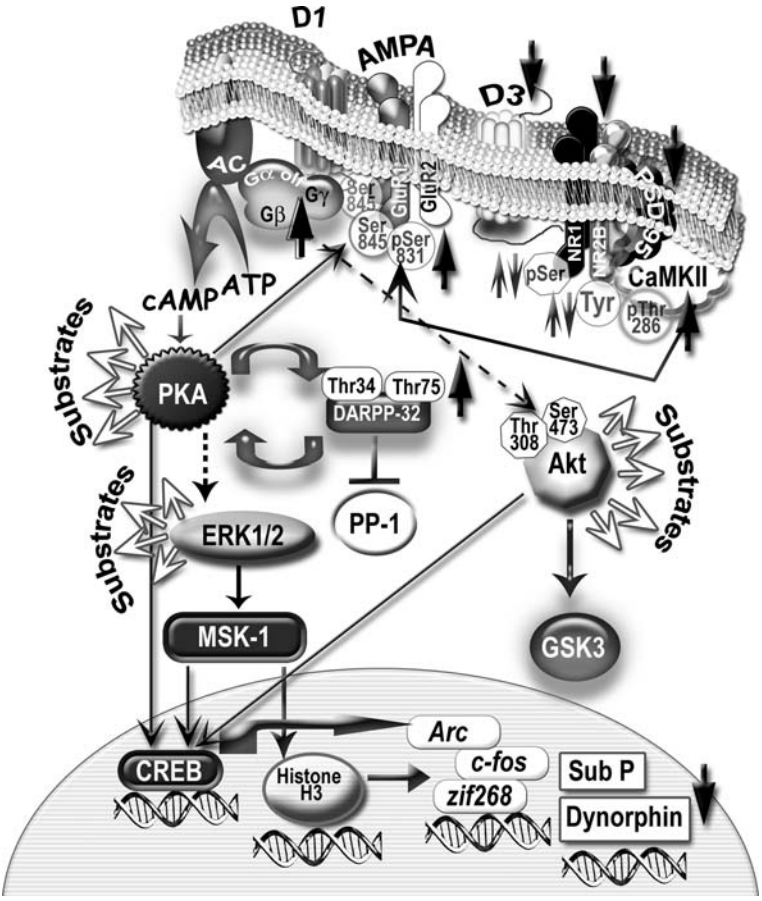

Fig. 18.2 Alterations in the dopamine D_1 receptor signaling following dopamine depletion. Schematic representation of the putative mechanisms of the D_1 receptor supersensitivity induced by dopamine depletion. Loss of D_1 receptor stimulation following DA depletion results in specific alterations of the expression of multiple proteins. *Short black arrows* pointing up or down next to the protein symbols indicate an increase or decrease in the basal expression; arrows next to the phosphorylation sites indicate changes in the basal phosphorylation level at the sites; pairs of up and down arrows indicate that there are published reports of up- as well as down-regulation. Many pathways with unaltered basal activity respond with a stronger signal to acute dopaminergic challenge. Known stimulatory signaling connections are indicated by *solid arrows* (the arrow between Akt and GSK3 depicts the Akt-mediated phosphorylation of GSK3, which inhibits the GSK3 activity); the inhibitory connection between DARPP-32 and PP-1 is indicated; *dashed arrows* indicate hypothetical connections. Since all signaling proteins have multiple substrates, the possibilities for the propagation of supersensitivity are indicated by *open arrows*. Note that there is no direct evidence that changes in NMDA receptors occur in D_1-bearing neurons. They are shown here because they follow the same general pattern as other D_1 receptor-mediated changes

circling behavior in hemiparkinsonian rats [77–83]. Elevated DA-stimulated accumulation of cAMP was found in the striatum of PD patients [81, 84]. Downstream targets of cAMP-dependent phosphorylation are also affected (Fig. 18.2). In 6-OHDA-lesioned rodents, the phosphorylation level of the DA- and cAMP-regulated phosphoprotein 32 kD (DARPP-32) at Thr34, which is the substrate of protein kinase A (PKA), in response to acute dopaminergic stimulation by L-DOPA is dramatically increased in the lesioned striatum without changes in the basal level of DARPP-32 phosphorylation [85]. The level of phosphorylation of another PKA substrate, the GluR1 subunit of the AMPA receptor, is also increased at the PKA site Ser845 in response to acute dopaminergic challenge without changes in the phosphorylation level at the calcium/ calmodulin-dependent protein kinase II (CaMKII) site Ser831.

DARPP-32 is an important component of the DA signaling, particularly in the striatum, where it is expressed at a high level in medium spiny neurons [86]. DARPP-32 activity is regulated by phosphorylation at multiple sites. It is phosphorylated by PKA at Thr34, which converts it into an inhibitor of protein phosphatase-1 [87, 88]. By inhibiting the dephosphorylation of proteins phosphorylated by PKA, DARPP-32 creates positive feedback for the D_1 receptor-mediated signaling. Stimulation of D_1 receptors enhances DARPP-32 phosphorylation at Thr34, and deactivation of DARPP-32 blunts the D_1-mediated signaling and suppresses the D_1 behavioral effects [88]. The DARPP-32-based positive feedback loop for the D_1 receptor signaling includes an additional component aimed at promoting PKA function. DARPP-32 is phosphorylated at Thr75 by cyclin-dependent kinase 5 (cdk5), which converts it into an inhibitor of PKA [89]. DA acting via D_1 receptors and PKA reduces DARPP-32 phosphorylation at Thr75 [90], thus removing the inhibition and promoting PKA-mediated signaling. In the DA-depleted striatum, the basal level of DARPP-32 phosphorylation at Thr75 is elevated [91], as should be expected. These data suggest that in the DA-depleted striatum the D_1 receptor-mediated signaling is enhanced at many levels, leading to exaggerated behavioral responses to D_1 drugs. D_2 receptor activation reduces the level of DARPP-32 phosphorylation at Thr34 via inhibition of PKA and activation of calcium/calcineurin signaling [92]. Loss of DARPP-32 results in a reduction in D_2 receptor-mediated behavioral effects, such as raclopride-induced catalepsy [88]. The fact that in the denervated striatum challenged with dopaminergic drugs the level of DARPP-32 phosphorylation is enhanced suggests that the D_1 supersensitivity predominates, outweighing the D_2 signaling, at least within this specific molecular pathway.

Dopaminergic activation induces a 2–3 times higher level of phosphorylation of extracellular signal-regulated kinases 1 and 2 (ERK1/2) in the DA depleted as compared to the intact striatum [18, 60, 76, 85]. L-DOPA does not alter ERK1/2 phosphorylation in the intact striatum but strongly activates it following dopaminergic denervation [60, 76, 85]. The supersensitive ERK1/2 response in the denervated striatum has been shown to be mediated by D_1 receptors [18, 93] and, presumably, by PKA. The ERK1/2 activation is influenced by DARPP-32, since ERK1/2 supersensitivity to acute L-DOPA is attenuated in DARPP-32 null mice [85]. The

dopaminergic denervation results in elevated phosphorylation of Akt (at both Thr34 and Ser375) and its substrate glycogen synthase kinase 3α and β (GSK3α,β) in response to acute administration of the D_1/D_2 agonist apomorphine in the 6-OHDA rat model of PD [60].

The denervation-induced supersensitivity of DA receptors can be demonstrated easily and reliably in the hemiparkinsonian rat model of PD. However, it is much more difficult to detect in other animal models of PD, and next to impossible in human patients. In the MPTP monkey model of PD, the lesion produced by systemic administration of the dopaminergic neurotoxin MPTP is bilateral and results in akinesia similar to that seen in advanced PD patients. Dopaminergic stimulation alleviates akinesia, thus producing an antiparkinsonian effect. Monkeys with unilateral lesions produced by a single injection of MPTP into the carotid artery exhibit contralateral circling behavior in response to L-DOPA or DA agonists [94] indicative of functional DA receptor supersensitivity on the lesioned side. Increased DA-stimulated adenylyl cyclase activity has been reported in patients with PD at postmortem [81, 84], but since all patients come for the autopsy after having been treated with L-DOPA for years or even decades, it cannot be ascertained whether this enhancement is due to denervation, L-DOPA treatment, or the combination of both. The degree of enhancement is usually not impressive, hovering around 50–60%.

In the MPTP monkey model, it is possible to separate the effects of L-DOPA treatment from those caused by DA denervation. Denervation leads to an enhanced stimulation of adenylyl cyclase activity by DA in L-DOPA-naïve MPTP-lesioned primates [81] and enhanced [^{35}S]GTPγS binding induced by the D_1 agonist SKF38393 [95] indicative of an increased D_1 receptor coupling to G protein. Similarly to the rodent data, ERK phosphorylation and expression is increased in the striatum of MPTP-treated monkeys [96], possibly as a result of receptor supersensitivity. In common marmosets unilaterally lesioned with 6-OHDA, L-DOPA elicits strong activation of *c-fos* and *c-jun* expression in the lesioned hemisphere, which is undetectable on the intact side [97]. However, the experiments necessary to detect DA receptor supersensitivity, such as acute injection of dopaminergic drugs and rapid detection of changes, are rarely performed in monkeys for practical reasons. Therefore, the supersensitivity of the multiple signaling pathways known to be super-responsive in hemiparkinsonian rats remains unproven in the parkinsonian primate brain, although there is no compelling reason to suppose that the situation there is substantially different than in the rodent.

18.1.3.2 The Effects of Dopamine Depletion on Transcription Factors

The lesion-induced supersensitivity of DA receptors causes strong activation of multiple genes in the striatum in response to acute dopaminergic stimulation. Many of these genes are transcription factors belonging to the *fos* (*c-fos, FosB, ΔFosB, fra-1, fra-2*) and *jun* (*c-jun*, *JunB*, *JunD*) gene families. The protein products of these genes, Fos and Jun, heterodimerize to form Fos/Jun, or homodimerize forming Jun/Jun complexes, collectively known as activator protein-1 (AP-1) transcription factors, which can bind to AP-1 consensus sites on target genes and regulate their

transcription [98]. The transcriptional activity of AP-1 depends heavily on the actual composition of the complex, posttranslational modifications, and interactions with regulatory proteins [99]. Many of these genes are termed *i*mmediate *e*arly *g*enes (IEG) because their transcription is rapidly induced by acute challenges and just as rapidly deactivated within a few hours. The activation of these genes can be easily detected by in situ hybridization or immunohistochemistry and has been extensively used as a general indicator of neuronal activation following various stimuli. It was found that the level of activation of the gene expression in the DA-deprived striatum of hemiparkinsonian rats is many times stronger than on the intact side [97, 100–102].

The cAMP response element-binding protein (CREB) is a transcription factor activated via cAMP- or Ca^{2+}-dependent phosphorylation at Ser133 by multiple protein kinases [103, 104]. Acute L-DOPA injection induces a substantially higher level of CREB phosphorylation in the lesioned than in the intact striatum [105, 106] indicative of supersensitivity. The supersensitive CREB response to L-DOPA is reproduced by D_1 but not D_2 agonist and blocked by D_1 antagonist, demonstrating the supersensitivity of D_1 receptors in the DA-depleted striatum [106]. The increased transcription of *c-fos* in the lesioned striatum induced by dopaminergic stimulation may reflect increased activation of CREB, since CREB activation has been shown to regulate drug-induced *c-fos* expression [104, 105, 107]. At the same time, it has been reported that in the lesioned striatum, in contrast to the intact brain, CREB does not participate in the induction of *c-fos* transcription [108], which would leave supersensitive *c-fos* induction unaccounted for. Interestingly, knockdown of CREB exacerbated the behavioral effects of L-DOPA in hemiparkinsonian rats [108], suggesting that supersensitive CREB activation is not the cause of behavioral supersensitivity but rather its negative regulator.

18.1.3.3 Changes in the Basal Activity or Expression of Signaling Proteins

In addition to the lesion-induced supersensitivity, which requires acute dopaminergic challenge to be revealed, the loss of DA causes changes in the basal level of activity or expression of various proteins. The increased level of DARPP-32 phosphorylation at Thr75 [91] is possibly mediated by loss of stimulation of the D_1 receptor, which normally inhibits Thr75 phosphorylation via activation of protein phosphatase 2A (PP2A) [90]. In the lesioned striatum, the basal level of autophosphorylation of calcium-, calmodulin-dependent protein kinase II (CaMKII) at Thr286 is elevated [91, 109]. The autophosphorylation of CaMKII at Thr286 enhances autonomous kinase activity and eventually leads to increased basal phosphorylation of the $GluR_1$ subunit of the AMPA receptor at the CaMKII substrate Ser831, although this effect does not occur until months after the dopaminergic denervation [91]. The increase in CaMKII autophosphorylation is mimicked by a D_1, but not D_2, antagonist and by a PKA inhibitor, whereas forskolin reverses it [109], suggesting that normally the D_1 receptor exerts a negative influence on CaMKII autophosphorylation.

The elevated CaMKII autophosphorylation enhances kinase association with the NMDA receptor subunits NR_{2A} and NR_{2B} [109]. The NMDA receptor is a heteromeric complex, most often comprising two NR_1 and two NR_2 subunits (identical or different), and the subunit composition is an important determinant of the receptor's functional properties [110] as is phosphorylation of the receptor subunits [111]. Many studies report alterations in the NMDA receptor subunit abundance, composition, and phosphorylation in the DA-depleted striatum. The results are, unfortunately, inconsistent. Different studies demonstrated either a decrease in the abundance of NR_1 and/or NR_{2B} subunits in the DA-depleted striatum in rats and monkeys [109, 112–114] or no change [115]. This discrepancy likely depends, at least partially, on the purity of the synaptic membrane fraction used in different studies, since no changes are usually seen in the total homogenate fraction but the availability of NMDA subunits is selectively reduced in synaptic membranes. The basal serine and tyrosine phosphorylation of NR_1 and NR_{2B} subunits was found to be decreased [112, 116] or increased [109, 115, 117] following dopaminergic denervation. It appears that when the reduction in the abundance of subunits is accounted for, most studies find a modest net increase in phosphorylation. Inhibitors of CaMKII reduce serine phosphorylation of NR_2 subunits [109, 115]. Additionally, the synaptic concentration of PSD95 in the DA-depleted striatum is reduced [116, 118, 119], and the interaction of NMDA receptor subunits with protein components of the postsynaptic density is suppressed, leading to altered subcellular localization of NMDA receptors [116]. Although the details remain obscure, there is little doubt that dopaminergic denervation causes multiple modifications in the functions of glutamate receptors brought about by changes in the subunit composition, their phosphorylation, and/or abundance or function of other components of the postsynaptic density. These modifications result in profound deficits in synaptic plasticity in striatal neurons [109, 120, 121] that might play an important role in parkinsonian symptoms.

It is important to remember that in most cases there is no evidence that these changes occur exclusively or even predominantly in the D_1 receptor-bearing neurons. The CaMKII hyper-phosphorylation and changes in the CaMKII-NMDA receptor interaction are likely due to D_1 receptor dysfunction, suggesting that the disturbances in the NMDA receptor function are also D_1 receptor dependent. The GABAergic transmission may also be modified in striatonigral neurons, although the extent of these modifications is uncertain. The down-regulation of the vesicular GABA transporter has been reported [122], but the level of the GABA biosynthetic enzyme glutamic acid decarboxylase 67kD (GAD67) was found to be unchanged [123] or increased [124].

18.1.3.4 Changes in D_2 Receptor-Mediated Signaling

The data discussed above demonstrate the supersensitivity of the D_1 receptor-dependent signaling pathways brought about by DA depletion (Fig. 18.2). The fact that rotational behavior in hemiparkinsonian rodents can be induced by both D_1 and D_2 selective agonists [60, 118, 125–129] suggests supersensitivity of both receptor

subtypes. Similarly, the behavioral supersensitivity in DA null mice reflects the supersensitivity of both D_1 and D_2 receptors, since both D_1 and D_2 agonists induce hyperactivity, and antagonists of either receptor partially block L-DOPA-induced hyperlocomotion [76]. A comparison of the relative efficacies of D_1 and D_2 selective agonists in eliciting behavioral (or molecular) responses, although frequently made, is quite problematic, since it is difficult to match doses of the drugs, which have different pharmacokinetic and pharmacodynamic properties.

The molecular evidence for D_2 receptor supersensitivity is less impressive than that for the D_1 receptor (Fig. 18.3). In addition to the up-regulation of D_2 receptor number, the coupling of D_2 receptors to $G\alpha_i$ may be enhanced by dopaminergic denervation [77, 130], although it was found to be unchanged in another study [82]. Early studies demonstrated that selective D_2 agonists, similarly to D_1 agonists, induce a supersensitive Fos response in the DA-depleted basal ganglia but in different regions: D_1 receptor stimulation induced Fos expression in striatal neurons, whereas D_2 stimulation caused Fos expression in the globus pallidus [128, 131, 132]. The latter effect may be due to excessive disinhibition of pallidal neurons by dopaminergic stimulation via supersensitive striatal D_2 receptors. Interestingly, the supersensitive ERK1/2 response in the DA-depleted striatum can be elicited by selective activation of D_2 receptors [129, 133]. Moreover, Cai and co-workers found that the D_1 agonist SKF38393 failed to induce ERK1/2 phosphorylation [129], which is directly contradictory to the findings of other teams [18, 85, 93]. The discrepancy cannot be readily attributed to any obvious difference in methods and, thus, remains a mystery. Importantly, inhibition of ERK1/2 activation or depletion of ERK1/2 by antisense oligonucleotide reduced the frequency of quinpirole-induced rotations [129] suggesting that ERK1/2 activation is part of the mechanism of D_2 receptor supersensitivity. The molecular mechanism by which D_2 receptor stimulation activates ERK1/2 involves inhibition of mitogen-activated protein kinase phosphatase (MKP), the enzyme responsible for ERK1/2 dephosphorylation [133]. Apparently, this coupling of the D_2 receptor with MPK activity is strongly enhanced by DA depletion, since no changes were seen in the intact striatum.

The presynaptic D_2 receptors localized to corticostriatal terminals, which inhibit the release of glutamate, were shown to do so more effectively in the DA-depleted striatum [134, 135], indicating that dopaminergic denervation does induce the supersensitivity of D_2 receptors. Loss of DA results in the loss of D_2 receptor-mediated inhibitory control over corticostriatal transmission and enhanced glutamatergic activity, and the same effect is produced by the loss of D_2 receptors [16]. The loss of DA profoundly alters GABAergic transmission in the striatopallidal neurons, which suggests the involvement of D_2 receptor-mediated signaling in the control of GABAergic activity. DA depletion induces the overexpression of GAD67 and vesicular GABA transporter in striatopallidal neurons, enhanced GAD enzymatic activity, and elevated GABA release [122, 123, 136] indicative of the overactivity of the indirect striatopallidal pathway. The dopaminergic lesion up-regulates the expression of enkephalin, which is co-expressed with D_2 receptors in striatal

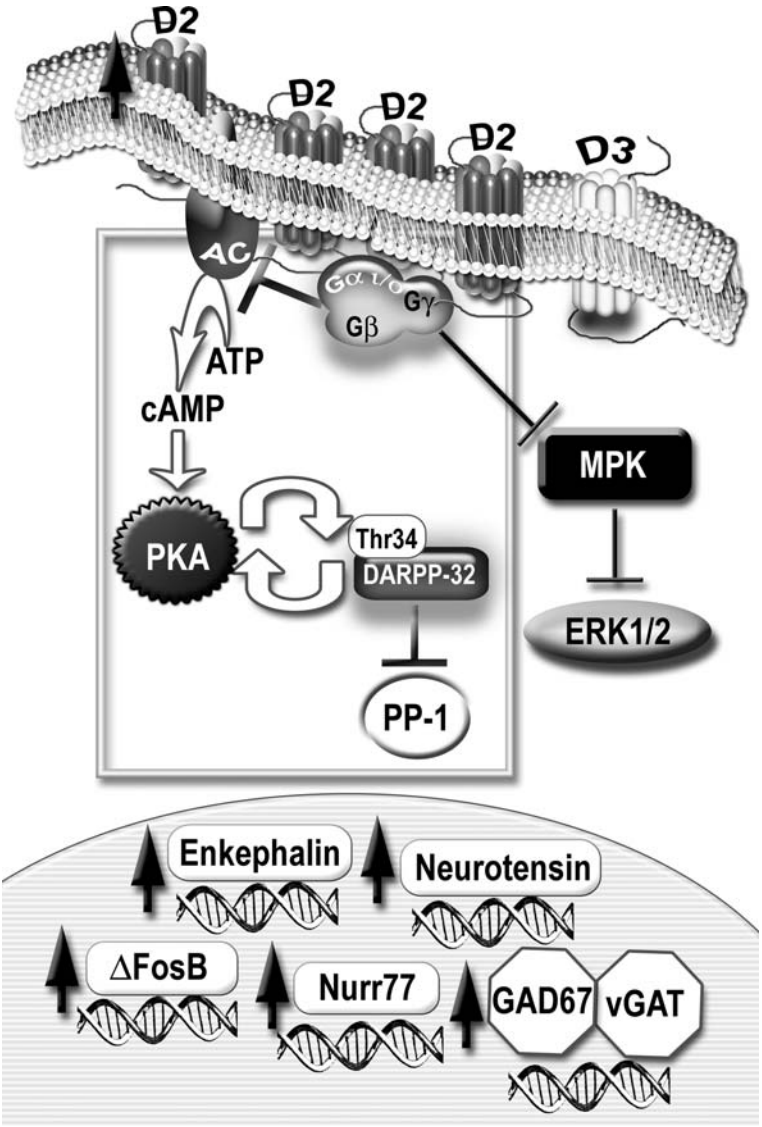

Fig. 18.3 Changes in the signaling pathways attributable to D_2 receptor activity. The loss of DA stimulation of D_2 receptors (the pathway in the box is inactive in the DA-depleted striatum) leads to constitutive upregulation of the expression of multiple genes (*short black arrows*). DA depletion enhances the coupling of the D_2 receptor to G_i. Acute stimulation of D_2 receptors with selective agonists induces supersensitive ERK1/2 activation in the DA-depleted striatum, presumably due to the D_2 receptor-mediated inhibition of the mitogen activated protein kinase phosphatase (MKP) (129)

medium spiny neurons [60, 118, 137–143]. These effects could be interpreted as a result of the removal of the inhibitory influence of the D_2 receptors.

DA depletion also increases the expression of Nurr77, an orphan member of the nuclear receptor family of transcription factors, as well as that of neurotensin in enkephalin-positive neurons [144, 145]. These effects are replicated by chronic treatment with the D_2 receptor antagonist haloperidol [146]. The data suggest that tonic stimulation of D_2 receptors in the intact striatum inhibits Nurr77 and neuropeptide expression, which is disinhibited when that stimulation is removed. The up-regulation of Nurr77 in D_2 receptor-bearing neurons after the lesion may serve as a compensatory mechanism aimed at reducing the consequences of DA loss, since Nurr77 null mice demonstrate exaggerated rotational behavior following DA depletion, accompanied by blunted enkephalin and neurotensin up-regulation [143].

It appears that D_1 receptors mediate acute responses to DA causing "positive" signals in multiple downstream signaling pathways. Consequently, the D_1 receptor supersensitivity resulting from the lack of D_1 receptor stimulation following the DA depletion manifests itself in exaggerated responses to acute dopaminergic challenge in numerous signaling pathways. At the same time, loss of D_1-mediated signaling leads to only a few changes in the basal levels of expression. The notable examples of D_1 receptor-dependent tonic regulation are the expression of dynorphin, substance P, and the D_3 DA receptor, all of which require periodic D_1 stimulation for maintenance. Conversely, D_2 receptors are more adapted for tonic inhibition of molecular pathways, and the removal of this influence results in basal up-regulation of a number of genes as well as of the corticostriatal glutamatergic and striatonigral GABAergic transmission (Fig. 18.3). The ERK1/2 activation appears to be the only example of D_2 receptor-dependent signaling supersensitivity in the DA-depleted striatum.

18.1.3.5 Possible Role of the Synergism Between D_1 and D_2 Receptors in Parkinson's Disease

One curious phenomenon, the synergism of D_1 and D_2 receptors, deserves mention here, since it may bear on the signaling mechanisms of PD. In many cases, concomitant administration of a D_1 and D_2 agonist produces much stronger behavioral or molecular effects than either drug alone. Thus, the addition of a low dose of the D_1 agonist SKF38393 potentiates rotations induced by the D_2 agonist quinpirole in hemiparkinsonian rats [128]. Similarly, quinpirole by itself induces minimal expression of Fos-like proteins in the DA-depleted caudate-putamen of 6-OHDA-lesioned rats, whereas the combined administration of quinpirole and SKF38393 induces much stronger Fos expression in striatonigral neurons than SKF38393 alone [17, 128].

The term "priming," or "reverse tolerance," is often used to describe an enhancement of a response to subsequent as compared to previous administrations of a drug. The behavioral sensitization to L-DOPA discussed above is one example of priming, and LID is another, since the probability of LID increases with each drug exposure. Usually, the term priming is applied to repeated treatment with the same drug such

as L-DOPA with administrations far enough apart to ensure washout of the previous dose. Thus, the priming is based on cumulative plastic adaptations in the brain caused by each drug dose, with the first dose applied to drug-naïve brain sensitized by loss of DA having a devastating effect [147]. Interestingly, D_1 and D_2 selective drugs can prime for each other. For example, a dose of quinpirole ineffective in a drug-naïve animal can induce robust rotations and Fos expression in striatonigral neurons if preceded by injections of the D_1/D_2 agonist apomorphine or the D_1 agonist SKF38393 [148, 149]. Interestingly, if priming is done with SKF38393 in combination with the ineffective dose of quinpirole, the result is equivalent to priming with a 10-fold higher dose of SKF38393 alone [148] illustrating the role of D_1–D_2 synergism in this "cross-priming" phenomenon. Similarly, SKF38393 alone does not induce Fos expression in the globus pallidus, but priming with quinpirole, which does, allows SKF38393 to induce Fos expression in this brain region [128]. Moreover, priming with apomorphine enhances the quinpirole-induced Fos stimulation in the globus pallidus [149], indicating that responses mediated by both D_1 and D_2 receptors are primed. The D_1–D_2 synergism and priming are likely to be mechanistically related. The synergism seen with concomitant administration of D_1 and D_2 drugs can be considered as "simultaneous" priming, since priming occurs even after a single administration of the priming agent. The D_1–D_2 synergism is inherent in the priming with L-DOPA, since DA produced from L-DOPA interacts with all DA receptors. The mechanisms of either phenomenon are unknown. The inhibition of NMDA receptors has been shown to counteract both synergism and priming, supporting an indirect mechanism dependent on glutamate release [128, 150, 151]. It is possible that D_1/D_5 and D_2/D_3 receptors co-expressed on the same neurons play a role. Alternatively, the effects could involve striatal interneurons, which are known to exert a powerful regulatory influence over striatal output neurons [26, 152].

It is important to remember that, although the striatum is the major recipient of dopaminergic projections and the site of massive alterations following DA depletion, extrastriatal areas also show changes. Thus, supersensitive activation of *c-jun* expression in the cortex of rats and monkeys unilaterally lesioned with 6-OHDA [92] suggests that the DA receptor supersensitivity is not restricted to the striatum. Similarly, the activation of the Akt pathway in the prefrontal cortex is supersensitive on the lesioned side in hemiparkinsonian rats, although the degree of supersensitivity is smaller than in the striatum [60]. The supersensitive D_1 receptor-mediated stimulation of adenylyl cyclase activity has been reported in the frontal cortex of PD patients at postmortem [84]. The functional significance of these phenomena in the clinical picture or progression of PD remains to be determined.

18.1.4 Molecular Mechanisms of the Dopamine Receptor Supersensitivity Induced by Dopaminergic Denervation

The data concerning G protein coupling and cAMP production seem to indicate that the DA receptor supersensitivity, particularly that of the D_1 receptor, involves

signaling events immediately downstream of receptor stimulation, specifically, the activation of G proteins. Thus, it is likely that many signaling pathways further downstream of the D_1 receptor-induced signaling would be supersensitive. There also might be a cumulative effect on behavior. The magnitude of supersensitivity reported for many signaling events is modest, whereas the behavioral supersensitivity is quite substantial. It is possible that minor changes amplified in multiple signaling pathways converge on behavior, producing severe changes in behavioral responsiveness to dopaminergic drugs. The supersensitivity of DA receptors caused by the loss of dopaminergic stimulation due to degeneration of dopaminergic neurons appears to be a logical and useful adaptation aimed at counteracting the declining availability of endogenous DA. However, the molecular mechanisms of this adaptation remain elusive.

Two types of mechanisms can conceivably enhance the signaling via any G protein-coupled receptor (GPCR), including all five DA receptors. Signal transduction can be facilitated by an increased number of receptors, their cognate G proteins, and/or effectors. Signaling can also be prolonged by suppressing the processes that turn it off at the receptor and/or G protein level. The mechanism that attracted much attention at first was an increase in the receptor concentration. Although the lesion-induced up-regulation of DA D_2 receptors is consistently detected in animal models and human patients [58, 60, 73, 95], the magnitude of the effect is clearly insufficient to account for the strong increase in responsiveness to dopaminergic stimulation normally observed. Moreover, D_1 receptors, the major striatal DA receptor subtype, are not increased [95], and D_3 receptors are consistently down-regulated in animals and humans [30, 58, 66, 67, 73]. Yet, the supersensitivity of D_1 receptors is consistently observed in DA-depleted animals. Besides, it is unclear how receptor number translates into the level of signaling. In most systems, the receptor itself is not rate limiting, and activation of a small proportion of the available receptor molecules is sufficient for a full functional response. In DA null mice, dopaminergic responses are strongly supersensitive but the concentrations of DA receptors in the striatum are unaltered [76], suggesting that the signaling can be effectively regulated without modifying the receptor number. Therefore, it seems unlikely that the DA receptor supersensitivity is due to changes in the receptor availability, although some contribution from the elevated number of D_2 receptors cannot be completely ruled out.

Another possible mechanism that attracted considerable attention is an increase in the availability of signaling molecules downstream of the receptors, particularly G proteins. A substantial increase in the availability of G proteins should enhance G protein-mediated signaling. An increase in the concentration of $G\alpha_{olf}$ and $G\gamma_7$ was detected in the putamen of human PD patients [78] and hemiparkinsonian rats [78, 153]. The D_1 receptor-mediated production of cAMP is absent in $G\alpha_{olf}$ knockout mice [154], indicating that the $G\alpha_{olf}$ isoform is required for D_1 receptor signaling in the neostriatum. $G\alpha_{olf}$ levels are modulated by DA via D_1 receptors; the $G\alpha_{olf}$ concentration is elevated in D_1 receptor knockout mice and decreased in DA transporter knockout mice, which have elevated extracellular DA [155]. Thus, loss of DA in the parkinsonian brain should be expected to elevate the level of $G\alpha_{olf}$. In rats and

humans, the magnitude of the increase is 50–60% [153]. Is this sufficient to produce the 4- to 5-fold greater behavioral response seen in DA null mice [76]? Hemizygous $G\alpha_{olf}$ mice that have approximately half of the normal level of $G\alpha_{olf}$ show a 2- to 3-fold lower locomotor response to amphetamine than wild-type mice [155], which suggests that a 50% reduction at the G protein level is translated into behavioral effects of similar magnitude. The elevation of the $G\alpha_{olf}$ concentration seems attractive as the mechanism of the D_1 receptor supersensitivity, since it is regulated by the receptor usage in the correct manner, and changes in the $G\alpha_{olf}$ availability have been shown to have signaling and behavioral consequences. However, another study found increased DA receptor coupling to their cognate G proteins in the denervated striatum without changes in the G protein levels [77]. No changes in the expression of other signaling molecules such as PKA, protein kinase C (PKC), CaMKII, or DARPP-32 in the denervated striatum have been reported [91, 156], which leave $G\alpha_{olf}$ as the only candidate.

Enhanced signaling via GPCRs such as DA receptors can also be achieved by suppressing the desensitization process. Upon activation by a ligand, GPCRs are phosphorylated by members of the G protein-coupled receptor kinase (GRK) family, and active phosphorylated receptors serve as preferred substrates for the binding of the uncoupling proteins arrestins (reviewed in [157]). Arrestin binding shields the cytoplasmic surface of the receptor and precludes further G protein activation. The receptors then need to be resensitized, which occurs in the endosomes upon receptor internalization. In the endosomes, the ligand dissociates, apparently causing conformational changes in the receptor protein resulting in the dissociation of arrestin. The receptor is then dephosphorylated and recycled back to the plasma membrane. If the desensitization process is suppressed or delayed, it would prolong the existence of the receptor state competent to activate G proteins and effectively enhance signaling.

A simple way to inhibit desensitization would be a reduction of the availability of arrestins and especially GRKs, since the phosphorylation step is rate limiting in the process [158, 159]. It has been demonstrated in many systems that a reduction in the availability of arrestins and GRKs inhibits receptor desensitization and facilitates GPCR signaling in cultured cells and live animals [160–167]. Specifically, the loss of the GRK6 isoform confers dopaminergic supersensitivity [163]. Analysis of the expression levels of GRK and arrestin isoforms in the hemiparkinsonian rat model demonstrated down-regulation of GRKs, but not arrestins, in the caudal striatum [118]. However, the GRK concentrations were unchanged or increased in the rostral subdivisions of the striatum. At the same time, the DA receptor supersensitivity, as measured by the responsiveness of downstream signaling pathways such as ERK and Akt, was just as strong in the rostral as in the caudal striatum [60]. Thus, the anatomical pattern of the GRK down-regulation does not match that of the DA receptor super-responsiveness, making it unlikely that the former is the sole cause of the latter. Moreover, GRK6 and arrestin2 were increased, rather than decreased, in the striatum of MPTP-lesioned monkeys [96]. No significant decrease in the concentrations of arrestins or GRKs was found in the striatum of patients with PD at postmortem [72], but it is important to remember that all patients had been treated

antemortem with dopaminergic drugs. Taken together, these data suggest that down-regulation of the components of the GPCR desensitization machinery is unlikely to be solely responsible for the DA receptor supersensitivity brought about by the loss of DA.

The arrestin- and GRK-mediated desensitization mechanism operates on many GPCRs regardless of the G proteins they couple to [157]. The $G\alpha_i$- or $G\alpha_q$-coupled GPCRs have alternative negative regulatory mechanisms responsible for the termination of signaling at the level of the G protein. Active GTP-liganded G protein α-subunits self-inactivate due to their intrinsic GTPase activity. The regulators of G protein signaling (RGS) proteins are GTPase-accelerating proteins (GAPs) that negatively modulate GPCR signaling by accelerating GTP hydrolysis by Gα subunits [168, 169]. Most RGS proteins act as GAPs toward the $G\alpha_{i/o}$ and some toward the $G\alpha_{q/11}$ subfamilies of G proteins, whereas no RGS for $G\alpha_s$ or $G\alpha_{12}$ has been described [168]. Thus, in the striatum RGS proteins could directly regulate signaling via D_2 or D_3 receptors. Similarly to the arrestin- and GRK-mediated desensitization discussed above, attenuation of the RGS-mediated signal termination should lead to receptor supersensitivity. Up-regulation of RGS proteins would increase RGS availability and promote RGS-mediated attenuation of signaling, whereas a reduced concentration of RGS proteins should be expected to suppress RGS-dependent turnoff and prolong signaling.

Multiple RGS isoforms have been identified with several subtypes (RGS4, 7, 8, and 9) highly expressed in the brain [170]. The splice variant of RGS9, RGS9-2, is enriched in striatal medium spiny neurons [171, 172]. Overexpression of RGS9-2 reduces D_2 receptor-mediated signaling in vitro [171, 173]. RGS9-2 expression is regulated by DA since chronic exposure to cocaine increases the level of the protein in the striatum [174]. The administration of L-DOPA or D_2 antagonists, or DA depletion, alters the expression level of multiple RGS isoforms in the striatum in an isoform- and cell-specific manner [26, 175, 176]. In particular, RGS9 mRNA is decreased following DA depletion by 6-OHDA lesion or reserpine treatment [176]. Mice lacking RGS9-2 are more sensitive to dopaminergic stimulation [173, 174]. Conversely, viral overexpression of RGS9-2 reduces locomotor responses to D_2, but not to D_1, agonists [174]. Thus, the modulation of RGS9-2 availability by DA depletion appears to be a good candidate mechanism for the supersensitivity of D_2 receptors. However, no changes in the expression of RGS9-2 were found in the MPTP monkey model of PD [177]. RGS9 was up-regulated in the striatum of PD patients at postmortem [178], but this could be the result of long-term dopaminergic treatment. Thus, we must conclude that if the RGS-mediated modulation of the D_2 receptor signaling is involved in the supersensitivity of D_2 receptors caused by DA depletion, it does not require changes in the RGS9-2 expression level.

To summarize, multiple alterations in striatal signaling mechanisms caused by the loss of DA have been described (Fig. 18.2 and 18.3). However, so far no coherent model of the molecular mechanisms responsible for the lesion-induced supersensitivity of DA receptors has been developed. The up-regulation of G proteins, leading to enhanced cAMP production, seems the most promising mechanism

for the D_1 receptor supersensitivity. This augmentation of the signal transduction at the G protein level can then be propagated to other cAMP-dependent mechanisms and, via various feedback loops, to cAMP-independent mechanisms, finally resulting in strong enhancement of behavioral responses to dopaminergic drugs. However, no analogous mechanism has been proposed for the D_2 receptor. It is equally unclear what molecular mechanism mediates the changes in the transcription of proteins caused by dopaminergic denervation. Changes in the mechanism of transcriptional control have been observed in the DA-depleted striatum. The up-regulation of specific ΔFosB proteins (43 and 45 kD) is induced by DA depletion in striatopallidal neurons, resulting in a corresponding increase in AP-1-binding activity [179], suggesting that these transcription factors may mediate some of the changes in gene expression observed in striatopallidal neurons following the loss of DA. Since the same Fos proteins are selectively elevated by chronic administration of the selective D_2 antagonist haloperidol [180], this up-regulation appears to be mediated by the reduced activity of D_2 receptors. Paradoxically, no change in transcriptional activity has so far been found in nigrostriatal D_2 receptor-bearing neurons.

The success of studies aimed at identifying the molecular mechanisms of signaling alterations caused by DA depletion will be largely determined by the sensitivity and spatial resolution of experimental methods. A global measurement of the expression of any signaling protein in the whole striatum could easily miss a dramatic change in a small subpopulation of striatal neurons. However, the detection of changes restricted to specific neuronal populations is challenging. Neurons are highly compartmentalized cells with elaborate geometry, where a significant change in the local concentration of a particular protein could be achieved without an overall change of expression. It is evident that subcellular localization of the proteins, their intracellular trafficking, and dynamic recruitment to specific cellular compartments have high functional significance, and yet all these events are difficult to observe experimentally. Finally, simultaneous modest increases of the receptor, G protein, and effector expression (each being below the level of reliable detection) in conjunction with a moderate decrease in the rates of signal termination at the receptor and G protein level could yield a dramatic manifold increase in signaling.

18.2 DA Receptors and Treatment of the Motor Symptoms of Parkinson's Disease

18.2.1 Dopamine Replacement Therapy and the Pathophysiology of L*-DOPA-Induced Dyskinesia*

Patients with PD are treated with the dopamine precursor L-DOPA with the goal of restoring lost DA. L-DOPA is normally produced from the amino acid tyrosine via hydroxylation by the enzyme tyrosine hydroxylase in dopaminergic and noradrenergic neurons. Exogenous L-DOPA bypasses this step and is converted to DA

by L-aromatic acid decarboxylase. L-DOPA was first introduced as a treatment for PD in the early 1960s [181], but remains the best available drug even today. L-DOPA is normally administered in combination with peripheral inhibitors of DOPA decarboxylase such as carbidopa or benzerazide to prevent peripheral conversion of L-DOPA to DA. In the brain, L-DOPA is converted into DA, and the newly produced DA acts at DA receptors, thereby restoring the balance within the striatal circuitry upset by the loss of endogenous DA. Acutely administered L-DOPA quickly restores movement in human PD patients and experimental animals. L-DOPA is a short-lived drug (half-life ~90 min). Therefore, repeated administration of L-DOPA results in surges of newly synthesized DA, stimulating DA receptors in a non-physiological pulsatile manner. In PD patients at early stages of the disease, the antiparkinsonian effect of acute L-DOPA lasts substantially longer than the L-DOPA life in the body, probably due to accumulation of DA in the synaptic vesicles of the surviving dopaminergic neurons. This storage process likely smoothes out the DA surges, producing a more physiological pattern of dopaminergic stimulation. L-DOPA therapy is remarkably efficacious at early stages of PD, but becomes less and less so as the disease progresses, necessitating higher L-DOPA doses to achieve adequate antiparkinsonian effect [182]. This is probably due to the continuing degeneration of dopaminergic neurons in PD patients, which increases the demand for DA and reduces the storage capacity for DA produced from exogenous L-DOPA. Moreover, multiple motor complications emerge within 5–7 years of L-DOPA treatment, such as motor fluctuations (on-off fluctuations, wearing off, delayed or no "on" response) and dyskinesia [182]. Since DA acts at all DA receptors, L-DOPA behaves as a non-selective agonist acting primarily through the most abundant D_1, D_2, and D_3 striatal receptors. It is important to bear in mind that an antiparkinsonian effect is produced by selective D_1 as well as D_2/D_3 agonists in humans and animals [183–188], although L-DOPA remains the most effective antiparkinsonian therapy [189].

L-DOPA-induced dyskinesia (LID) is a debilitating condition characterized by involuntary purposeless movements involving the limbs, trunk, or face. The most common forms of LID are peak-dose dyskinesia, diphasic dyskinesia, and off-period dystonia. Peak-dose dyskinesia occurs at the peak plasma level of L-DOPA, diphasic dyskinesia correlates with the rise and fall of the L-DOPA plasma level, and off-period dystonia correlates with the akinesia that precedes the L-DOPA effect [190]. These forms are not mutually exclusive and can occur in the same patient. Since peak-dose dyskinesia is the easiest to reproduce in animal models, most of the mechanistic data available apply to this form of LID. LID poses a significant clinical challenge, since, as the disease progresses, it becomes increasingly difficult or impossible to produce adequate antiparkinsonian effects without triggering dyskinesia [191].

The neural mechanisms of LID remain obscure. The pathophysiological model of PD posits that akinesia is the result of excessive inhibition of the cortico-thalamic loop by the striatum. DA depletion shifts the balance within the striatal circuitry in favor of the indirect pathway, which is a negative feedback mechanism, causing hyperactivity of the striatal output nuclei and enhanced net inhibitory striatal output

to the thalamus [1]. L-DOPA produces its antiparkinsonian effect via restoration of normal dopaminergic control over the striatal circuitry, which reduces the overall inhibitory striatal output and relieves the inhibition of the motor cortical activity, making movement possible. At first, based on studies of hyperkinetic disorders such as Huntington's disease and ballism, LID was considered a simple reversal of the parkinsonian condition; strong stimulation of DA receptors by DA produced from L-DOPA reduces the striatal output too much by facilitating transmission via the direct pathway at the expense of the indirect pathway [192, 193]. The lack of proper striatal inhibitory control disinhibits the motor cortex, causing hyperactivation and dyskinetic movements. Whereas the model of PD is well supported by the experimental data and is remarkably successful as a heuristic tool, the model of LID is much less satisfactory in this regard. The experimental support for the key prediction of the model, hypoactivity of the gateway structure of the striatal circuit, the globus pallidus internal, was never strong. The final blow to the model was dealt by the discovery that the ablation of the globus pallidus internal alleviates both parkinsonian symptoms (where it is hyperactive) and dyskinesia, where this nucleus, according to the model, is supposed to be hypoactive [190]. This result suggests that the difference between the parkinsonian and dyskinetic states is not a simple opposition between high (parkinsonian) and low (dyskinetic) output of the striatal circuit, but likely involves complex alterations of activity patterns throughout the striatal system.

18.2.2 The Effects of L-DOPA Treatment on Dopaminergic Signaling

The evidence seems to indicate that in the normal striatum DA serves to dampen signaling and behavioral responses to itself via some sort of desensitization mechanism. When this endogenous check is removed in PD, the responsiveness greatly increases. Can this supersensitivity be reversed by the application of dopaminergic stimulation to mimic the action of endogenous DA? Most importantly, does L-DOPA suppress supersensitive responses? The answer is yes and no.

18.2.2.1 Signaling Consequences of Dopamine Depletion Normalized by L-DOPA

L-DOPA certainly suppresses the supersensitivity of some signaling pathways (Fig. 18.4). In human PD patients and animal models of PD, L-DOPA reverses the denervation-induced up-regulation of D_2 receptors [47, 51, 52, 54, 60–62], supporting the explanation that this up-regulation is caused by the loss of DA. In DA null mice, chronic L-DOPA treatment eliminates exaggerated locomotor, *c-fos*, and ERK responses [76, 194]. L-DOPA also reverses the increase in the $G\alpha_{olf}$ concentration in hemiparkinsonian rats [78]. Similarly, the supersensitivity of the ERK pathway is reversed by L-DOPA in both 6-OHDA-lesioned rats and MPTP-lesioned monkeys [60, 96], as is the exaggerated basal phosphorylation of CaMKII [91, 109]

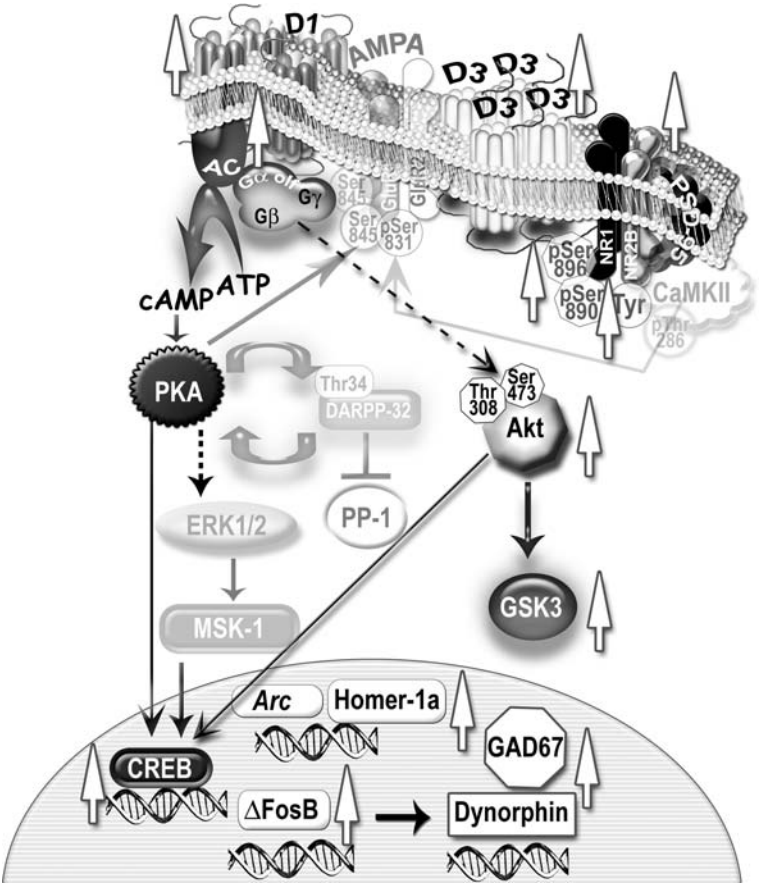

Fig. 18.4 Signaling mechanisms of L-DOPA-induced dyskinesia. Most proposed mechanisms of LID pertain to D_1 receptor-mediated signaling. Chronic L-DOPA treatment reverses the denervation-induced supersensitivity of several signaling pathways (shown *dimmed*). *Short open arrows* facing up indicate the L-DOPA-induced augmentation of the expression or activity/phosphorylation of proteins beyond the pre-lesion level. The open arrow between adenylyl cyclase (AC) and $G\alpha_{olf}$ indicates increased coupling of D_1 receptors to G_{olf}. Note that increased Akt-mediated phosphorylation of GSK3 translates into decreased activity of the enzyme. *Solid arrows* denote known stimulatory connections in signaling pathways; *dashed arrows* indicate hypothetical connections

and DARPP-32 at Thr75 [91]. The lesion-induced super-responsiveness of many IEGs (*c-fos*, *junB*, *junD*, *c-Jun*) is reversed or at least significantly ameliorated by chronic L-DOPA administration [100, 101, 180]. L-DOPA also reverses the supersensitivity of presynaptic D_2 receptors on corticostriatal terminals and the resulting glutamatergic hyperactivity in the striatum [135]. However, the exact functional role of the signaling changes observed in the DA-deprived striatum in the motor deficits caused by the loss of DA is unclear. Equally, it is not known whether the

reversal of these changes by L-DOPA plays a role in its therapeutic activity. The hyper-phosphorylation of CaMKII seems to be involved in the lesion-induced motor abnormalities, since intrastriatal administration of a selective CaMKII inhibitor rescues the motor performance in 6-OHDA-leisoned rats [109], thus mimicking the action of L-DOPA. It is possible that other effects are also part of the molecular mechanisms responsible for the therapeutic antiparkinsonian activity of L-DOPA, but their exact roles remain to be determined.

18.2.2.2 Molecular Consequences of Dopamine Depletion Unchanged or Augmented by L-DOPA

L-DOPA does not reverse the up-regulation of enkephalin induced by the dopaminergic lesion in hemiparkinsonian rats and MPTP-lesioned monkeys [60, 137, 139–141, 195, 196], and may even enhance enkephalin expression in the 6-OHDA-lesioned rat, MPTP-lesioned monkey, and human dyskinetic PD patients [138, 141, 197]. Neither the L-DOPA nor the long-lived DA agonist pergolide is successful in reversing the effect of DA depletion on the expression of arrestins and GRKs in the 6-OHDA-lesioned rat [118]. On the other hand, in MPTP-lesioned monkeys L-DOPA actually reverses the up-regulation of arrestin2 and GRK6 caused by the lesion [96]. Apparently, some alterations in signaling mechanisms induced by the loss of DA are permanent and, once developed, cannot be reversed by DA. It is also possible that the dose, duration of treatment, or administration mode used is inadequate to produce the reversal in some cases.

It stands to reason that the molecular aberrations caused by DA loss that are not reversed by the therapeutically active mode of L-DOPA administration likely underlie L-DOPA-induced motor fluctuations. Importantly, L-DOPA exaggerates some of the abnormalities in dopaminergic signaling in the striatum caused by DA depletion and/or induces de novo signaling perturbations beyond those already present due to DA depletion (Fig. 18.4). Acute L-DOPA administration induces contralateral rotations in hemiparkinsonian rats. Chronic L-DOPA treatment leads to a progressive increase in the frequency of the rotations [60, 66, 67, 118, 144], the phenomenon termed behavioral sensitization to L-DOPA.

At the molecular level, the down-regulation of D_3 receptors and dynorphin caused by DA depletion in hemiparkinsonian rats are reversible by L-DOPA, but in the course of chronic treatment both increase substantially beyond the pre-lesion level [60, 65–67, 137, 198]. Chronic L-DOPA also aggravates the lesion-induced hyper-phosphorylation of CREB in response to dopaminergic challenge [105]. Our data also demonstrate that the Akt-GSK3 pathway in the lesioned rat striatum is supersensitive to dopaminergic stimulation but shows little or no change in basal activity [60]. Chronic L-DOPA enhances the basal constitutive activity of the pathway to a level that is even higher than that induced by acute dopaminergic stimulation in drug-naïve hemiparkinsonian rats, and this level is no longer increased by dopaminergic stimulation. In the normal striatum, the Akt-GSK3 pathway is under the negative influence of DA acting via D_2 and D_3 receptors [199, 200]. It appears that the loss of DA brings forward a different, positive, mode of Akt regulation by

DA, which may be D_1 receptor mediated. $G\alpha_q$ and $G\alpha_s$-coupled receptors can activate Akt via Ca^{2+} or other second messengers [201]. The D_1 receptor can couple to $G\alpha_{q/11}$ [202]. DA can activate Akt via D_1 receptors in a phosphoinositide-3-kinase-independent manner, possibly involving ERK1/2. Loss of Akt activation impairs phosphorylation of CREB [203]. Thus, in the lesioned and L-DOPA-treated striatum, persistent Akt activation may contribute to the persistent phosphorylation of CREB.

L-DOPA-induced perturbation of the glutamatergic and GABAergic transmission may play a role in LID. L-DOPA treatment increases the expression of GAD67 and vesicular GABA transporter in striatonigral neurons and the release of GABA in the substantia nigra reticulata [122, 124, 204, 205], indicative of hyperactivity of the direct striatonigral pathway. L-DOPA reverses the down-regulation of NMDA receptor subunits seen in parkinsonian monkeys and causes excessive expression of selected subunits in dyskinetic monkeys and PD patients [113, 206, 207]. L-DOPA also causes exaggerated tyrosine phosphorylation of NR_2 [112, 115, 117] and serine phosphorylation of NR_1 [112] subunits that correlate with L-DOPA-induced sensitization. The D_1 receptor regulates the subcellular trafficking of the NMDA receptor, and its activation rapidly recruits the NMDA receptor, with the NR_{2A} and NR_{2B} subunits phosphorylated at tyrosines, to the synaptic membrane [208–210]. Loss of protein kinase Fyn abolishes tyrosine phosphorylation, the D_1 receptor-mediated membrane recruitment of the NMDA receptor, and behavioral sensitization to L-DOPA (the interpretation of that result is equivocal because of less extensive DA depletion in Fyn null mice) [209]. Interestingly, the NR_2 subunit tyrosine phosphorylation is DARPP-32 independent [209]. Thus, tyrosine phosphorylation of the NMDA receptor subunits and D_1-induced synaptic recruitment may play an important role in the development of LID.

18.2.2.3 Effects of L-DOPA in "Dyskinetic" Versus "Non-dyskinetic" Animal

The data cited above are based on effects detected in the entire population of animals treated chronically with L-DOPA. The importance of these signaling effects for LID is supported by their development over time in parallel with behavioral sensitization to L-DOPA. However, when people or animals are treated with L-DOPA, their response to the drug varies substantially. Between 40 and 80% of PD patients, according to different estimates, develop dyskinesia within 5 years of treatment, but some develop it earlier and some later [211]. Similarly, MPTP-lesioned monkeys treated with L-DOPA develop dyskinesia at individual rates [30, 96]. Hemiparkinsonian rats demonstrate different rotation frequencies following the first L-DOPA administration and the individual propensity for behavioral sensitization to L-DOPA varies widely [125, 212]. In addition to rotations, hemiparkinsonian rats show abnormal involuntary movements (AIMs) of the limbs, tongue, jaw, and trunk that have been proposed to model human LID with better fidelity than behavioral sensitization [213]. Rats show remarkably different propensities to develop AIMs in response to L-DOPA. Thus, parkinsonian animals, rodents and primates, can be classified into "dyskinetic" and "non-dyskinetic" categories based on the presence

of AIMs. It should be noted that, since the probability of dyskinesia is roughly proportional to the L-DOPA dose and duration of treatment (cumulative dose) [125], dyskinesia is inducible in "non-dyskinetic" animals by a higher dose or longer treatment. Therefore, this classification simply describes the situation at a given moment in the treatment schedule. Nonetheless, to elucidate the molecular mechanisms underlying dyskinesia, it is still important to explore signaling alterations that are present in overtly dyskinetic individuals, but absent in non-dyskinetic subjects (Fig. 18.4). Another possible approach would be to correlate the severity of dyskinesia (e.g., AIMs scores) with the level of expression or activation of signaling proteins and/or pathways.

Importantly, in some studies the distinction between "dyskinetic" and "non-dyskinetic" animals has no apparent correlation with the degree of DA depletion [214–216]. However, since experimental animals in those studies usually had extensive loss of DA, it is possible that above a certain level, individual differences in the degree of DA depletion no longer matter. It is a well-established fact that in human PD patients the risk of dyskinesia increases with the disease severity [217, 218]. When a wider range of DA depletion is considered in animal models, it is also found that the propensity to LID is proportionate to the degree of DA loss [216, 219].

Using this experimental approach, it has been determined that the level of D_1 receptor supersensitivity (measured by D_1 agonist-stimulated GTPγS binging to striatal sections) induced by loss of DA is further enhanced in dyskinetic MPTP-lesioned monkeys as compared to L-DOPA-naïve MPTP monkeys, whereas in non-dyskinetic animals L-DOPA reverses the supersensitivity [95]. The level of D_1 receptor supersensitivity in these animals correlates positively with the severity of LID and is accompanied by increased expression of DARPP-32 and cdk5 [95]. In dyskinetic monkeys, the expression of the D_3 DA receptor is dramatically increased, whereas the receptor was down-regulated by the MPTP lesion and restored to the normal level in non-dyskinetic animals [30]. Moreover, a partial D_3 agonist significantly ameliorates dyskinesia without reducing the antiparkinsonian effect of L-DOPA. These data suggest that in some individuals (eventually in all) the supersensitivity of DA receptors is not reversed or is even aggravated by L-DOPA and that leads to LID.

Supersensitive DA receptors convey signaling to downstream signaling pathways that are also selectively enhanced in "dyskinetic" animals (Fig. 18.4). The up-regulation of $G\alpha_{olf}$ is reversed by L-DOPA in hemiparkinsonian rats, but it is detectable in human PD patients in spite of decades of dopaminergic therapy, with the highest levels seen in patients with severe antemortem dyskinesia [78]. Although hyper-phosphorylation of DARPP-32 by PKA (at Thr34) is generally reduced by chronic L-DOPA treatment, it persists in 6-OHDA-lesioned mice and rats with a high frequency of AIMs [85, 120]. Moreover, the level of DARPP-32 phosphorylation positively correlates with the AIMs scores. Conversely, AIMs are attenuated in DARPP-32 knockout mice [85]. These data suggest that the failure of L-DOPA to suppress the DARPP-32 hyper-phosphorylation induced by DA loss contributes substantially to the induction of LID, possibly by virtue of maintaining the lesion-induced supersensitivity of D_1 receptors.

Hyper-phosphorylation of ERK1/2 proportional to the AIM frequency was observed in "dyskinetic" mice [85, 220] and rats [93]. The suppression of AIMs in DARPP-32 knockout mice was accompanied by reduced phosphorylation of ERK1/2 [85], suggesting that ERK1/2 activation is PKA mediated. Furthermore, direct blockade of ERK1/2 phosphorylation also reduced AIMs [85]. However, we found that the elevated level of ERK1/2 phosphorylation and expression was reversed by L-DOPA treatment in both "dyskinetic" and "non-dyskinetic" MPTP-lesioned monkeys [96]. We also found that both the L-DOPA and the D_2 agonist pergolide suppress the lesion-induced supersensitive ERK1/2 response equally well, in spite of their different ability to produce behavioral sensitization [60]. Lesion-induced supersensitive phosphorylation of the $GluR_1$ subunit of the AMPA receptor is also attenuated by chronic L-DOPA to a lesser degree in "dyskinetic" versus "non-dyskinetic" hemiparkinsonian rats [85]. These data implicate the enhanced responsiveness of the D_1 receptor-PKA pathway in LID (Fig. 18.4).

L-DOPA-induced abnormalities in NMDA receptor-mediated transmission develop (or, at least, appear to be more severe) in dyskinetic animals. Dyskinetic animals have higher synaptic availability of the NR_{2A} [113, 116] and lower availability of the NR_{2B} subunit than L-DOPA-treated non-dyskinetic animals [114, 116]. Since the D_1 receptor has been implicated in the control of NMDA receptor trafficking [209, 210], these changes are likely mediated by overactive D_1 signaling. The importance of mislocalization of the NR_{2B} subunit for dyskinesia is highlighted by the fact that artificial disruption of the NR_{2B} interaction with proteins in the postsynaptic density induces AIMs in non-dyskinetic rats [116]. The redistribution of NMDA receptors between the synaptic and the extrasynaptic compartments, in addition to changes in the subunit composition, may have important functional consequences. The signaling of synaptic and extrasynaptic NMDA receptors is different; calcium entry through synaptic NMDA receptors enhances CREB activation and CREB-dependent gene expression, whereas activation of extrasynaptic NMDA receptors suppresses CREB activity [221]. The exact nature and functional consequences of modifications in the expression, composition, trafficking, and signaling of NMDA receptors in LID are still poorly understood. NMDA antagonists generally ameliorate LID, and the noncompetitive NMDA antagonist amantadine is the only drug proven effective against LID in clinical practice [190]. This suggests that elevated activity of NMDA receptors, possibly the NR_{2B}-containing receptors in the "wrong" non-synaptic compartments, induced by intermittent stimulation of D_1 receptors may be critical for LID development.

18.2.2.4 Effects of L-DOPA on Immediate Early Genes and Transcription Factors

The supersensitive activation in the DA-depleted striatum of some members of the Fos and Jun gene families such as c-Fos, JunB, c-Jun, and JunD is reduced by chronic treatment with L-DOPA [100–102, 183], although it takes a fairly prolonged treatment to substantially downregulate the response. For example, 5-day

treatment with 10 mg/kg/day of L-DOPA does not perceptibly reduce the supersensitive response in the DA-depleted striatum in unilaterally 6-OHDA-lesioned rats or common marmosets [97].

Some members of the Fos family are longer lived than immediate early gene types such as c-Fos. The 35 and 37 kDa isoforms of the ΔFosB protein are not induced or are induced only slightly by acute stimulation but slowly accumulate in response to chronic stimuli and persist in the brain longer than other Fos proteins (weeks to months) [180]. These proteins are induced by an incredible variety of stimuli, including drugs of abuse, antipsychotics, some forms of antidepressant treatment, stress, etc. [180]. The expression of ΔFosB isoforms is induced in the DA-depleted striatum by acute and is further promoted by chronic treatment with L-DOPA, with the 35 and 37 kDa isoforms being the most affected by chronic L-DOPA [93, 101, 102, 179, 212, 220]. Moreover, ΔFosB proteins are found in the striatum of PD patients at postmortem and in MPTP-lesioned monkeys, and the levels of expression correlate with the degree of DA depletion and symptoms of dyskinesia [178, 179]. The severity of AIMs in the rodent model of PD correlates with the ΔFosB expression [222]. The inhibition of ΔFosB accumulation by the infusion of ΔFosB antisense oligonucleotides into the striatum reduces the severity of dyskinesia in hemiparkinsonian rats [222, 223]. The accumulation of ΔFosB occurs in dynorphin-expressing striatonigral neurons, and overexpression of dynorphin upon chronic L-DOPA treatment correlates well with dyskinesia scores [205, 222]. The DARPP-32-based amplification loop may be involved in the ΔFosB accumulation, since removal of DARPP-32 attenuates the ΔFosB buildup induced by dopaminergic stimulation [224, 225].

The accumulation of ΔFosB during L-DOPA treatment may contribute to changes in the expression of proteins controlled by ΔFosB-responsive promoters [180]. In particular, ΔFosB regulates the expression of dynorphin [223], and dynorphin overexpression induced by L-DOPA in the lesioned striatum correlates well with indices of LID [60, 205, 212, 223]. However, the exact functional role of the ΔFosB accumulation in LID remains unclear. From the data on LID, it seems that the ΔFosB-mediated transcription acts as the positive feedback in the L-DOPA signaling. At the same time, ΔFosB knockout mice have no cocaine-induced accumulation of the ΔFosB 35 and 37 kDa isoforms, but demonstrate enhanced responsiveness to cocaine (place preference, locomotion), which suggests that ΔFosB accumulation functions as a *negative*, rather than positive, feedback in the dopaminergic signaling [226]. However, virus-mediated overexpression of ΔFosB in the nucleus accumbens sensitizes the animals to the locomotor and rewarding effects of cocaine [227]. To reconcile these data, it has been suggested that ΔFosB, which is induced by chronic cocaine treatment, is responsible for the long-term increase in sensitivity [227], whereas loss of ΔFosB results in a short-term increase in sensitivity but precludes sensitization to repeated treatment.

The L-DOPA-induced elevation of the expression of *a*ctivity *r*egulated *c*ytoskeletal-associated (*Arc*) and *Homer-1a* genes shows a very similar pattern to that of dynorphin, being more prominent in dyskinetic than in non-dyskinetic rats [212]. The basal level of expression of these genes was not altered by the

DA depletion but increased with L-DOPA treatment, more substantially so in rats that developed AIMs. The up-regulation of *Arc* is largely restricted to dynorphin-expressing neurons, suggesting that this is a downstream effect of the D1 receptor activation. The exact function of the protein product of the *Arc* gene, Arc (also referred to as *a*ctivity *r*egulated *g*ene 3.1 protein homolog, Arg3.1), is incompletely understood, but its enrichment in dendrites and recruitment to dendritic synaptic regions upon stimulation [228] suggests its involvement in synaptic plasticity. Recently, the Arc protein has been reported to regulate trafficking of the AMPA glutamate receptor by promoting its activity-dependent endocytosis [228, 229]. Overexpression of Arc reduces the AMPA receptor surface expression and AMPA-dependent synaptic transmission, whereas Arc knockout enhanced both [230, 231]. Thus, the up-regulation of Arc in dyskinetic animals may actually promote the reduction of AMPA activity, which is enhanced in LID, as evidenced by the antidyskinetic efficacy of AMPA antagonists [190, 232].

Homer proteins are part of postsynaptic densities and are involved in regulating the functions of metabotropic glutamate receptors [233]. The short Homer-1a isoform is rapidly induced by neuronal stimulation and acts as a negative regulator of the signaling via $mGluR_{1/5}$ metabotropic glutamate receptors [233, 234]. Transgenic mice expressing Homer-1a in striatal neurons are akinetic with impaired performance in motor tasks [235], which is similar to the parkinsonian phenotype. Interestingly, mice that express Homer-1a in the striosome striatal compartment (but not in the matrix) show behavioral and molecular supersensitivity to amphetamine [235], which is consistent with peak-dose dyskinesia. The striosome–matrix organization of the striatum has been identified based on differential staining with neurochemical markers and connectivity [236]. The striosomes (patches) have high density of μ-opioid receptor but low calbindin immunoreactivity and receive dopaminergic projections mostly from the dorsal tier of the substantia nigra pars compacta. In contrast, matrix is innervated largely by the ventral tier [236, 237], although this innervation topography is less evident in primates than in rodents [238]. The functional role of the striosome-matrix dichotomy in PD and LID remains almost completely unexplored. Interestingly, apomorphine- or L-DOPA-induced expression of c-Fos and FosB in the DA-depleted striatum, which is uniform after acute injection, concentrates in the striosomes upon chronic administration [101, 239]. Thus, this limited evidence points to a special importance of the striosomal compartment in LID.

Such paradoxical effects of L-DOPA of the type that upset the balance in DA receptor signaling instead of restoring it may be responsible for the L-DOPA side effects, including LID. Interestingly, in DA null mice, even low concentrations of L-DOPA restore normal feeding behavior and suppress supersensitive behavioral and molecular responses without noticeable paradoxical effects. The difference between these mice and parkinsonian animals or humans is that the mice have intact dopaminergic neurons and terminals that are capable of packaging and releasing DA in the appropriately regulated manner. In contrast, in the parkinsonian brain, dopaminergic neurons are reduced in number or are no longer present, and complete, functionally appropriate restoration of the DA signaling is not possible.

18.2.3 Dopamine Agonists in the Treatment of Parkinson's Disease

18.2.3.1 Dyskinesia-Inducing Properties of DA Agonists

It has been established that both D_1- and D_2/D_3-selective agonists produce antiparkinsonian effects in experimental animals and human patients [184, 185, 240–242]. Similarly, both classes of drugs produce dyskinesia, although their propensity to do so is a complex function of the pharmacological profile and pharmacokinetic properties. One of the important features of L-DOPA is its short life (90 min half-life in the body), resulting in rapid changes in the DA concentration in the DA-depleted striatum following L-DOPA administration. Short-lived nonselective DA and selective D_1 or D_2/D_3 agonists induce LID in humans and motor complications in animals [184, 186, 243]. Whether DA agonists have the same dyskinetic potential as L-DOPA is a matter of some controversy. Several studies reported that in therapeutically effective doses short-lived DA agonists induce the same level of dyskinesia as L-DOPA [184, 185, 244], whereas others found DA agonists less prone to induce dyskinesia than L-DOPA regardless of their properties [187, 245]. The data regarding the propensity of D_1/D_5 or D_2/D_3 selective agonists to induce dyskinesia is also highly controversial, with some studies favoring D_1-targeting agonist [246, 247] and some D_2-targeting agonists [183, 248]. The comparison is not easy to make due to differences in pharmacokinetic properties of the drugs and the higher propensity of D_1 agonists to induce tolerance [183, 249, 250]. It is often reported that in animal models D_1 antagonists block behavioral sensitization to L-DOPA [66, 67], although they may just reduce the absolute rotation frequency rather than block the sensitization process per se [145]. Agonists selective for the D_2 receptor are able to induce behavioral sensitization [60, 247].

A number of longer-lived DA agonists proved to possess antiparkinsonian properties combined with a lower propensity to induce dyskinesia. Many such drugs are now used in clinical practice, and their clinical and chemical properties have been extensively reviewed [240–242]. Their half-life ranges from 6 h (bromocriptine) to 76–100 h (cabergoline). All clinically used DA agonists are D_2/D_3 selective. Importantly, DA agonists that have a lower propensity to induce dyskinesia also demonstrate lower antiparkinsonian efficacy than L-DOPA. Most are currently used for treatment of early PD with the aim of delaying the introduction of L-DOPA, but eventually all PD patients require L-DOPA to achieve adequate antiparkinsonian effects [241, 251, 252]. The importance of long half-life for reduced dyskinetic potential is best supported by the data that continuous infusion of the same drug (L-DOPA, apomorphine, etc.) causes less dyskinesia than intermittent administration [253, 254].

The long-lived DA agonists, in contrast to L-DOPA, cause minimal behavioral sensitization and a smaller increase in rotation frequency upon repeated administration [60, 118, 125], and they do not induce AIMs [125]. Short-lived agonists such as apomorphine induce behavioral sensitization and AIMs similarly to L-DOPA [137, 247]. Long-lived DA agonists do not induce some of the signaling effects characteristic of L-DOPA, such as up-regulation of dynorphin [60, 138, 142, 196, 255],

substance P [195, 196], or D_3 receptors [60] in the lesioned striatum. We showed that the clinically effective DA agonist pergolide (half-life ~21 h), in contrast to L-DOPA, does not induce constitutive activity of the Akt pathway [60]. The long-lived D_2 agonist cabergoline does not up-regulate ΔFosB in MPTP-lesioned monkeys, but instead reverses the up-regulation of the basal level of ΔFosB induced by the lesion [179]. The DA agonists also reverse the lesion-induced up-regulation of enkephalin [60, 138, 141, 195, 196], which is not reversed by L-DOPA. In our experiments, pergolide was more successful than L-DOPA in normalizing the expression of GRKs altered by the dopaminergic lesion [118]. The fact that these signaling changes correlate with the presence or absence of dyskinesia argues for their role in LID. The continuous administration of L-DOPA via pump also does not produce LID in PD patients [252, 254] or animal models of PD [67]. Thus, long-lived DA agonists do not, as a rule, exaggerate the signaling effects produced by DA depletion or induce them de novo. They are often capable of reversing those effects of the DA depletion that are not normalized by L-DOPA. These properties might be related to their lower propensity to induce dyskinesia in humans and experimental animals, but the exact signaling mechanisms remain to be elucidated.

18.2.3.2 Why Are Clinically Used DA Agonists Less Efficacious than L-DOPA?

One possibility is that they target only D_2/D_3 receptors, whereas L-DOPA obviously targets all DA receptor subtypes. The dysfunctions of the D_1 receptor-mediated signaling in the DA-depleted striatum are well documented. Thus, the exclusion of D_1 receptors may reduce the efficacy of these drugs. The mixed $D_3/D_2/D_1$ agonist rotigotine has recently been shown to be effective in animal models and early [256] and advanced PD [257], although its signaling effects are yet to be explored. The synergism of the D_1/D_2 activity in the striatum and the ability of D_1 and D_2 drugs to prime for each other (discussed in Section 18.1.3) suggest that the absence of D_1 action is likely to reduce the amplitude of the responses elicited by DA agonists, as well as limit the cells and brain regions involved.

Another possibility is that the same property of these drugs that ensures their lower propensity to cause dyskinesia, i.e., longer life, also contributes to their lower efficacy. Continuous receptor stimulation often reduces responses to subsequent stimuli, a phenomenon known as tolerance. Long-lived or short-lived continuously administered DA agonists induce behavioral tolerance in parkinsonian animals [59, 250, 258]. GPCRs desensitize upon continuous stimulation via GRK-mediated phosphorylation followed by arrestin binding, which uncouples the receptor from G proteins and precludes further signal transduction. The receptor is then internalized into the endosomes and needs to be relieved of arrestin and dephosphorylated before it can be recycled to the plasma membrane and reused [157]. If the stimulation persists, the receptor is sent to the lysosomes and degraded, which down-regulates the available cell surface receptors. DA agonists may differ from L-DOPA in the way they engage the receptor desensitization machinery.

The homologous desensitization system is aimed at rapid termination of the signaling to ensure high temporal resolution. It is specifically designed to allow the cell to ignore persistent background stimulation to ensure high sensitivity to changing stimuli. Thus, longer-lived DA agonists are likely to induce more pronounced receptor desensitization, internalization, and down-regulation than short-lived drugs such as L-DOPA.

There are reports that DA agonists down-regulate D_2 receptors in the striatum of PD patients more effectively than L-DOPA [259, 260]. In live animals, it is difficult to detect desensitization because it is a rapid process with minute kinetics, and internalization can only be seen at the resolution of electron microscopy. Down-regulation, however, can be easily detected by receptor-binding techniques, and overall inhibition of signaling can also be easily documented. Increased cytoplasmic localization of D_1 receptors has been found at postmortem in the putamen of PD patients chronically treated with L-DOPA [261], possibly indicative of increased internalization. It is impossible to establish whether this is indeed so from this type of experiment, but if it is, it means that L-DOPA promotes receptor desensitization, which makes sense. However, studies in MPTP-treated monkeys demonstrated increased recruitment of D_1 receptors to the plasma membrane both in drug-naïve MPTP-lesioned and in L-DOPA-treated dyskinetic animals [262], suggesting *a deficit* in receptor internalization in LID rather than excessive internalization. This result is in agreement with the evidence that L-DOPA down-regulates the expression of arrestins and GRKs in MPTP-lesioned monkeys and tends to do so slightly more in animals with overt dyskinesia [96]. In hemiparkinsonian rats, pergolide, but not L-DOPA, was able to reverse the lesion-induced down-regulation of GRK isoforms in the caudal striatum [72], which could promote the desensitization of DA receptors and a reduction of the signaling caused by the drug [60]. The limited available data confirm that long-lived DA agonists tend to suppress signaling and reduce the receptor number. We have demonstrated that pergolide reduced the concentration of D_3 receptors in the lesioned and even in the intact hemisphere of hemiparkinsonian rats, whereas L-DOPA significantly up-regulated them [60]. This effect of pergolide could be indicative of D_3 receptor desensitization by the drug. Interestingly, long-lived agonists selectively targeting D_1 receptors rapidly lose their antiparkinsonian effect due to the development of tolerance, and dose escalation usually fails to restore the response [183, 249, 250]. This is one of the reasons why no selective D_1 drug has reached the clinic in spite of demonstrated antiparkinsonian efficacy. Molecular studies found that D_1 receptors readily desensitize in an arrestin- and GRK-dependent manner [263], whereas D_2 and especially D_3 receptors are relatively resistant to the homologous desensitization [264–266]. These data agree well with preclinical and clinical evidence on the activity of D_1- and D_2-selective agonists in PD.

Another property of L-DOPA that DA agonists do not have is that L-DOPA is converted to DA, which is then packaged in synaptic vesicles and released by neurons upon stimulation in a phasic spike-dependent manner. Agonists are not packaged and provide task-independent continuous stimulation of DA receptors. Therefore, long-lived DA agonists mimic well the tonic mode of DA action but are unable

to act in the spike-dependent phasic mode. Thus, they should be less able to support goal-oriented behavior than DA. The potential importance of this feature in the treatment of PD is highlighted by studies of DA null mice. They are hypoactive and hypophagic and cannot survive without forced feeding. The mice can be rescued by administration of L-DOPA, which supports proper feeding behavior, but not by D_1, D_2, or mixed D_1/D_2 agonists [267]. Apparently, the mode of DA release and receptor stimulation is critical in determining downstream signaling events, which are indispensable for the regulation of complex behaviors.

18.2.3.3 Continuous Versus Pulsatile Stimulation of DA Receptors

The lower propensity of DA agonists to induce dyskinesia is believed to be due to their longer life, which translates into more even stimulation of DA receptors. The successful use of long-lived DA agonists and infusion of L-DOPA and short-lived agonists gave rise to the concept that continuous stimulation of DA receptors should be the goal in the treatment of PD [252, 253, 268]. The idea that continuous stimulation of DA receptors is physiological is supported by the evidence that dopaminergic neurons fire constantly at a low rate, inducing tonic release of DA [269], which maintains a constant or nearly constant DA concentration in the striatum and an ongoing tonic modulatory influence of DA on striatal neurons [270, 271]. D_2 antagonists cause multiple signaling events in striatal neurons [180], which is easy to interpret as stemming from the removal of the constant inhibition of these pathways via D_2 receptors. Apparently, the numerous extrasynaptic DA receptors found on striatal neurons [15] are meant to mediate tonic spike-independent DA activity in the striatum. However, this continuous tonic mode is not the only type of DA release in the striatum, as evidenced by phasic massive DA release during task performance in animals and humans [271, 272].

Normally, efficient reuptake of DA by dopaminergic terminals rapidly reduces the DA concentration to basal. L-DOPA causes manifold higher elevation of DA in the lesioned than in the intact striatum [273–275], likely due to the reduced clearance capacity of the sparse dopaminergic terminals. Even by itself, without specific postsynaptic changes, such a huge short-term increase in the synaptic DA concentration might contribute to peak-dose dyskinesia. Therefore, for the prevention of LID it might be beneficial to smooth out such fluctuations in the DA availability. Partially this could be achieved via co-administration of catechol-*O*-methyl transferase (COMT) (entacapone, tolcapone) or monoamine oxidase (MAO) inhibitors (deprenyl) with L-DOPA to slow down the degradation of DA and prolong its effects. These drugs have been introduced into clinical practice and proved beneficial in patients at different stages of the disease [276].

When drug-naïve PD patients are treated with long-lived DA agonists, they experience a lower incidence of dyskinesia [240, 241]. A somewhat different question is whether continuous stimulation of DA receptors with continuously administered L-DOPA/short-lived DA agonists or with long-lived DA agonists can reverse already established LID. Clinical trials so far failed to unequivocally demonstrate an antidyskinetic effect of DA agonists on previously established LID [251], although

there is strong enthusiasm for continuous dopaminergic stimulation as the strategy to provide antiparkinsonian benefits while controlling LID [253, 268]. If the lower efficacy of clinically used DA agonists is due to the lack of D_1 activity, continuously administered L-DOPA should be just as efficacious as regular L-DOPA but without the motor fluctuations. It remains unclear whether this is the case, since no large, long-term, double-blind studies have been conducted so far. Tolerance might be less of a problem with L-DOPA, even continuously administered, than with long-lived DA agonists, since DA synthesized from L-DOPA can be packaged and released in a phasic manner. Very few studies have examined the signaling effects of continuous L-DOPA administration, and those that have show similar results to those with long-lived DA agonists [67], specifically, that continuous L-DOPA does not induce the effects typical for intermittent L-DOPA administration.

18.2.4 Molecular Mechanisms of L-DOPA-Induced Dyskinesia

Multiple biochemical changes discussed above are induced by prolonged L-DOPA treatment, and some of them appear to correlate with the severity of LID in humans and animals. However, the functional role of most of these effects in LID remains unknown. Unfortunately, even the most robust correlation does not mean cause-and-effect relationship. The correlation of a molecular process with LID by itself does not help to establish the place of the process in the chain of events that ultimately produces LID. Since L-DOPA has antiparkinsonian as well as dyskinetic activity, classifying molecular consequences of L-DOPA treatment as antiparkinsonian or dyskinetic is a formidable task. Moreover, to understand the molecular mechanisms of LID, it is critical to separate the core processes from downstream events. What we already know about the molecular mechanisms of GPCR regulation might be very helpful in achieving these goals. Here we attempt to reconstruct molecular events that might bring about modification of the DA receptor signaling underlying LID and point out the gaps in our knowledge.

18.2.4.1 Critical Elements in the Development of L-DOPA-Induced Dyskinesia

Three considerations are important for understanding the molecular mechanisms of LID. First, L-DOPA is taken by PD patients that have lost at least half of their dopaminergic neurons, which means that L-DOPA acts on the background of the DA depletion and the signaling changes already induced by it (Figs. 18.2 and 18.3). The requirement of dopaminergic degeneration for the induction of LID is well supported by clinical and experimental data [125, 218]. Normal people or animals do not respond to doses of L-DOPA that produce dyskinesia in PD patients or lesioned animals. The probability of LID increases with the severity of dopaminergic degeneration in PD patients and experimental animals. It is conceivable that the lesion-induced supersensitivity of DA receptors is a prerequisite for LID induction. This view is supported by the evidence that a single administration of L-DOPA

induces multiple persistent proteomic changes in the DA-depleted, but not the normal, monkey striatum [147]. These effects may underlie the priming phenomenon, which is believed to be critical for the development of LID.

The second point is that L-DOPA is taken for a long time, and its clinical effects change over time. LID emerges after a few years of L-DOPA therapy and progresses thereafter. One reason for the delay may simply be the continuing degeneration of dopaminergic neurons that eventually reaches the LID threshold. Another possibility is that chronic L-DOPA induces plastic changes in the striatal signaling pathways that require time and chronic drug administration to develop, irrespective of the progression of the degeneration. In human PD patients, both processes are likely at work. Using animal models of PD, which have stable and often quite extensive loss of dopaminergic neurons, we can distinguish between lesion-induced changes and plastic effects that require chronic L-DOPA administration.

The third point is that, because of its short life, L-DOPA causes rapid elevation of the DA concentration followed by rapid clearance, resulting in a pulsatile mode of DA receptor stimulation: strong stimulation followed by periods of little or no stimulation.

18.2.4.2 DA Receptor Supersensitivity and L-DOPA-Induced Dyskinesia

If the presence of a particular signaling process is necessary and sufficient for the induction of LID, it would prove that this process is part of the molecular mechanism underlying LID. Since LID undoubtedly involves multiple mechanisms, it will likely be possible to demonstrate only that the process significantly contributes to LID severity. The behavioral sensitization phenomenon in the 6-OHDA-lesioned hemiparkinsonian rat is the most frequently used animal model of LID, particularly in molecular studies. Although the L-DOPA-induced rotations in this model do not resemble LID in the least, the progressive increase in the rotation frequency upon chronic administration of L-DOPA mimics the progressive nature of LID quite well. The progressive behavioral sensitization to L-DOPA in animals with stable depletion of dopaminergic neurons argues for the role of plastic signaling changes produced by chronic L-DOPA in LID induction. Since rotations induced by dopaminergic stimulation are attributed to the supersensitivity of DA receptors in the striatum, the increased rotation frequency due to behavioral sensitization suggests that receptor responsiveness is further increased by chronic L-DOPA administration. However, different signaling pathways respond differentially to the L-DOPA treatment. As discussed in Section 18.2.2, the sensitivity of several pathways is actually reverted to normal levels by L-DOPA, suggesting that these pathways are probably not responsible for the behavioral sensitization to L-DOPA. The signaling abnormalities caused by DA loss that are aggravated by L-DOPA are more likely candidates (Fig. 18.4).

The pathway(s) leading to LID appear to start from the supersensitivity of DA receptors, apparently both D_1 and D_2 subtypes. The molecular mechanism of this supersensitivity may include increased availability of D_1 receptors at the plasma membrane [262] and increased D_1 receptor coupling to G proteins [95], although

the mechanisms that bring about these changes remain undefined. The up-regulation of $G\alpha_{olf}$ that has been proposed to mediate the lesion-induced supersensitivity of D_1 receptors is actually reversed by chronic L-DOPA [78]. A number of downstream members of various pathways have been shown to be hyper-activated in the dyskinetic striatum as compared to non-dyskinetic L-DOPA-treated animals. Enhanced cAMP-mediated signaling has been implicated in LID, including enhanced activity of the DARPP-32-based amplification loop and ERK1/2 [85]. The substrate of ERK1/2, mitogen- and stress-activated kinase 1 (MSK-1), is also hyperphosphorylated in the striatum of dyskinetic animals [85]. LID is accompanied by enhanced PKA-mediated phosphorylation of the GluR1 subunit of the AMPA receptor [85] indicative of elevated AMPA transmission. However, the supersensitivity of these pathways is attenuated by L-DOPA, although to a lesser extent in dyskinetic animals.

Conversely, the accumulation of ΔFosB seems to have a causal connection with LID [223]. ΔFosB is known to regulate dynorphin expression [223], and strong up-regulation of dynorphin by L-DOPA closely parallels the time course of behavioral sensitization to L-DOPA [66, 144, 205, 223]. The D_3 receptor is also strongly up-regulated by L-DOPA, with a time course parallel to that of behavioral sensitization, and abolition of the D_3 receptor up-regulation by blockade of D_1 receptors inhibits the behavioral response [60, 66, 67]. Moreover, treatment of dyskinetic monkeys with a partial D_3 receptor agonist co-administered with L-DOPA reduces LID without affecting the antiparkinsonian benefits, presumably via a judicious reduction of the D_3 receptor-mediated signaling [30]. Enhanced constitutive activity of the Akt-GSK3 pathway also parallels behavioral sensitization to L-DOPA [60]. These signaling pathways might provide some mechanistic foundation for the enhanced dopaminergic responsiveness of the dyskinetic brain. Thus, it appears that dopamine depletion-induced supersensitivity of some, but not all, DA receptor-dependent pathways is further exacerbated or fixed by L-DOPA, thereby leading to LID (Fig. 18.4). It remains unclear how differential sensitivity of several pathways initiated by the same receptor can be achieved mechanistically. It is equally unclear which exaggerated molecular responses are the cause and which are the consequence of the abnormal dopaminergic signaling.

18.2.4.3 Molecular Mechanisms of the Dopaminergic Supersensitivity in L-DOPA-Induced Dyskinesia

The key point in this model that remains completely unexplained is the origin of the L-DOPA-induced supersensitivity of DA receptor-mediated signaling pathways. Strictly speaking, we do not know how DA depletion produces supersensitivity, but we understand even less the L-DOPA-induced sensitization of dopaminergic responses. Additionally, there is no ready explanation of why L-DOPA normalizes some signaling pathways while further upsetting the others. The lesion-induced receptor supersensitivity can be understood as one of many compensatory responses designed to maintain dopaminergic signaling upon the loss of DA. Mechanistically,

it could be explained by up-regulation of G protein(s) that enhances the G protein-mediated signaling, which is then propagated and amplified along the signaling pathways.

Assuming that striatal DA controls the expression of signaling proteins, the removal of that control upon DA depletion might be responsible for the $G\alpha_{olf}$ up-regulation via activation of specific transcription factors. L-DOPA, by replenishing striatal DA, should be expected to reverse that effect, and it does just that. Thus, no mechanistic explanation for the further enhancement of the signaling via D_1 receptors by chronic L-DOPA administration is currently available. It is possible that L-DOPA augments the signaling via suppression of receptor desensitization. We found that L-DOPA, in contrast to the long-lived DA agonist pergolide, was largely unsuccessful in reversing the down-regulation of GRK isoforms induced by DA depletion in the caudal striatum [72]. The reduction in the availability of GRKs in the motor regions of the striatum, which is not reversible by L-DOPA, could actually contribute to LID or provide a favorable background for the development of LID. Lower concentrations of all GRK isoforms would impede receptor desensitization, and any factor that enhances the signaling would do it more effectively when the normal desensitization process is partially disabled. If this is the case, an increased GRK concentration should suppress LID. We have found that overexpression of GRK isoforms in the lesioned striatum using lentivirus-mediated gene transfer suppresses behavioral sensitization to L-DOPA and L-DOPA-induced AIMs in hemiparkinsonian rats [277].

Enhanced RGS-mediated attenuation of D_2 receptor signaling may also be beneficial in LID. Indeed, overexpression of RGS9-2 was found to ameliorate LID in the rodent and monkey models of PD, whereas the loss of RGS9-2 augmented it [177]. Therefore, desensitization of D_1 and D_2 receptors appears to be beneficial for controlling LID without compromising the antiparkinsonian effect of L-DOPA. This is understandable, because facilitated desensitization ensures rapid signal shutoff but does not prevent the signal from going through, thus preserving antiparkinsonian activity. The efficacy of techniques promoting the desensitization process as antidyskinetic measures supports the idea that abnormally enhanced signaling by DA receptors is responsible for LID. This evidence suggests that the mechanisms of DA receptor desensitization are intimately involved in the development of LID and should be targeted for therapeutic purposes.

To date, numerous questions concerning the mechanism of LID remain unanswered. Many studies of LID mechanisms rely on the distinction between "dyskinetic" and "non-dyskinetic" animals. It has been repeatedly reported that in non-dyskinetic animals, L-DOPA reverses various signaling effects produced by DA depletion, whereas these changes persist or are even augmented in dyskinetic animals [85, 95, 121]. At first glance, these data suggest that the effects of L-DOPA are different or even opposite in the two categories of subjects. However, it is only a matter of time and/or L-DOPA dose before non-dyskinetic subjects become dyskinetic. The AIMs frequency in hemiparkinsonian rodents depends directly on the L-DOPA dose and the duration of treatment [121, 125, 216], and all MPTP-lesioned monkeys can be rendered dyskinetic and achieve similar dyskinetic scores with

prolonged and/or higher dose L-DOPA treatment [177, 278]. Similarly, a very large proportion, if not all human PD patients eventually develop LID [218]. Thus, it is much more likely that L-DOPA (as well as other antiparkinsonian treatments) induces qualitatively similar changes in signaling in all subjects, and the difference lies in the extent and/or the time course of these changes. For example, if ERK1/2 hyper-phosphorylation induced by DA depletion is reversed in non-dyskinetic animals, it is likely reversed in *all* animals to different degrees. Indeed, the data show that there is a reversal of the lesion-induced super-responsiveness of ERK1/2, DARPP-32, and $GluR_1$ in both dyskinetic and non-dyskinetic groups [60, 154]. The difference between the drug-naïve and the L-DOPA-treated hemiparkinsonian animals is actually greater than the difference between dyskinetic and non-dyskinetic ones. It should be emphasized that these data do not mean that the residual supersensitivity does not contribute to LID. In particular, DARPP-32 acts as an amplifier of the PKA-mediated signaling, and its removal may suppress dyskinesia by blunting the signaling via multiple pathways [88]. However, the pathways that respond to chronic L-DOPA in this manner are unlikely to be at the core of the LID pathology. A few "master" modifications propagated along the signaling network, eventually fixing the overactive state of DA receptors are likely to be responsible for LID. The candidate pathways should be progressively distorted by L-DOPA to a varying degree in all individuals. A few signaling effects described so far meet this criterion: accumulation of ΔFosB, *Arc*, *Homer-1a*, GAD67, dynorphin, and D_3 receptors, as well as sensitization of CREB phosphorylation and the Akt pathway (Fig. 18.4). Technically, the dynorphin and D_3 receptor effects could be considered a reversal of the lesion-induced down-regulation, but the L-DOPA-induced expression of both proteins overshoots the pre-lesion levels very rapidly in all animals. The molecular processes that elicit these disparate effects and the specific roles of each in the generation of LID need to be determined.

It is obvious that L-DOPA exerts multiple effects on signaling mechanisms in all individuals. Some of these actions may be beneficial and the others detrimental from the clinical standpoint. The beneficial action mimics the normal effects of DA, thus reversing the signaling deficits induced by DA depletion. The detrimental action likely represents a further distortion of the signaling already damaged by DA depletion, rather than a simple reversion of the depletion effects. It is likely that even a single dose of L-DOPA has damaging potential, which is reflected in the well-known "priming" phenomenon. Upon prolonged treatment or administration of higher doses, these detrimental signaling effects of L-DOPA might grow strong enough to block and/or overshadow its beneficial effects, which restore normal signaling. These effects also seem to become permanent or semi-permanent, maintaining sensitized signaling even after a prolonged "drug holiday." Due to individual differences, the detrimental activities of L-DOPA might be stronger in some individuals and weaker in others, which is reflected in the different L-DOPA doses and treatment times required to induce overt LID in different individuals. The goal should be to identify the alterations induced by L-DOPA that persist or increase with time and/or dose and have the potential to establish a positive feedback mechanism,

further elevating the DA receptor signaling. Unfortunately, most studies have been performed with a single dose of L-DOPA administered for a fixed time. If we are to identify the mechanisms of LID, it is crucial to quantitatively evaluate the dose and time dependence of L-DOPA-induced signaling changes in animals with both low and high propensities for LID.

18.2.5 Mechanisms of L*-DOPA-Induced Motor Fluctuations*

Treatment with L-DOPA, in addition to dyskinesia, gives rise to a number of effects collectively referred to as motor fluctuations. Different types of motor fluctuations are known but all describe a short, uncertain, or unstable therapeutic effect of a dose of the drug, which can appear or disappear without connection to the actual presence of the drug in the body. The therapeutic action of L-DOPA becomes shorter (wearing off) as the disease progresses. Occasionally, the therapeutic relief is delayed or does not appear at all (delayed or no "on" effect), and sometimes a person alternates between the "off" and the "on" stages (on-off fluctuations), even though the concentration of the drug in the body remains stable. In the worst case, the person may alternatively experience the "off" state and dyskinesia without any positive "on" effect at all [182].

If LID represents an unwanted response to L-DOPA that emerges with time due to some sort of sensitization, motor fluctuations reflect a loss of the therapeutic effect of the drug present in the system and may be due to tolerance [251]. The shortened response to L-DOPA is likely to be partially due to progressive loss of dopaminergic terminals and the capacity for storage of DA. Since the response to DA agonists, which are not stored in the brain, is also shortened with repeated administration [185, 279–282], additional explanation involving modifications of postsynaptic striatal machinery is necessary. It is important to bear in mind that therapeutic, appropriate motor responses to L-DOPA become larger with shorter latency to peak [279, 281–284] at the same time as they become shorter in duration. The increased amplitude of the responses is analogous to the sensitization mechanism involved in LID, which also becomes more severe with time [284], whereas the reduced duration seems to reflect the opposite process of tolerance.

What are the possible molecular mechanisms of L-DOPA-induced motor fluctuations, and how do they relate to those of dyskinesia? The common feature of dyskinesia and motor fluctuations is that they seem to be based largely on postsynaptic modifications of the responsiveness of striatal neurons. These modifications are probably initiated by alterations in the DA receptor signaling caused by DA depletion and L-DOPA treatment and then spread to other signaling systems. The development of LID and motor fluctuations go hand in hand, and many patients have both [211, 217], although phenotypically LID and motor fluctuations, or insufficient "on" response, appear to be the opposite ends of the spectrum. In the clinic, it has been a challenge to control LID while maintaining adequate antiparkinsonian response, which suggests that the mechanisms of LID and the antiparkinsonian

action are intertwined. Only a handful of drugs have been found to ameliorate LID without reducing the antiparkinsonian effect [251]. Thus, the clinical practice indicates that LID and motor complications cannot be entirely suppressed at the same time, and only a trade-off between LID and motor complications can be achieved. The mechanistic implication of this conclusion is that, although both LID and motor complications develop as a result of the signaling changes induced by chronic L-DOPA treatment on the background of dopamine denervation, these phenomena rely on molecular mechanisms opposite in nature and yet co-existing and interconnected. The current challenge is to identify these mechanisms.

A possibility has been suggested that continuous stimulation of DA receptors by mixed DA agonists or L-DOPA delivered constantly (via transdermal patches or intraintestinally) may provide antiparkinsonian benefits without inducing dyskinesia, thus avoiding the Scylla and Charybdis of LID and motor fluctuations [251, 253, 268]. This idea is based on the tonic mode of DA release in the striatum, the low dyskinetic potential of long-lived DA agonists, and the experimental evidence that dyskinesia is more easily avoided with continuous than with intermittent delivery of the same drugs [243, 244]. However, round the clock delivery of L-DOPA or short-lived DA agonists is frequently associated with psychiatric problems such as hallucinations [251]. Similarly, long-lived DA agonists cause psychiatric problems, such as hallucinations, pathological gambling, hypersexuality, compulsive behaviors, and addiction to dopaminergic medication [285–287]. This necessitates the delivery to be confined to daytime hours, which usually alleviates the problems [191]. If the dopaminergic stimulation is not continuous, troughs of the stimulation seem to be particularly detrimental [254]. So far, no large, randomized clinical studies have been conducted to prove the benefits of continuous stimulation. The presence of dyskinesia combined with modest antiparkinsonian effects in patients with fetal nigral transplants, which, supposedly, provide continuous release of DA, is at odds with the idea that continuous stimulation is the optimal therapy [288–290].

Very few mechanistic studies of motor fluctuations have been performed due to the paucity of animal models of this phenomenon. In hemiparkinsonian rats, chronic treatment with L-DOPA induced a progressively shorter rotational response [105, 291], the behavioral process closely resembling wearing off in PD patients. The effect may require specific conditions to emerge, since it was not noted in other labs [125]. Longer treatment with L-DOPA is needed to induce the response shortening (approximately 3 weeks) than behavioral sensitization (a few days), which mimics the time course of these complications in PD patients [292]. This effect is associated with sensitized CREB phosphorylation in response to acute challenge with L-DOPA [105]. D_1, but not D_2, agonists mimic the elevation. The increase in CREB responsiveness parallels the time course of the shortening of the rotational response, which remains decreased for several weeks after L-DOPA withdrawal and returns to normal by the sixth week. Moreover, intrastriatal administration of a PKA inhibitor or CREB antisense oligonucleotide restored the normal duration of the rotational response. Up-regulation of PKC activity by viral gene transfer of the PKC catalytic subunit accelerates the shortening of the response in the course of chronic L-DOPA treatment, a process apparently mediated by enhanced phosphorylation of the $GluR_1$

subunit of the AMPA receptor at the PKC site Ser831. Inhibition of PKC in animals overexpressing PKC attenuates both $GluR_1$ phosphorylation and response shortening. Interestingly, the reversal of the response shortening during chronic L-DOPA treatment by an $adenosine_{A2A}$ receptor antagonist was concomitant with a further increase in the expression of dynorphin as compared to L-DOPA alone [293], supporting opposing mechanisms for LID and motor fluctuations and providing a certain mechanistic basis for the increase in LID by suppressing motor fluctuations. However, the relationship between the shortening of the response and the severity of LID was not addressed in these studies. In some cases, the same drugs that relieved LID also restored the normal response duration [232, 294–296], implicating similar mechanisms in both types of L-DOPA-induced motor complications. In spite of superficial similarity, it remains unclear whether the shortening of the motor response to L-DOPA in hemiparkinsonian rats is mechanistically analogous to L-DOPA-induced motor fluctuations in human patients. The amount of available data is grossly insufficient to identify the overlapping or contrasting molecular processes underlying LID and motor fluctuations.

18.3 Dopamine Receptors and Neuroprotection in Parkinson's Disease

Dopaminergic drugs used in the symptomatic treatment of PD, such as L-DOPA and DA agonists, exert their therapeutic effects via interaction with DA receptors in the striatum. However, the dopaminergic neurons of the substantia nigra pars compacta that provide DA to the striatum express DA receptors, suggesting that DA and dopaminergic drugs can exert direct regulatory influence on these neurons. The expression of D_2 and D_3 receptors in the substantia nigra pars compacta and, specifically, in dopaminergic neurons of the rodent and primate brain is well documented [7, 11, 13, 28, 297, 298]. D_4 receptors are abundant in the neuropil and are detected in GABAergic cells of the substantia nigra pars reticulata [40, 299]. D_4 receptors are present in the small proportion of tyrosine hydroxylase (TH)-positive terminals in the nucleus accumbens [300] suggesting that a fraction of dopaminergic neurons may express these receptors. Most studies failed to detect D_1 receptor protein or mRNA in neurons of the substantia nigra compacta [6, 7, 301]. In contrast, immunohistochemical and in situ hybridization experiments demonstrated the presence of D_5 receptors in dopaminergic neurons [42, 44, 302]. D_1 receptors are abundant in the neuropil in the substantia nigra reticulata, presumably localizing on striatonigral terminals of the direct pathway [6, 24] including terminals forming synapses on TH-positive dendrites [301]. In the monkey brain, occasional D_1 receptor-positive perikarya are detected in the substantia nigra [24].

Since dopaminergic neurons, which progressively die in PD, possess DA receptors, it is possible that dopaminergic drugs affect viability of these neurons. By the time PD is diagnosed, approximately 50–70% of the dopaminergic cells of the substantia nigra have been lost. The remaining cells are believed to slowly degenerate as the disease progresses, contributing to the worsening of the symptoms and

of the side effects of L-DOPA therapy. Therefore, the welfare of the surviving dopaminergic neurons is of prime concern for the success of the PD treatment.

The effect of DA replacement therapy on the survival of these neurons is a highly controversial subject. In the course of its normal metabolism, DA produces a number of reactive oxygen species that can cause oxidative stress. L-DOPA, being a close relative of DA, can do the same, as can DA synthesized from L-DOPA. Since L-DOPA administration creates surges of L-DOPA and DA in the DA-depleted brain, this situation may be toxic for dopaminergic cells, which are vulnerable to oxidative stress [303], and promote cell death. Numerous in vitro studies have demonstrated L-DOPA toxicity in dopaminergic cell lines and cultured mesencephalon neurons [304–311]. The opposite view holds that L-DOPA can be neuroprotective under specific circumstances [304, 309, 312, 313]. L-DOPA induces oxidative stress and at the same time protects from it by engaging cellular antioxidative mechanisms, since inhibition of L-DOPA-induced oxidative stress also inhibits neuroprotection [304, 309]. These L-DOPA effects might be based solely on L-DOPA metabolism and independent of DA receptors [313]. The stimulation of DA receptors by DA may also be toxic to neurons [314, 315]. The most important question is, of course, whether L-DOPA is toxic in vivo in humans and animals in the situation when nigral neurons are under oxidative stress as occurs in PD. The answer to this question has immediate clinical implications for whether or not to delay L-DOPA introduction. Most in vivo studies in animals have found so far that L-DOPA is harmless [125, 316] or even neuroprotective [317, 318]. The large ELLDOPA clinical study aimed at resolving the issue failed to do so [319, 320]. The patients on L-DOPA showed a better clinical picture after the 2-week L-DOPA washout period than those on the placebo, but the concern was raised that long-term drug effects might have been responsible. The imaging studies included in the trial added insult to injury by demonstrating a greater decline in nigrostriatal biomarkers in the L-DOPA as compared to the placebo group. Thus, the problem of the potential toxicity of L-DOPA remains unresolved.

In contrast to L-DOPA, DA agonists are generally believed to be neuroprotective, although hard evidence remains scarce. Clinical studies have demonstrated better preservation of the nigrostriatal system in patients treated with DA agonists than in those treated with L-DOPA [321, 322]. Many DA agonists have been found to protect dopaminergic neurons against neurotoxins in animal models of PD [323–330]. The mechanism of the neuroprotection provided by DA agonists is puzzling. The neuroprotection by DA agonists may be independent of DA receptors, relying instead on the free radical scavenging properties of the drugs [327]. However, the action at DA receptors may also be involved. In vitro, DA agonists protect dopaminergic neurons from death induced by a variety of insults via stimulation of D2-like [327, 331–333] or D1-like [334] DA receptors. In particular, a case has been made for the prime role of the D_3 receptor in the neuroprotective activity of DA agonists [335]. Indeed, most clinically used DA agonists have a higher affinity for the D_3 than for the D_2 receptor [242]. Furthermore, the neuroprotective activity of the D_3/D_2 agonist pramipexole against the MPTP toxicity is attenuated in mice lacking D_3 receptors and by co-administration of a selective D_3 antagonist, the

effect of which is abrogated in D_3 knockout mice [336]. The neuroprotective effect of pramipexole and another D_3/D_2 agonist, S32504, against the MPTP toxicity in terminally differentiated neuroblastoma SH-SY5Y cells depends on the recruitment of brain-derived neurotrophic factor (BDNF) [333, 337], glial-derived neurotrophic factor (GDNF) [337], and the Akt pathway [333]. To summarize, a number of studies have demonstrated the neuroprotective activity of D_2/D_3 DA agonists in vitro and in vivo. However, the signaling mechanisms mediating this effect remain largely unexplored, and no convincing mechanistic model has emerged so far. The inherent difficulties of these complex studies are further compounded by the obscure signaling pathways of the D_3 receptor, even if the D_3 receptor does play the starring role in neuroprotection in PD.

18.4 Conclusions

The available evidence strongly indicates that the hallmark feature of PD, DA depletion, results in profound changes in several signaling pathways. These alterations, including the supersensitivity of DA receptor signaling, apparently first emerge as adaptive responses to the reduced supply of endogenous DA. Administration of L-DOPA and DA agonists in parkinsonian patients and animal models of PD brings about additional changes in cell signaling. Certain therapy-induced modulations appear to represent the reversal of disease-induced changes and a rebalancing of signal transduction at or near normal levels, whereas others constitute further deviations that are likely involved in the pathogenesis of dyskinesia and other motor complications arising in the process of chronic treatment.

Our understanding of the cellular and molecular mechanisms underlying different aspects of Parkinson's pathology and motor complications is very uneven. For example, our current model of the circuit effects translating DA depletion into akinesia is fairly well developed, whereas the mechanisms producing another typical feature of Parkinson's pathology, tremor, remain obscure. At the molecular level, G protein-mediated signaling via D_1 receptors and its connection with PKA activity and modulation of its targets are much better documented than the signaling via the D_2 and D_3 receptors that are also affected in PD. To improve the effectiveness of existing therapies and, especially to devise novel treatments for PD, we urgently need to elucidate how the network of cellular signaling pathways is regulated by each of the DA receptors in the normal and diseased brain. The field needs to take full advantage of the available molecular approaches and the opportunities opened by the creation of knockout and transgenic mice to identify all the key players in DA signaling via each of the DA receptors. In each pathway, we need to establish which proteins are up- or downstream of each other and which players are rate limiting in the normal and diseased striatum. Manipulation of the most upstream and rate limiting elements usually changes the pathway output more effectively, which would make these signaling proteins the most promising therapeutic targets. Although this approach would require a lot of additional experimentation, it offers the best hope for selective enhancement of the antiparkinsonian effects of L-DOPA or DA agonists with simultaneous suppression of the signaling events responsible

for dyskinesia and other unwanted side effects of currently used treatments. Only a mechanism-based approach can lead to the "holy grail" of PD therapy, the preservation of dopaminergic neurons using molecular tools to enhance pro-survival and suppress pro-apoptotic signaling in the substantia nigra, which would make DA replacement strategies obsolete.

References

1. Albin RL, Young AB, Penney JB. The functional anatomy of basal ganglia disorders. Trends Neurosci 1989;12:366–75.
2. Bergman H, Deuschl G. Pathophysiology of Parkinson's disease: from clinical neurology to basic neuroscience and back. Mov Disord 2002;17:S28–S40.
3. Wilms H, Sievers J, Deuschl G. Animal models of tremor. Mov Disord 1999;14:557–71.
4. Aubert I, Ghorayeb I, Normand E, Bloch B. Phenotypical characterization of the neurons expressing the D1 and D2 dopamine receptors in the monkey striatum. J Comp Neurol 2000;418:22–32.
5. Gerfen CR. Dopamine-mediated gene regulation in models of Parkinson's disease. Ann Neurol 2000;47(Suppl 1):S42–S50.
6. Yung KK, Bolam JP, Smith AD, Hersch SM, Ciliax BJ, Levey AI. Immunocytochemical localization of D1 and D2 dopamine receptors in the basal ganglia of the rat: light and electron microscopy. Neuroscience 1995;65:709–30.
7. Hurd YL, Suzuki M, Sedvall GC. D1 and D2 dopamine receptor mRNA expression in whole hemisphere sections of the human brain. J Chem Neuroanat 2001;22:127–37.
8. Meador-Woodruff JH, Damask SP, Wang J, Haroutunian V, Davis KL, Watson SJ. Dopamine receptor mRNA expression in human striatum and neocortex. Neuropsychopharmacology 1996;15:17.
9. Le Moine C, Bloch B. D1 and D2 dopamine receptor gene expression in the rat striatum: sensitive cRNA probes demonstrate prominent segregation of D1 and D2 mRNAs in distinct neuronal populations of the dorsal and ventral striatum. J Comp Neurol 1995;355:418–26.
10. Nadjar A, Brotchie JM, Guigoni C, et al. Phenotype of striatofugal medium spiny neurons in parkinsonian and dyskinetic nonhuman primates: A call for a reappraisal of the functional organization of the basal ganglia. J Neurosci 2006;26:8653–61.
11. Gurevich EV, Joyce JN. Distribution of dopamine D3 receptor expressing neurons in the human forebrain: comparison with D2 receptor expressing neurons. Neuropsychopharmacology 1999;20:60–80.
12. Gurevich EV, Himes JW, Joyce JN. Developmental regulation of expression of the D3 dopamine receptor in rat nucleus accumbens and islands of Calleja. J Pharmacol Exp Ther 1999;289:587–98.
13. Sesack SR, Aoki C, Pickel VM. Ultrastructural localization of D2 receptor-like immunoreactivity in midbrain dopamine neurons and their striatal targets. J Neurosci 1994;14:88–106.
14. Levey AI, Hersch SM, Rye DB, et al. Localization of D1 and D2 dopamine receptors in brain with subtype-specific antibodies. Proc Natl Acad Sci USA 1993;90:8861–5.
15. Wang H, Pickel VM. Dopamine D2 receptors are present in prefrontal cortical afferents and their targets in patches of the rat caudate-putamen nucleus. J Comp Neurol 2002;442:392–404.
16. Cepeda C, Hurst RS, Altemus KL, et al. Facilitated glutamatergic transmission in the striatum of D2 dopamine receptor-deficient mice. J Neurophysiol 2001;85:659–70.
17. Gerfen CR, Keefe KA, Gauda EB. D1 and D2 dopamine receptor function in the striatum: Coactivation of D1- and D2-dopamine receptors on separate populations of neurons results in potentiated immediate early gene response in D1-containing neurons. J Neurosci 1995;15:8167–76.

18. Gerfen CR, Miyachi S, Paletzki R, Brown P. D1 dopamine receptor supersensitivity in the dopamine-depleted striatum results from a switch in the regulation of ERK1/2 kinase. J Neurosci 2002;22:5042–54.
19. Aizman O, Brismar H, Uhlen P, et al. Anatomical and physiological evidence for D1 and D2 dopamine receptor colocalization in neostriatal neurons. Nat Neurosci 2000;3:226.
20. Surmeier DJ, Song WJ, Yan Z. Coordinated expression of dopamine receptors in neostriatal medium spiny neurons. J Neurosci 1996;16:6579–91.
21. Levesque M, Parent A. The striatofugal fiber system in primates: A reevaluation of its organization based on single-axon tracing studies. Proc Natl Acad Sci USA 2005;102: 11888–93.
22. Wu Y, Richard S, Parent A. The organization of the striatal output system: a single-cell juxtacellular labeling study in the rat. Neurosci Res 2000;38:49–62.
23. Mizuno T, Schmauss C, Rayport S. Distinct roles of presynaptic dopamine receptors in the differential modulation of the intrinsic synapses of medium-spiny neurons in the nucleus accumbens. BMC Neurosci 2007; (in press).
24. Bergson C, Mrzljak L, Smiley JF, Pappy M, Levenson R, Goldman-Rakic PS. Regional, cellular, and subcellular variations in the distribution of D1 and D5 dopamine receptors in primate brain. J Neurosci 1995;15:7821–36.
25. Yan Z, Song W-J, Surmeier DJ. D2 dopamine receptors reduce N-type Ca2+ currents in rat neostriatal cholinergic interneurons through a membrane-delimited, protein-kinase-C-insensitive pathway. J Neurophysiol 1997;77:1003–15.
26. Ding J, Guzman JN, Tkatch T, et al. RGS4-dependent attenuation of M4 autoreceptor function in striatal cholinergic interneurons following dopamine depletion. Nat Neurosci 2006;9:832–42.
27. Meador-Woodruff JH, Grandy DK, Van Tol HH, et al. Dopamine receptor gene expression in the human medial temporal lobe. Neuropsychopharmacology 1994;10:239–48.
28. Bouthenet ML, Souil E, Martres MP, Sokoloff P, Giros B, Schwartz JC. Localization of dopamine D3 receptor mRNA in the rat brain using in situ hybridization histochemistry: comparison with dopamine D2 receptor mRNA. Brain Res 1991;564:203–19.
29. Sokoloff P, Giros B, Martres MP, Bouthenet ML, Schwartz JC. Molecular cloning and characterization of a novel dopamine receptor (D3) as a target for neuroleptics. Nature 1990;347:146–51.
30. Bezard E, Ferry S, Mach U, et al. Attenuation of levodopa-induced dyskinesia by normalizing dopamine D3 receptor function. Nature Med 2003;9:762–7.
31. Murray AM, Ryoo HL, Gurevich E, Joyce JN. Localization of dopamine D3 receptors to mesolimbic and D2 receptors to mesostriatal regions of human forebrain. Proc Natl Acad Sci USA 1994;91:11271–5.
32. Suzuki M, Hurd YL, Sokoloff P, Schwartz J-C, Sedvall G. D3 dopamine receptor mRNA is widely expressed in the human brain. Brain Res 1998;779:58–74.
33. Le Moine C, Bloch B. Expression of the D3 dopamine receptor in peptidergic neurons of the nucleus accumbens: comparison with the D1 and D2 dopamine receptors. Neuroscience 1996;73:131–43.
34. Perachon S, Schwartz JC, Sokoloff P. Functional potencies of new antiparkinsonian drugs at recombinant human dopamine D1, D2 and D3 receptors. Eur J Pharmacol 1999;366: 293–300.
35. Gurevich EV, Bordelon Y, Shapiro RM, Arnold SE, Gur RE, Joyce JN. Mesolimbic dopamine D3 receptors and use of antipsychotics in patients with schizophrenia. A postmortem study. Arch Gen Psychiatry 1997;54:225–32.
36. Diaz J, Ridray S, Mignon V, Griffon N, Schwartz JC, Sokoloff P. Selective expression of dopamine D3 receptor mRNA in proliferative zones during embryonic development of the rat brain. J Neurosci 1997;17:4282–92.
37. Araki KY, Sims JR., Bhide PG. Dopamine receptor mRNA and protein expression in the mouse corpus striatum and cerebral cortex during pre- and postnatal development. Brain Res 2007;1156:31–45.

38. Gurevich EV, Joyce JN. Dopamine D(3) receptor is selectively and transiently expressed in the developing whisker barrel cortex of the rat. J Comp Neurol 2000;420:35–51.
39. Gurevich EV, Robertson RT, Joyce JN. Thalamo-cortical afferents control transient expression of the dopamine D(3) receptor in the rat somatosensory cortex. Cereb Cortex 2001;11:691–701.
40. Rivera A, Trías S, Peñafiel A, et al. Expression of D4 dopamine receptors in striatonigral and striatopallidal neurons in the rat striatum. Brain Res 2003;989:35–41.
41. Rivera A, Cuéllar B, Girón FJ, Grandy DK, de la Calle A, Moratalla R. Dopamine D4 receptors are heterogeneously distributed in the striosomes/matrix compartments of the striatum. J Neurochem 2002;80:219–29.
42. Ciliax BJ, Nash N, Heilman C, et al. Dopamine D_5 receptor immunolocalization in rat and monkey brain. Synapse 2000;37:125–45.
43. Centonze D, Grande C, Usiello A, et al. Receptor subtypes involved in the presynaptic and postsynaptic actions of dopamine on striatal interneurons. J Neurosci 2003;23: 6545–54.
44. Khan ZU, Gutiérrez A, Martín R, Peñafiel A, Rivera A, de la Calle A. Dopamine D5 receptors of rat and human brain. Neuroscience 2000;100:689–99.
45. Centonze D, Grande C, Saulle E, et al. Distinct roles of D1 and D5 dopamine receptors in motor activity and striatal synaptic plasticity. J Neurosci 2003;23:8506–12.
46. Rinne JO, Laihinen A, NÂgren K, et al. PET demonstrates different behaviour of striatal dopamine D-1 and D-2 receptors in early Parkinson's disease. J Neurosci Res 1990;27: 494–9.
47. Rinne UK, Laihinen A, Rinne JO, NaÂgren K, Bergman J, Ruotsalainen U. Positron emission tomography demonstrates dopamine D_2 receptor supersensitivity in the striatum of patients with early Parkinson's disease. Mov Disord 1990;5:55–9.
48. Brooks DJ, Ibanez V, Sawle GV, et al. Striatal D_2 receptor status in patients with Parkinson's disease, striatonigral degeneration, and progressive supranuclear palsy, measured with [^{11}C]-raclopride and positron emission tomography. Ann Neurol 1992;31:184–92.
49. Laulumaa V, Kuikka JT, Soininen H, Bergstrom K, Lansimies E, Riekkinen P. Imaging of D2 dopamine receptors of patients with Parkinson's disease using single photon emission computed tomography and iodobenzamide I 123. Arch Neurol 1993;50:509–12.
50. SawleGV, Playford ED, Brooks DJ, Quinn N, Frackowiak RSJ. Asymmetrical pre-synaptic and post-synaptic changes in the striatal dopamine projection in dopa naive Parkinsonism. Diagnostic implications of the D2 receptor status. Brain 1993;116:853–67.
51. Antonini A, Schwarz J, Oertel WH, Pogarell O, Leenders KL. Long-term changes of striatal dopamine D_2 Receptors in patients with Parkinson's disease: A study with positron emission tomography and [^{11}C]Raclopride. Mov Disord 1997;12:33–8.
52. Turjanski N, Lees AJ, Brooks DJ. In vivo studies on striatal dopamine D1 and D2 site binding in L- dopa-treated Parkinson's disease patients with and without dyskinesias. Neurology 1997;49:717–23.
53. Kim YJ, Ichise M, Ballinger JR, et al. Combination of dopamine transporter and D2 receptor SPECT in the diagnostic evaluation of PD, MSA, and PSP. Mov Disord 2002;17: 303–12.
54. Thobois S, Vingerhoets F, Fraix V, et al. Role of dopaminergic treatment in dopamine receptor down-regulation in advanced Parkinson disease: A positron emission tomographic study. Arch Neurol 2004;61:1705–9.
55. Bokobza B, Ruberg M, Scatton B, Javoy-Agid F, Agid Y. [3H]spiperone binding, dopamine and HVA concentrations in Parkinson's disease and supranuclear palsy. Eur J Pharmacol 1984;99:167.
56. Joyce JN. Differential response of striatal dopamine and muscarinic cholinergic receptor subtypes to the loss of dopamine. III. Results in Parkinson's disease cases. Brain Res 1993;600:156.
57. Piggott MA, Marshall EF, Thomas N, et al. Striatal dopaminergic markers in dementia with Lewy bodies, Alzheimer's and Parkinson's diseases: rostrocaudal distribution. Brain 1999;122:1449–68.

58. Ryoo HL, Pierrotti D, Joyce JN. Dopamine D3 receptor is decreased and D2 receptor is elevated in the striatum of Parkinson's disease. Mov Disord 1998;13:788–97.
59. Goulet M, Morissette M, Calon F, et al. Continuous or pulsatile chronic D2 dopamine receptor agonist (U91356A) treatment of drug-naive 4-phenyl-1,2,3,6-tetrahydropyridine monkeys differentially regulates brain D1 and D2 receptor expression: in situ hybridization histochemical analysis. Neuroscience 1997;79(2):497.
60. Bychkov E, Ahmed MR., Dalby KN, Gurevich EV. Dopamine depletion and subsequent treatment with L-DOPA, but not the long-lived dopamine agonist pergolide, enhances activity of the Akt pathway in the rat striatum. J Neurochem 2007;102:699–711.
61. Herrero MT, Augood SJ, Asensi H, et al. Effects of -DOPA-therapy on dopamine D2 receptor mRNA expression in the striatum of MPTP-intoxicated parkinsonian monkeys. Mol Brain Res 1996;42:149.
62. Morissette M, Goulet M, Calon F, et al. Changes of D1 and D2 dopamine receptor mRNA in the brains of monkeys lesioned with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine: Correction with chronic administration of L-3,4-dihydroxyphenylalanine. Mol Pharmacol 1996;50:1073–9.
63. Bezard E, Dovero S, Prunier C, et al. Relationship between the appearance of symptoms and the level of nigrostriatal degeneration in a progressive 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-lesioned macaque model of Parkinson's disease. J Neurosci 2001;21:6853–61.
64. Shinotoh H, Inoue O, Hirayama K, et al. Dopamine D1 receptors in Parkinson's disease and striatonigral degeneration: A positron emission tomography study. J Neurol Neurosurg Psychiat 1993;56:467–72.
65. Lévesque D, Martres M-P, Diaz J, et al. A paradoxical regulation of the dopamine D3 receptor expression suggests the involvement of an anterograde factor from dopamine neurons. Proc Nat Acad Sci USA 1995;92:1719–23.
66. Bordet R, Ridray S, Carboni C, Diaz J, Sokoloff P, Schwartz JC. Induction of dopamine D3 receptor expression as a mechanism of behavioral sensitization to levodopa. Proc Natl Acad Sci USA 1997;94:3363–7.
67. Bordet R, Ridray S, Schwartz JC, Sokoloff P. Involvement of the direct striatonigral pathway in levodopa-induced sensitization in 6-hydroxydopamine-lesioned rats. Eur J Neurosci 2000;12:2117–23.
68. Quik M, Police S, He L, Di Monte DA, Langston JW. Expression of D(3) receptor messenger RNA and binding sites in monkey striatum and substantia nigra after nigrostriatal degeneration: effect of levodopa treatment. Neuroscience 2000;98:263–73.
69. Hirsch E, Graybiel AM, Agid YA. Melanized dopaminergic neurons are differentially susceptible to degeneration in Parkinson's disease. Nature 1988;334:345–8.
70. Goto S, Hirano A, Matsumoto S. Subdivisional involvement of nigrostriatal loop in idiopathic Parkinson's disease and striatonigral degeneration. Ann Neurol 1989;26: 766–70.
71. Gibb WR, Lees AJ. Anatomy, pigmentation, ventral and dorsal subpopulations of the substantia nigra, and differential cell death in Parkinson's disease. J Neurol Neurosurg Psychiatry 1991;54:388–96.
72. Bychkov E, Gurevich VV, Joyce JN, Benovic JL, Gurevich EV. Arrestins and two receptor kinases are upregulated in Parkinson's disease with dementia. Neurobiol Aging 2008;29:379–96.
73. Joyce JN, Ryoo HL, Beach TB, et al. Loss of response to levodopa in Parkinson's disease and co-occurrence with dementia: role of D3 and not D2 receptors. Brain Res 2002;955: 138–52.
74. Kaasinen V, Aalto S, Någren K, Hietala J, Sonninen P, Rinne JO. Extrastriatal dopamine D2 receptors in Parkinson's disease: a longitudinal study. J Neural Transm 2003;110:591–601.
75. Ungerstedt U. Postsynaptic supersensitivity after 6-hydroxydopamine induced degeneration of the nigrostriatal dopamine system. Acta Physiol Scand Suppl 1971;367:69–93.

76. Kim DS, Szczypka MS, Palmiter RD. Dopamine-deficient mice are hypersensitive to dopamine receptor agonists. J Neurosci 2000;20:4405–13.
77. Cai G, Wang HY, Friedman E. Increased dopamine receptor signaling and dopamine receptor-G protein coupling in denervated striatum. J Pharmacol Exp Ther 2002;302: 1105–12.
78. Corvol J-C, Muriel M-P, Valjent E, et al. Persistent increase in olfactory type G-protein alpha subunit levels may underlie D1 receptor functional hypersensitivity in Parkinson disease. J Neurosci 2004;24:7007–14.
79. Mishra RK, Gardner EL, Katzman R, Makman MH. Enhancement of dopamine-stimulated adenylate cyclase activity in rat caudate after lesions in substantia nigra: evidence for denervation supersensitivity. Proc Natl Acad Sci USA 1974;71:3883–7.
80. Mishra RK, Marshall AM, Varmuza SL. Supersensitivity in rat caudate nucleus: effects of 6-hydroxydopamine on the time course of dopamine receptor and cyclic AMP changes. Brain Res 1980;200:47–57.
81. Pifl C, Nanoff C, Schingnitz G, Schütz W, Hornykiewicz O. Sensitization of dopamine-stimulated adenylyl cyclase in the striatum of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-treated rhesus monkeys and patients with idiopathic Parkinson's disease. J Neurochem 1992;58:1997–2004.
82. Pifl C, Reither H, Hornykiewicz O. Functional sensitization of striatal dopamine D1 receptors in the 6-hydroxydopamine-lesioned rat. Brain Res 1992;572:87–93.
83. Satoh H, Satoh Y, Notsu Y, Honda F. Adenosine 3′,5′-cyclic monophosphate as a possible mediator of rotational behaviour induced by dopaminergic receptor stimulation in rats lesioned unilaterally in the substantia nigra. Eur J Pharmacol 1976;39:365–77.
84. Tong J, Fitzmaurice PS, Ang LC, Furukawa Y, Guttman M, Kish SJ. Brain dopamine-stimulated adenylyl cyclase activity in Parkinson's disease, multiple system atrophy, and progressive supranuclear palsy. Ann Neurol 2004;55:125–9.
85. Santini E, Valjent E, Usiello A, et al. Critical involvement of cAMP/DARPP-32 and extracellular signal-regulated protein kinase signaling in L-DOPA-induced dyskinesia. J Neurosci 2007;27:6995–7005.
86. Ouimet CC, Greengard P. Distribution of DARPP-32 in the basal ganglia: an electron microscopic study. J Neurocytol 1990;19:39–52.
87. Hemmings HCJ, Williams RR, Konigsberg WH, Greengard P. DARPP-32, a dopamine- and adenosine 3′:5′-monophosphate-regulated neuronal phosphoprotein. I. Amino acid sequence around the phosphorylated threonine. J Biol Chem 1984;259:14486–90.
88. Fienberg AA, Hiroi N, Mermelstein PG, et al. DARPP-32: Regulator of the efficacy of dopaminergic neurotransmission. Science 1998;281:838–42.
89. Bibb JA, Snyder GL, Nishi A, et al. Phosphorylation of DARPP-32 by Cdk5 modulates dopamine signalling in neurons. Nature 1999;402:669.
90. Nishi A, Bibb JA, Snyder GL, Higashi H, Nairn AC, Greengard P. Amplification of dopaminergic signaling by a positive feedback loop. Proc Natl Acad Sci USA 2000;97: 12840–5.
91. Brown A, Deutch AY, Colbran RJ. Dopamine depletion alters phosphorylation of striatal proteins in a model of Parkinsonism. Eur J Neurosci 2005;22:247–56.
92. Svenningsson P, Lindskog M, Ledent C, et al. Regulation of the phosphorylation of the dopamine- and cAMP-regulated phosphoprotein of 32 kDa in vivo by dopamine D1, dopamine D2, and adenosine A2A receptors. Proc Natl Acad Sci USA 2000;97:1856–60.
93. Westin JE, Vercammen L, Strome EM, Konradi C, Cenci MA. Spatiotemporal pattern of striatal ERK1/2 phosphorylation in a rat model of L-DOPA-induced dyskinesia and the role of dopamine D1 receptors. Biol Psychiatry 2007;62:800–10.
94. Emborg-Knott ME, Domino EF. MPTP-induced hemiparkinsonism in nonhuman primates 6–8 years after a single unilateral intracarotid dose. Exp Neurol 1998;152:214–20.
95. Aubert I, Guigoni C, Hakansson K, et al. Increased D1 dopamine receptor signaling in levodopa-induced dyskinesia. Ann Neurol 2005;57:17–26.

96. Bezard E, Gross CE, Qin L, Gurevich VV, Benovic JL, Gurevich EV. L-DOPA reverses the MPTP-induced elevation of the arrestin2 and GRK6 expression and enhanced ERK activation in monkey brain. Neurobiol Dis 2005;18:323–35.
97. Svenningsson P, Arts J, Gunne L, Andren PE. Acute and repeated treatment with -DOPA increase c-jun expression in the 6-hydroxydopamine-lesioned forebrain of rats and common marmosets. Brain Res 2002;955:8–15.
98. Hess J, Angel P, Schorpp-Kistner M.AP-1 subunits: quarrel and harmony among siblings. J Cell Sci 2004;117:5965–73.
99. Chinenov Y, Kerppola TK. Close encounters of many kinds: Fos-Jun interactions that mediate transcription regulatory specificity. Oncogene 2001;20:2438–52.
100. Berke JD, Paletzki R, Aronson GJ, Hyman SE, Gerfen CR. A complex program of striatal gene expression induced by dopaminergic stimulation. J Neurosci 1998;18:5301–10.
101. Cenci MA, Tranberg A, Andersson M, Hilbertson A. Changes in the regional and compartmental distribution of FosB- and JunB-like immunoreactivity induced in the dopamine-denervated rat striatum by acute or chronic L-DOPA treatment. Neuroscience 1999;94:515.
102. Valastro B, Andersson M, Lindgren HS, Cenci MA. Expression pattern of JunD after acute or chronic L-DOPA treatment: Comparison with DeltaFosB. Neuroscience 2007;144:198–207.
103. Shaywitz AJ, Greenberg ME. CREB: A stimulus-induced transcription factor activated by a diverse array of extracellular signals. Ann Rev Biochem 1999;68:821–61.
104. Mayr B, Montminy M. Transcriptional regulation by the phosphorylation-dependent factor CREB. Nat Rev Mol Cell Biol 2001;2:599–609.
105. OhJD, Chartisathian K, Ahmed SM, Chase TN. Cyclic AMP responsive element binding protein phosphorylation and persistent expression of levodopa-induced response alterations in unilateral nigrostriatal 6-OHDA lesioned rats. J Neurosci Res 2003;72:768–80.
106. Cole DG, Kobierski LA, Konradi C, Hyman SE. 6-Hydroxydopamine lesions of rat substantia nigra up-regulate dopamine-induced phosphorylation of the cAMP-response element-binding protein in striatal neurons. Proc Natl Acad Sci USA 1994;91:9631–5.
107. Konradi C, Cole RL, Heckers S, Hyman SE. Amphetamine regulates gene expression in rat striatum via transcription factor CREB. J Neurosci 1994;14:5623–34.
108. Andersson M, Konradi C, Cenci MA. cAMP response element-binding protein is required for dopamine-dependent gene expression in the intact but not the dopamine-denervated striatum. J Neurosci 2001;21:9930–43.
109. Picconi B, Gardoni F, Centonze D, et al. Abnormal Ca2+-calmodulin-dependent protein kinase II function mediates synaptic and motor deficits in experimental parkinsonism. J Neurosci 2004;24:5283–91.
110. Cull-Candy S, Brickley S, Farrant M. NMDA receptor subunits: diversity, development and disease. Curr Opin Neurobiol 2001;11:327.
111. Chen B-S, Roche KW. Regulation of NMDA receptors by phosphorylation. Neuropharmacology 2007;53:362.
112. Dunah AW, Wang Y, Yasuda RP, et al. Alterations in subunit expression, composition, and phosphorylation of striatal N-methyl-D-aspartate glutamate receptors in a rat 6-hydroxydopamine model of Parkinson's disease. Mol Pharmacol 2000;57: 342–52.
113. Hallett PJ, Dunah AW, Ravenscroft P, et al. Alterations of striatal NMDA receptor subunits associated with the development of dyskinesia in the MPTP-lesioned primate model of Parkinson's disease. Neuropharmacology 2005;48:503–16.
114. Fiorentini C, Rizzetti MC, Busi C, et al. Loss of synaptic D1 dopamine/N-methyl-D-aspartate glutamate receptor complexes in L-DOPA-induced dyskinesia in the rat. Mol Pharmacol 2006;69:805–12.
115. Oh JD, Vaughan CL, Chase TN. Effect of dopamine denervation and dopamine agonist administration on serine phosphorylation of striatal NMDA receptor subunits. Brain Res 1999;821:433–42.
116. Gardoni F, Picconi B, Ghiglieri V, et al. A Critical Interaction between NR2B and MAGUK in L-DOPA Induced Dyskinesia. J Neurosci 2006;26:2914–22.

117. Oh JD, Russell DS, Vaughan CL, Chase TN. Enhanced tyrosine phosphorylation of striatal NMDA receptor subunits: effect of dopaminergic denervation and L-DOPA administration. Brain Res 1998;813:150–9.
118. Ahmed MR, Bychkov E, Gurevich VV, Benovic JL, Gurevich EV. Altered expression and subcellular distribution of GRK subtypes in the dopamine-depleted rat basal ganglia is not normalized by L-DOPA treatment. J Neurochem 2007;104:1622–36.
119. Nash JE, Johnston TH, Collingridge GL, Garner CC, Brotchie JM. Subcellular redistribution of the synapse-associated proteins PSD-95 and SAP97 in animal models of Parkinson's disease and L-DOPA-induced dyskinesia. FASEB J 2005;19:583–5.
120. Picconi B, Centonze D, Hakansson K, et al. Loss of bidirectional striatal synaptic plasticity in L-DOPA-induced dyskinesia. Nat Neurosci 2003;6:501–6.
121. Picconi B, Paille V, Ghiglieri V, et al. L-DOPA dosage is critically involved in dyskinesia via loss of synaptic depotentiation. Neurobiol Dis 2008;29:327.
122. Wang H, Katz J, Dagostino P, Soghomonian JJ. Unilateral 6-hydroxydopamine lesion of dopamine neurons and subchronic L-DOPA administration in the adult rat alters the expression of the vesicular GABA transporter in different subsets of striatal neurons and in the substantia nigra, pars reticulata. Neuroscience 2007;145:727–37.
123. Soghomonian J-J, Laprade N. Glutamate decarboxylase (GAD67 and GAD65) gene expression is increased in a subpopulation of neurons in the putamen of parkinsonian monkeys. Synapse 1997;27:122–32.
124. Carta AR, Fenu S, Pala P, Tronci E, Morelli M. Selective modifications in GAD67 mRNA levels in striatonigral and striatopallidal pathways correlate to dopamine agonist priming in 6-hydroxydopamine-lesioned rats. Eur J Neurosci 2003;18:2563–72.
125. Lindgren HS, Rylander D, Ohlin KE, Lundblad M, Cenci MA. The "motor complication syndrome" in rats with 6-OHDA lesions treated chronically with L-DOPA: relation to dose and route of administration. Behav Brain Res 2007;177:150–9.
126. Engber TM, Marin C, Susel Z, Chase TN. Differential effects of chronic dopamine D1 and D2 receptor agonists on rotational behavior and dopamine receptor binding. Eur J Pharmacol 1993;236:385–93.
127. Löschmann PA, Wüllner U, Heneka MT, et al. Differential interaction of competitive NMDA and AMPA antagonists with selective dopamine D-1 and D-2 agonists in a rat model of Parkinson's disease. Synapse 1997;26:381–91.
128. Paul ML, Graybiel AM, David JC, Robertson HA. D1-like and D2-like dopamine receptors synergistically activate rotation and c-fos expression in the dopamine-depleted striatum in a rat model of Parkinson's disease. J Neurosci 1992;12:3729–42.
129. Cai G, Zhen X, Uryu K, Friedman E. Activation of extracellular signal-regulated protein kinases is associated with a sensitized locomotor response to D(2) dopamine receptor stimulation in unilateral 6-hydroxydopamine-lesioned rats. J Neurosci 2000;20:1849–57.
130. Newman-Tancredi A, Cussac D, Brocco M, et al. Dopamine D2 receptor-mediated G-protein activation in rat striatum: functional autoradiography and influence of unilateral 6-hydroxydopamine lesions of the substantia nigra. Brain Res 2001;920:41.
131. LaHoste GJ, Marshall JF. Rapid development of D1 and D2 dopamine receptor supersensitivity as indicated by striatal and pallidal Fos expression. Neurosci Lett 1994;179:153–6.
132. Marshal JF, Cole BN, LaHoste GJ. Dopamine D2 receptor control of pallidal fos expression: comparisons between intact and 6-hydroxydopamine-treated hemispheres. Brain Res 1993;632:308–13.
133. Zhen X, Torres C, Cai G, Friedman E. Inhibition of protein tyrosine/mitogen-activated protein kinase phosphatase activity is associated with D2 dopamine receptor supersensitivity in a rat model of Parkinson's disease. Mol Pharmacol 2002;62:1356–63.
134. Bamford NS, Robinson S, Palmiter RD, Joyce JA, Moore C, Meshul CK. Dopamine modulates release from corticostriatal terminals. J Neurosci 2004;24:9541–52.

135. Picconi B, Centonze D, Rossi S, Bernardi G, Calabresi P. Therapeuticdoses of L-dopa reverse hypersensitivity of corticostriatal D2-dopamine receptors and glutamatergic overactivity in experimental parkinsonism. Brain 2004;127:1661–9.
136. Lindefors N. Dopaminergic regulation of glutamic acid decarboxylase mRNA expression and GABA release in the striatum: A review. Progr Neuro-Psychopharmacol Biol Psychiatry 1993;17:887.
137. Gerfen CR, McGinty JF, Young WS. Dopamine differentially regulates dynorphin, substance P, and enkephalin expression in striatal neurons: in situ hybridization histochemical analysis. J Neurosci 1991;11:1016–31.
138. Henry B, Crossman AR, Brotchie JM. Effect of repeated L-DOPA, bromocriptine, or lisuride administration on preproenkephalin-A and preproenkephalin-B mRNA levels in the striatum of the 6-hydroxydopamine-lesioned rat. Exp Neurol 1999;155:204–20.
139. Herrero MT, Augood SJ, Hirsch EC, et al. Effects of L-DOPA on preproenkephalin and preprotachykinin gene expression in the MPTP-treated monkey striatum. Neuroscience 1995;68:1189–98.
140. Morissette M, Goulet M, Soghomonian JJ, et al. Preproenkephalin mRNA expression in the caudate-putamen of MPTP monkeys after chronic treatment with the D2 agonist U91356A in continuous or intermittent mode of administration: comparison with L-DOPA therapy. Brain Res Mol Brain Res 1997;49:55–62.
141. Morissette M, Grondin R, Goulet M, Bédard PJ, Di Paolo T. Differential regulation of striatal preproenkephalin and preprotachykinin mRNA levels in MPTP-lesioned monkeys chronically treated with dopamine D1 or D2 agonists. J Neurochem 1999;72: 682–92.
142. Westin JE, Andersson M, Lundblad M, Cenci MA. Persistent changes in striatal gene expression induced by long-term L-DOPA treatment in a rat model of Parkinson's disease. Eur J Neurosci 2001;14:1171–6.
143. St-Hilaire M, Bourhis E, Lévesque D, Rouillard C. Impaired behavioural and molecular adaptations to dopamine denervation and repeated L-DOPA treatment in Nur77-knockout mice. Eur J Neurosci 2006;24:795–805.
144. St-Hilaire M, Landry E, Levesque D, Rouillard C. Denervation and repeated L-DOPA induce a coordinate expression of the transcription factor NGFI-B in striatal projection pathways in hemi-parkinsonian rats. Neurobiol Dis 2003;14:98–109.
145. St-Hilaire M, Landry E, Levesque D, Rouillard C. Denervation and repeated L-DOPA induce complex regulatory changes in neurochemical phenotypes of striatal neurons: Implication of a dopamine D1-dependent mechanism. Neurobiol Dis 2005;20:450.
146. Ethier I, Beaudry G, St-Hilaire M, Milbrandt J, Rouillard C, Lévesque D. The transcription factor NGFI-B (Nur77) and retinoids play a critical role in acute neuroleptic-induced extrapyramidal effect and striatal neuropeptide gene expression. Neuropsychopharmacology 2004;29:335–46.
147. Scholz B, Svensson M, Alm H, et al. Striatal proteomic analysis suggests that first L-Dopa dose equates to chronic exposure. PLoS ONE 2008;3:e1589.
148. Pollack AE, Yates TM. Prior D1 dopamine receptor stimulation is required to prime D2-mediated striatal Fos expression in 6-hydroxydopamine-lesioned rats. Neuroscience 1999;94:505–14.
149. Pollack AE, Turgeon SM, Fink JS. Apomorphine priming alters the response of striatal outflow pathways to D2 agonist stimulation in 6-hydroxydopamine-lesioned rats. Neuroscience 1997;79:79.
150. Pollack AE, Strauss JB. Time dependence and role of N-methyl-D-aspartate glutamate receptors in the priming of D2-mediated rotational behavior and striatal Fos expression in 6-hydroxydopamine lesioned rats. Brain Res 1999;827:160–8.
151. Paul ML, Currie RW, Robertson HA. Priming of a D1 dopamine receptor behavioural response is dissociated from striatal immediate-early gene activity. Neuroscience 1995;66:347–59.

152. Wang Z, Kai L, Day M, et al. Dopaminergic control of corticostriatal long-term synaptic depression in medium spiny neurons is mediated by cholinergic interneurons. Neuron 2006;50:443–52.
153. Herve D, Levi-Strauss M, Marey-Semper I, et al. Golf and Gs in rat basal ganglia: possible involvement of Golf in the coupling of dopamine D1 receptor with adenylyl cyclase. J Neurosci 1993;13:2237–48.
154. Corvol JC, Studler JM, Schonn JS, Girault JA, Herve D. Gaolf is necessary for coupling D1 and A2a receptors to adenylyl cyclase in the striatum. J Neurochem 2001;76:1585–8.
155. Herve D, Le Moine C, Corvol J-C, et al. Gaolf levels are regulated by receptor usage and control dopamine and adenosine action in the striatum. J Neurosci 2001;21:4390–9.
156. Nishino N, Kitamura N, Hashimoto T, Tanaka C. Transmembrane signalling systems in the brain of patients with Parkinson's disease. Rev Neurosci 1993;4:213–22.
157. Gurevich VV, Gurevich EV. The structural basis of arrestin-mediated regulation of G-protein-coupled receptors. Pharmacology & Therapeutics 2006;110:465–502.
158. Krasel C, Vilardaga JP, Bunemann M, Lohse MJ. Kinetics of G-protein-coupled receptor signalling and desensitization. Biochem Soc Trans 2004;32:1029–31.
159. Violin JD, DiPilato LM, Yildirim N, Elston TC, Zhang J, Lefkowitz RJ. β2-Adrenergic receptor signaling and desensitization elucidated by quantitative modeling of real time cAMP dynamics. J Biol Chem 2008;283:2949–61.
160. Bohn LM, Gainetdinov RR, Sotnikova TD, et al. Enhanced rewarding properties of morphine, but not cocaine, in beta-arrestin-2 knock-out mice. J Neurosci 2003;23(32):10265–73.
161. Bohn LM, Lefkowitz RJ, Caron MG. Differential mechanisms of morphine antinociceptive tolerance revealed in beta-arrestin-2 knock-out mice. J Neurosci 2002;22:10494–500.
162. Bohn LM, Lefkowitz RJ, Gainetdinov RR, Peppel K, Caron MG, Lin FT. Enhanced morphine analgesia in mice lacking beta-arrestin2. Science 1999;286:2495–8.
163. Gainetdinov RR, Bohn LM, Sotnikova TD, et al. Dopaminergic supersensitivity in G protein-coupled receptor kinase 6-deficient mice. Neuron 2003;38:291–303.
164. Gainetdinov RR, Bohn LM, Walker JK, et al. Muscarinic supersensitivity and impaired receptor desensitization in G protein-coupled receptor kinase 5-deficient mice. Neuron 1999;24:1029–36.
165. Pan L, Gurevich EV, Gurevich VV. The nature of the arrestin x receptor complex determines the ultimate fate of the internalized receptor. J Biol Chem 2003;278:11623–32.
166. Willets JM, Nash MS, Challiss RA, Nahorski SR. Imaging of muscarinic acetylcholine receptor signaling in hippocampal neurons: evidence for phosphorylation-dependent and -independent regulation by G-protein-coupled receptor kinases. J Neurosci 2004;24:4157–62.
167. Willets JM, Parent JL, Benovic JL, Kelly E. Selective reduction in A2 adenosine receptor desensitization following antisense-induced suppression of G protein-coupled receptor kinase 2 expression. J Neurochem 1999;73:1781–9.
168. Berman DM, Gilman AG. Mammalian RGS proteins: Barbarians at the gate. J Biol Chem 1998;273:1269–72.
169. Ross EM, Wilkie TM. GTPase-activating proteins for heterotrimeric G proteins: regulators of G protein signaling (RGS) and RGS-like proteins. Annu Rev Biochem 2000;69:795–827.
170. Gold SJ, Ni YG, Dohlman HG, Nestler EJ. Regulators of G-protein signaling (RGS) proteins: Region-specific expression of nine subtypes in rat brain. J Neurosci 1997;17: 8024–37.
171. Granneman JG, Zhai Y, Zhu Z, et al. Molecular characterization of human and rat RGS 9L, a novel splice variant enriched in dopamine target regions, and chromosomal localization of the RGS 9 gene. Mol Pharmacol 1998;54:687–94.
172. Rahman Z, Gold SJ, Potenza MN, et al. Cloning and characterization of RGS9-2: A striatal-enriched alternatively spliced product of the RGS9 gene. J Neurosci 1999;19: 2016–26.
173. Kovoor A, Seyffarth P, Ebert J, et al. D2 dopamine receptors colocalize regulator of G-protein signaling 9-2 (RGS9-2) via the RGS9 DEP domain, and RGS9 knock-out mice develop dyskinesias associated with dopamine pathways. J Neurosci 2005;25:2157–65.

174. Rahman Z, Schwarz J, Gold SJ, et al. RGS9 modulates dopamine signaling in the basal ganglia. Neuron 2003;38:941.
175. Geurts M, Hermans E, Maloteaux JM. Opposite modulation of regulators of G protein signalling-2 RGS2 and RGS4 expression by dopamine receptors in the rat striatum. Neurosci Lett 2002;333:146–50.
176. Geurts M, Maloteaux JM, Hermans E. Altered expression of regulators of G-protein signaling (RGS) mRNAs in the striatum of rats undergoing dopamine depletion. Biochem Pharmacol 2003;66:1163–70.
177. Gold SJ, Hoang CV, Potts BW, et al. RGS9 2 negatively modulates L-3,4-dihydroxyphenylalanine-Induced dyskinesia in experimental Parkinson's disease. J Neurosci 2007;27:14338–48.
178. Tekumalla PK, Calon F, Rahman Z, et al. Elevated levels of DeltaFosB and RGS9 in striatum in Parkinson's disease. Biol Psychiatry 2001;50:813–6.
179. Doucet JP, Nakabeppu Y, Bedard PJ, et al. Chronic alterations in dopaminergic neurotransmission produce a persistent elevation of deltaFosB-like protein(s) in both the rodent and primate striatum. Eur J Neurosci 1996;8:365–81.
180. McClung CA, Ulery PG, Perrotti LI, Zachariou V, Berton O, Nestler EJ. DeltaFosB: a molecular switch for long-term adaptation in the brain. Brain Res Mol Brain Res 2004;132:146–54.
181. Hornykiewicz O. Basic research on dopamine in Parkinson's disease and the discovery of the nigrostriatal dopamine pathway: the view of an eyewitness. Neurodegener Dis 2008;5: 114–7.
182. Marsden CD, Parkes JD. Success and problems of long-term levodopa therapy in Parkinson's disease. Lancet 1977;309:345–9.
183. Asin KE, Bednarz L, Nikkel A, Perner R. Rotation and striatal c-fos expression after repeated, daily treatment with selective dopamine receptor agonists and levodopa. J Pharmacol Exp Ther 1995;273:1483–90.
184. Rascol O, Nutt JG, Blin O, et al. Induction by dopamine D1 receptor agonist ABT-431 of dyskinesia similar to levodopa in patients with Parkinson disease. Arch Neurol 2001;58: 249–54.
185. Blanchet PJ, Grondin R, Bédard PJ. Dyskinesia and wearing-off following dopamine D1 agonist treatment in drug-naive 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-lesioned primates. Mov Disord 1996;11:91–4.
186. Blanchet PJ, Konitsiotis S, Chase TN. Motor response to a dopamine D3 receptor preferring agonist compared to apomorphine in levodopa-primed 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine monkeys. J Pharmacol Exp Ther 1997;283:794–9.
187. Pearce RKB, Jackson M, Britton DR, Shiosaki K, Jenner P, Marsden CD. Actions of the D1 agonists A-77636 and A-86929 on locomotion and dyskinesia in MPTP-treated L-dopa-primed common marmosets. Psychopharmacology 1999;142:51–60.
188. Rascol O, Blin O, Thalamas C, et al. ABT-431, a D1 receptor agonist prodrug, has efficacy in Parkinson's disease. Ann Neurol 1999;45:736–41.
189. Mercuri NB, Giorgio Bernardi G. The 'magic' of L-dopa: why is it the gold standard Parkinson's disease therapy? Trends Pharmacol Sci 2005;26:341–4.
190. FabbriniG, Brotchie JM, Grandas F, Nomoto M, Goet ZCG. Levodopa-induced dyskinesias. Mov Disord 2007;22:1379–89.
191. Olanow CW, Obeso JA, Stocchi F. Continuous dopamine-receptor treatment of Parkinson's disease: scientific rationale and clinical implications. Lancet Neurol 2006;5:677.
192. Obeso JA, Rodriguez-Oroz MC, Rodrigue ZM, DeLong MR, Olanow CW. Pathophysiology of levodopa-induced dyskinesias in Parkinson's disease: problems with the current model. Ann Neurol 2000;47:S22–32.
193. Crossman AR. A hypothesis on the pathophysiological mechanisms that underlie levodopa- or dopamine agonist-induced dyskinesia in Parkinson's disease: Implications for future strategies in treatment. Mov Disord 1990;5:100–8.

194. Kim DS, Palmiter RD, Cummins A, Gerfen CR. Reversal of supersensitive striatal dopamine D1 receptor signaling and extracellular signal-regulated kinase activity in dopamine-deficient mice. Neuroscience 2006;137:1381–8.
195. Granata R, Wenning GK, Jolkkonen J, Jenner P, Marsden CD. Effect of repeated administration of dopamine agonists on striatal neuropeptide mRNA expression in rats with a unilateral nigral 6-hydroxydopamine lesion. J Neural Transm 1996;103:249–60.
196. Tel BC, Zeng BY, Cannizzaro C, Pearce RK, Rose S, Jenner P. Alterations in striatal neuropeptide mRNA produced by repeated administration of L-DOPA, ropinirole or bromocriptine correlate with dyskinesia induction in MPTP-treated common marmosets. Neuroscience 2002;115:1047–58.
197. Calon F, Birdi S, Rajput AH, Hornykiewicz O, Bédard PJ, Di Paolo T. Increase of preproenkephalin mRNA levels in the putamen of Parkinson disease patients with levodopa-induced dyskinesias. J Neuropathol Exp Neurol 2002;61:186–96.
198. Gerfen CR, Engber TM, Mahan L, et al. D1 and D2 dopamine receptor-regulated gene expression of striatonigral and striatopallidal neurons. Science 1990;250: 1429–32.
199. Beaulieu J-M, Tirotta E, Sotnikova TD, et al. Regulation of Akt signaling by D2 and D3 dopamine receptors in vivo. J Neurosci 2007;27:881–5.
200. Beaulieu JM, Gainetdinov RR, Caron MG. The Akt-GSK-3 signaling cascade in the actions of dopamine. Trends Pharmacol Sci 2007;28:166–72.
201. NewDC, Wu K, Kwok AWS, Wong YH. G protein-coupled receptor-induced Akt activity in cellular proliferation and apoptosis. FEBS J 2007;274:6025–36.
202. Rashid AJ, O'Dowd BF, Verma V, George SR. Neuronal Gq/11-coupled dopamine receptors: an uncharted role for dopamine. Trends Pharmacol Sci 2007;28:551–5.
203. Brami-Cherrier K, Valjent E, Garcia M, Pages C, Hipskind RA, Caboche J. Dopamine induces a PI3-Kinase-Independent activation of Akt in striatal neurons: A new route to cAMP response element-binding protein phosphorylation. J Neurosci 2002;22:8911–21.
204. Nielsen KM, Soghomonian JJ. Normalization of glutamate decarboxylase gene expression in the entopeduncular nucleus of rats with a unilateral 6-hydroxydopamine lesion correlates with increased gabaergic input following intermittent but not continuous levodopa. Neuroscience 2004;123:31.
205. Cenci MA, Lee CS, Björklund A.L-DOPA-induced dyskinesia in the rat is associated with striatal overexpression of prodynorphin- and glutamic acid decarboxylase mRNA. Eur J Neurosci 1998;10:2694–706.
206. Hurley MJ, Jackson MJ, Smith LA, Rose S, Jenner P. Immunoautoradiographic analysis of NMDA receptor subunits and associated postsynaptic density proteins in the brain of dyskinetic MPTP-treated common marmosets. Eur J Neurosci 2005;21:3240–50.
207. Calon F, Rajput AH, Hornykiewicz O, Bédard PJ, Di Paolo T. Levodopa-induced motor complications are associated with alterations of glutamate receptors in Parkinson's disease. Neurobiol Dis 2003;14:404–16.
208. Dunah AW, Standaert DG. Dopamine D1 receptor-dependent trafficking of striatal NMDA glutamate receptors to the postsynaptic membrane. J Neurosci 2001;21:5546–58.
209. Dunah AW, Sirianni AC, Fienberg AA, Bastia E, Schwarzschild MA, Standaert DG. Dopamine D1-dependent trafficking of striatal N-methyl-D-aspartate glutamate receptors requires Fyn protein tyrosine kinase but not DARPP-32. Mol Pharmacol 2004;65:121–9.
210. Hallett PJ, Spoelgen R, Hyman BT, Standaert DG, Dunah AW. Dopamine D1 activation potentiates striatal NMDA receptors by tyrosine phosphorylation-dependent subunit trafficking. J Neurosci 2006;26:4690–700.
211. Ahlskog JE, Muenter MD. Frequency of levodopa-related dyskinesias and motor fluctuations as estimated from the cumulative literature. Movement Disorders 2001;16:448–58.
212. Sgambato-Faure V, Buggia V, Gilbert F, Levesque D, Benabid AL, Berger F. Coordinated and spatial upregulation of arc in striatonigral neurons correlates with L-dopa-induced behavioral sensitization in dyskinetic rats. J Neuropathol Exp Neurol 2005;64: 936–47.

213. Cenci MA, Whishaw IQ, Schallert T. Animal models of neurological deficits: how relevant is the rat? Nat Rev Neurosci 2002;3:574–9.
214. Carta M, Lindgren HS, Lundblad M, Stancampiano R, Fadda F, Cenci MA. Role of striatal L-DOPA in the production of dyskinesia in 6-hydroxydopamine lesioned rats. J Neurochem 2006;96:1718–28.
215. Guigoni C, Dovero S, Aubert I, et al. Levodopa-induced dyskinesia in MPTP-treated macaques is not dependent on the extent and pattern of nigrostrial lesioning. Eur J Neurosci 2005;22:283–7.
216. Putterman DB, Munhall AC, Kozell LB, Belknap JK, Johnson SW. Evaluation of levodopa dose and magnitude of dopamine depletion as risk factors for levodopa-induced dyskinesia in a rat model of Parkinson's disease. J Pharmacol Exp Ther 2007;323:277–84.
217. Hauser RA, McDermott MP, Messing S. Factors associated with the development of motor fluctuations and dyskinesias in Parkinson disease. Arch Neurol 2006;63:1756–60.
218. Schrag A, Quinn N. Dyskinesias and motor fluctuations in Parkinson's disease: a community-based study. Brain 2000;123:2297–305.
219. Winkle RC, Kirik D, Björklund A, Cenci MA.L-DOPA-induced dyskinesia in the intrastriatal 6-hydroxydopamine model of Parkinson's disease: relation to motor and cellular parameters of nigrostriatal function. Neurobiol Dis 2002;10:165–86.
220. Pavon N, Martin AB, Mendialdua A, Moratalla R. ERK phosphorylation and FosB expression are associated with L-DOPA-induced dyskinesia in hemiparkinsonian mice. Biol Psychiatry 2006;59:64–74.
221. Hardingham GH, Fukunaga Y, Hilmar Bading H. Extrasynaptic NMDARs oppose synaptic NMDARs by triggering CREB shut-off and cell death pathways. Nat Neurosci 2002;5:405–14.
222. Andersson M, Hilbertson A, Cenci MA. Striatal fosB expression is causally linked with L-DOPA-induced abnormal involuntary movements and the associated upregulation of striatal prodynorphin mRNA in a rat model of Parkinson's disease. Neurobiol Dis 1999;6:461–74.
223. Cenci MA. Transcription factors involved in the pathogenesis of L-DOPA-induced dyskinesia in a rat model of Parkinson's disease. Amino Acids 2002;23:105–9.
224. Zachariou V, Sgambato-Faure V, Sasaki T, et al. Phosphorylation of DARPP-32 at Threonine-34 is required for cocaine action. Neuropsychopharmacology 2006;31:555–62.
225. Hiroi N, Fienberg AA, Haile CN, et al. Neuronal and behavioural abnormalities in striatal function in DARPP-32-mutant mice. Eur J Neurosci 1999;11:1114–8.
226. Hiroi N, Brown JR, Haile CN, Ye H, Greenberg ME, Nestler EJ. FosB mutant mice: loss of chronic cocaine induction of Fos-related proteins and heightened sensitivity to cocaine's psychomotor and rewarding effects. Proc Natl Acad Sci USA 1997;94:10397–402.
227. Kelz MB, Chen J, Carlezon WA, et al. Expression of the transcription factor DeltaFosB in the brain controls sensitivity to cocaine. Nature 1999;401:272.
228. Tzingounis AV, Nicoll RA. Arc/Arg3.1: linking gene expression to synaptic plasticity and memory. Neuron 2006;52:403–7.
229. Chowdhury S, Shepherd JD, Okuno H, et al. Arc/Arg3.1 interacts with the endocytic machinery to regulate AMPA receptor trafficking. Neuron 2006;52:445.
230. Shepherd JD, Rumbaugh G, Wu J, et al. Arc/Arg3.1 mediates homeostatic synaptic scaling of AMPA receptors. Neuron 2006;52:475.
231. Rial Verde EM, Lee-Osbourne J, Worley PF, Malinow R, Cline HT. Increased expression of the immediate-early gene Arc/Arg3.1 reduces AMPA receptor-mediated synaptic transmission. Neuron 2006;52:461.
232. Bibbiani F, Oh JD, Kielaite A, Collins MA, Smith C, Chase TN. Combined blockade of AMPA and NMDA glutamate receptors reduces levodopa-induced motor complications in animal models of PD. Exp Neurol 2005;196:422.
233. Shiraishi-Yamaguchi Y, Furuichi T. The Homer family proteins. Genome Biol 2007;8:206.
234. Kammermeier PJ, Worley PF. Homer 1a uncouples metabotropic glutamate receptor 5 from postsynaptic effectors. Proc Natl Acad Sci USA 2007;104:6055–60.

235. Tappe A, Kuner R. Regulation of motor performance and striatal function by synaptic scaffolding proteins of the Homer1 family. Proc Natl Acad Sci USA 2006;103:774–9.
236. Gerfen CR. The neostriatal mosaic: multiple levels of compartmental organization. Trends Neurosci 1992;15:133–9.
237. Prensa L, Parent A. The nigrostriatal pathway in the rat: A single-axon study of the relationship between dorsal and ventral tier nigral neurons and the striosome/matrix striatal compartments. J Neurosci 2001;21:7247–60.
238. Joel D, Weiner I. The connections of the dopaminergic system with the striatum in rats and primates: an analysis with respect to the functional and compartmental organization of the striatum. Neuroscience 2000;96:451.
239. Saka E, Elibol B, Erdem S, Dalkara T. Compartmental changes in expression of c-Fos and FosB proteins in intact and dopamine-depleted striatum after chronic apomorphine treatment. Brain Res 1999;825:104–14.
240. Bonuccelli U.Comparing dopamine agonists in Parkinson's disease. Curr Opin Neurol 2003;16(Suppl 1):S13–9.
241. Bonuccelli U, Pavese N. Dopamine agonists in the treatment of Parkinson's disease. Expert Rev Neurother 2006;6:81–9.
242. Foley P, Gerlach M, Double KL, Riederer P. Dopamine receptor agonists in the therapy of Parkinson's disease. J Neural Transm 2004;111:1375–446.
243. BibbianiF, Costantini LC, Pate lR, Chase TN. Continuous dopaminergic stimulation reduces risk of motor complications in parkinsonian primates. Exp Neurol 2005;192:7378.
244. Blanchet PJ, Calon F, Martel JC, et al. Continuous administration decreases and pulsatile administration increases behavioral sensitivity to a novel dopamine D2 agonist (U-91356a) in MPTP- exposed monkeys. J Pharmacol Exp Ther 1995;272:854–9.
245. Maratos EC, Jackson MJ, Pearce RK, Cannizzaro C, Jenner P. Both short- and long-acting D-1/D-2 dopamine agonists induce less dyskinesia than L-DOPA in the MPTP-lesioned common marmoset (Callithrix jacchus). Exp Neurol 2003;179:90–102.
246. Delfino M, Kalisch R, Czisch M, et al. Mapping the effects of three dopamine agonists with different dyskinetogenic potential and receptor selectivity using pharmacological functional magnetic resonance imaging. Neuropsychopharmacology 2007;32:1911.
247. Delfino MA, Stefano AV, Ferrario JE, Taravini IRE, Murer MG, Gershanik OS. Behavioral sensitization to different dopamine agonists in a parkinsonian rodent model of drug-induced dyskinesias. Behav Brain Res 2004;152:297.
248. Blanchet P, Bedard PJ, Britton DR, Kebabian JW. Differential effect of selective D-1 and D-2 dopamine receptor agonists on levodopa-induced dyskinesia in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine- exposed monkeys. J Pharmacol Exp Ther 1993;267:275–9.
249. Bianchi BR, Miller TR, Stashko MA, et al. Persistent activation of the dopamine D1 receptor contributes to prolonged receptor desensitization: Studies with A-77636. J Pharmacol Exp Ther 1996;276:1022–9.
250. Blanchet PJ, Grondin R, Bedard PJ, Shiosaki K, Britton DR. Dopamine D1 receptor desensitization profile in MPTP-lesioned primates. Eur J Pharmacol 1996;309:13.
251. Nutt JG. Continuous dopaminergic stimulation: Is it the answer to the motor complications of Levodopa? Mov Disord 2007;22:1–9.
252. Stocchi F, Olanow CW. Continuous dopaminergic stimulation in early and advanced Parkinson's disease. Neurology 2004;62(Suppl 1):S56–63.
253. Olanow CW, Obeso JA, Stocchi F. Drug insight: Continuous dopaminergic stimulation in the treatment of Parkinson's disease. Nat Clin Pract Neurol 2006;2:382–92.
254. Stocchi F, Vacca L, Ruggieri S, Olanow CW. Intermittent vs continuous levodopa administration in patients with advanced Parkinson disease: a clinical and pharmacokinetic study. Arch Neurol 2005;62:905–10.
255. Ravenscroft P, Chalon S, Brotchie JM, Crossman AR. Ropinirole versus L-DOPA effects on striatal opioid peptide precursors in a rodent model of Parkinson's disease: implications for dyskinesia. Exp Neurol 2004;185:36–46.

256. Jenner P. A novel dopamine agonist for the transdermal treatment of Parkinson's disease. Neurology 2005;65:S3–S5.
257. LeWitt PA, Lyons KE, Pahwa R, on behalf of the SPSG. Advanced Parkinson disease treated with rotigotine transdermal system: PREFER Study. Neurology 2007;68:1262–7.
258. Winkler JD, Weiss B. Reversal of supersensitive apomorphine-induced rotational behavior in mice by continuous exposure to apomorphine. J Pharmacol Exp Ther 1986;238:242–7.
259. Linazasoro G, Obeso JA, Gómez JC, Martínez M, Antonini A, Leenders KL. Modification of dopamine D2 receptor activity by pergolide in Parkinson's disease: An in vivo study by PET. Clin Neuropharmacol 1999;22:277–80.
260. Goulet M, Grondin R, Morissette M, et al. Regulation by chronic treatment with cabergoline of dopamine D1 and D2 receptor levels and their expression in the striatum of Parkinsonian-monkeys. Progr Neuro-Psychopharmacol Biol Psychiatry 2000;24:607.
261. Muriel MP, Bernard V, Levey AI, et al. Levodopa induces a cytoplasmic localization of D1 dopamine receptors in striatal neurons in Parkinson's disease. Ann Neurol 1999;46: 103–11.
262. Guigoni C, Doudnikoff E, Li Q, Bloch B, Bezard E. Altered D1 dopamine receptor trafficking in parkinsonian and dyskinetic non-human primates. Neurobiol Dis 2007;26:452.
263. Kim OJ, Gardner BR, Williams DB, et al. The role of phosphorylation in D1 dopamine receptor desensitization: evidence for a novel mechanism of arrestin association. J Biol Chem 2004;279:7999–8010.
264. Kim KM, Valenzano KJ, Robinson SR, Yao WD, Barak LS, Caron MG. Differential regulation of the dopamine D2 and D3 receptors by G protein-coupled receptor kinases and beta-arrestins. J Biol Chem 2001;276:37409–14.
265. Kim KM, Gainetdinov RR, Laporte SA, Caron MG, Barak LS. G protein-coupled receptor kinase regulates dopamine D3 receptor signaling by modulating the stability of a receptor-filamin-beta-arrestin complex. A case of autoreceptor regulation. J Biol Chem 2005;280:12774–80.
266. Cho DI, Beom S, Van Tol HH, Caron MG, Kim KM. Characterization of the desensitization properties of five dopamine receptor subtypes and alternatively spliced variants of dopamine D2 and D4 receptors. Biochem Biophys Res Commun 2006;350:634–40.
267. Szczypka MS, Rainey MA, Kim DS, et al. Feeding behavior in dopamine-deficient mice. Proc Natl Acad Sci USA 1999;96:12138–43.
268. Steige RM. Constant dopaminergic stimulation by transdermal delivery of dopaminergic drugs: a new treatment paradigm in Parkinson's disease. Eur J Neurol 2008;15:6–15.
269. Grace AA. Phasic versus tonic dopamine release and the modulation of dopamine system responsivity: A hypothesis for the etiology of schizophrenia. Neuroscience 1991;41:1.
270. West AR, Grace AA. Opposite influences of endogenous dopamine D1 and D2 receptor activation on activity states and electrophysiological properties of striatal neurons: studies combining in vivo intracellular recordings and reverse microdialysis. J Neurosci 2002;22:294–304.
271. Grace AA. The tonic/phasic model of dopamine system regulation and its implications for understanding alcohol and psychostimulant craving. Addiction 2000;95(Suppl 2):S119–S28.
272. Schultz W. Predictive reward signal of dopamine neurons. J Neurophysiol 1998;80:1–27.
273. de la Fuente-Fernández R, Sossi V, Huang Z, et al. Levodopa-induced changes in synaptic dopamine levels increase with progression of Parkinson's disease: implications for dyskinesias. Brain 2004;127:2747–54.
274. Miller DW, Abercrombie ED. Role of high-affinity dopamine uptake and impulse activity in the appearance of extracellular dopamine in striatum after administration of exogenous L-DOPA: studies in intact and 6-hydroxydopamine-treated rats. J Neurochem 1999;72: 1516–22.
275. Tedroff J, Pedersen M, Aquilonius SM, Hartvig P, Jacobsson G, Långström B. Levodopa-induced changes in synaptic dopamine in patients with Parkinson's disease as measured by [^{11}C]raclopride displacement and PET. Neurology 1996;46:1430–6.

276. Brooks DJ, Sagar H. Entacapone is beneficial in both fluctuating and non-fluctuating patients with Parkinson's disease: a randomised, placebo controlled, double blind, six month study. J Neurol Neurosurg Psychiatry 2003;74:1071–9.
277. Ahmed MR, Bychkov E, Gurevich EV. Lentivirus-mediated overexpression of GRK3 and 5 suppresses behavioral sensitization to L-DOPA in the 6-OHDA rat model of Parkinson's disease. Soc Neurosci Abstr San Diego, 2007.
278. Maratos EC, Jackson MJ, Pearce RKB, Jenner P. Antiparkinsonian activity and dyskinesia risk of ropinirole and L-DOPA combination therapy in drug naive MPTP-lesioned common marmosets (Callithrix jacchus). Mov Disord 2001;16:631–41.
279. Verhagen Metman L, Locatelli ER, Bravi D, Mouradian MM, Chase TN. Apomorphine responses in Parkinson's disease and the pathogenesis of motor complications. Neurology 1997;48:369–72.
280. Grandas F, Gancher ST, Rodriguez M, Lera G, Nutt JG, Obeso JA. Differences in the motor response to apomorphine between untreated and fluctuating patients with Parkinson's disease. Clin Neuropharmacol 1992;15:13–8.
281. Bravi D, Mouradian MM, Roberts JW, Davis TL, Sohn YH, Chase TN. Wearing-off fluctuations in Parkinson's disease: Contribution of postsynaptic mechanisms. Ann Neurol 1994;36:27–31.
282. Colosimo C, Merello M., Hughes AJ, Sieradzan K, Lees AJ. Motor response to acute dopaminergic challenge with apomorphine and levodopa in Parkinson's disease: implications for the pathogenesis of the on-off phenomenon. J Neurol Neurosurg Psychiatry 1996;60:634–7.
283. Harder S, Baas H. Concentration-response relationship of levodopa in patients at different stages of Parkinson's disease. Clin Pharmacol Ther 1998;64:183–91.
284. Nutt JG, Carter JH, Lea ES, Sexton GJP. Evolution of the response to levodopa during the first 4 years of therapy. Ann Neurol 2002;51:686–93.
285. Voon V, Potenza MN, Thomsen T. Medication-related impulse control and repetitive behaviors in Parkinson's disease. Curr Opin Neurol 2007;20:484–92.
286. Nirenberg MJ, Waters C. Compulsive eating and weight gain related to dopamine agonist use. Mov Disord 2006;21:524–9.
287. Weintraub D, Potenza MN. Impulse control disorders in Parkinson's disease. Curr Neurol Neurosci Rep 2006;6:302–6.
288. Freed CR, Greene PE, Breeze RE, et al. Transplantation of embryonic dopamine neurons for severe Parkinson's disease. N Engl J Med 2001;344:710–9.
289. Hagell P, Piccini P, Björklund A, et al. Dyskinesias following neural transplantation in Parkinson's disease. Nat Neurosci 2002;5:627–8.
290. Olanow WC, Goetz CG, Kordower JH, et al. A double-blind controlled trial of bilateral fetal nigral transplantation in Parkinson's disease. Ann Neurol 2003;54:403–14.
291. OhJD, Geller AI, Zhang G, Chase TN. Gene transfer of constitutively active protein kinase C into striatal neurons accelerates onset of levodopa-induced motor response alterations in parkinsonian rats. Brain Res 2003;971:18–30.
292. Mazzella L, Yahr MD, Marinelli L, Huang N, Moshier E, Di Rocco A. Dyskinesias predict the onset of motor response fluctuations in patients with Parkinson's disease on L-dopa monotherapy. Parkinsonism Relat Disord 2005;11:151–5.
293. Bové J, Serrats J, Mengod G, Cortés R, Aguilar E, Marin C. Reversion of levodopa-induced motor fluctuations by the A2A antagonist CSC is associated with an increase in striatal preprodynorphin mRNA expression in 6-OHDA-lesioned rats. Synapse 2006;59:435–44.
294. Wessell RH, Ahmed SM, Menniti FS, Dunbar GL, Chase TN, Oh JD. NR2B selective NMDA receptor antagonist CP-101,606 prevents levodopa-induced motor response alterations in hemi-parkinsonian rats. Neuropharmacology 2004;47:184–94.
295. Bibbiani F, Oh JD, Petzer JP, et al. A2A antagonist prevents dopamine agonist-induced motor complications in animal models of Parkinson's disease. Exp Neurol 2003;184: 285–94.

296. Oh JD, Bibbiani F, Chase TN. Quetiapine attenuates levodopa-induced motor complications in rodent and primate parkinsonian models. Exp Neurol 2002;177:557–64.
297. Hurley MJ, Jenner P. What has been learnt from study of dopamine receptors in Parkinson's disease? Pharmacol Ther 2006;111:715.
298. Meador-Woodruff JH, Damask SP, Watson SJJ. Differential expression of autoreceptors in the ascending dopamine systems of the human brain. Proc Natl Acad Sci USA 1994;91:8297–301.
299. Mrzljak L, Bergson C, Pappy M, Huff R, Levenson R, Goldman-Rakic PS. Localization of dopamine D4 receptors in GABAergic neurons of the primate brain. Nature 1996;381: 245–8.
300. Svingos AL, Periasamy S, Pickel VM. Presynaptic dopamine D(4) receptor localization in the rat nucleus accumbens shell. Synapse 2000;36:222–32.
301. Caillé I, Dumartin B, Bloch B. Ultrastructural localization of D1 dopamine receptor immunoreactivity in rat striatonigral neurons and its relation with dopaminergic innervation. Brain Res 1996;730:17–31.
302. Beischlag TV, Marchese A, Meador-Woodruff JH, et al. The human dopamine D5 receptor gene: cloning and characterization of the 5′-flanking and promoter region. Biochemistry 1995;34:5960–70.
303. Moore DJ, West AB, Dawson VL, Dawson TM. Molecular pathophysiology of Parkinson's disease. Annu Rev Neurosci 2005;28:57–87.
304. Mytilineou C, Han SK, Cohen G. Toxic and protective effects of L-dopa on mesencephalic cell cultures. J Neurochem 1993;61:1470–8.
305. Michel PP, Hefti F. Toxicity of 6-hydroxydopamine and dopamine for dopaminergic neurons in culture. J Neurosci Res 1990;26:428–35.
306. Steece-Collier K, Collier TJ, Sladek CD, Sladek JRJ. Chronic levodopa impairs morphological development of grafted embryonic dopamine neurons. Exp Neurol 1990;110: 201–8.
307. Mena MA, Pardo B, Paino CL, De Yebenes JG. Levodopa toxicity in foetal rat midbrain neurones in culture: modulation by ascorbic acid. Neuroreport 1993;4:438–40.
308. Pardo B, Mena MA, Casarejos MJ, Paíno CL, De Yébenes JG. Toxic effects of L-DOPA on mesencephalic cell cultures: protection with antioxidants. Brain Res 1995;682:133–43.
309. Han SK, Mytilineou C, Cohen G. L-DOPA up-regulates glutathione and protects mesencephalic cultures against oxidative stress. J Neurochem 1996;66:501–10.
310. Ling ZD, Pieri SC, Carvey PM. Comparison of the neurotoxicity of dihydroxyphenylalanine stereoisomers in cultured dopamine neurons. Clin Neuropharmacol 1996;19:360–5.
311. MenaMA, Casarejos MJ, Carazo A, Paíno CL, García de Yébenes J. Glia protect fetal midbrain dopamine neurons in culture from L-DOPA toxicity through multiple mechanisms. J Neural Transm 1997;104:317–28.
312. Mena MA, Davila V, Sulzer D. Neurotrophic effects of L-DOPA in postnatal midbrain dopamine neuron/cortical astrocyte cocultures. J Neurochem 1997;69:1398–408.
313. Mena MA, Davila V, Bogaluvsky J, Sulzer D. A synergistic neurotrophic response to L-dihydroxyphenylalanine and nerve growth factor. Mol Pharmacol 1998;54:678–86.
314. Chen J, Wersinger C, Sidhu A. Chronic stimulation of D1 dopamine receptors in human SK-N-MC neuroblastoma cells induces nitric-oxide synthase activation and cytotoxicity. J Biol Chem 2003;278:28089–100.
315. Chen J, Rusnak M, Luedtke RR, Sidhu A. D1 Dopamine Receptor Mediates Dopamine-induced Cytotoxicity via the ERK Signal Cascade. J Biol Chem 2004;279:39317–30.
316. Mytilineou C, Walker RH, JnoBaptiste R, Olanow CW. Levodopa is toxic to dopamine neurons in an in vitro but not an in vivo model of oxidative stress. J Pharmacol Exp Ther 2003;304:792–800.
317. Murer M, Dziewczapolski G, Menalled LB, et al. Chronic levodopa is not toxic for remaining dopamine neurons, but instead promotes their recovery, in rats with moderate nigrostriatal lesions. Ann Neurol 1998;43:561–75.

318. Datla KP, Blunt SB, Dexter DT. Chronic L-DOPA administration is not toxic to the remaining dopaminergic nigrostriatal neurons, but instead may promote their functional recovery, in rats with partial 6-OHDA or FeCl3 nigrostriatal lesions. Mov Disord 2001;16: 424–34.
319. Fahn S. A new look at levodopa based on the ELLDOPA study. J Neural Transm Suppl 2006;70:419–26.
320. Fahn S, the Parkinson Study Group. Does levodopa slow or hasten the rate of progression of Parkinson's disease? J Neurol 2005;252(Suppl 4):IV37–IV42.
321. Group PS. Dopamine transporter brain imaging to assess the effects of pramipexole vs levodopa on Parkinson disease progression. JAMA 2002;287:1653–61.
322. Whone AL, Watts RL, Stoessl AJ, et al. Slower progression of Parkinson's disease with ropinirole versus levodopa: The REAL-PET study. Ann Neurol 2003;54:93–101.
323. Scheller D, Chan P, Li Q, et al. Rotigotine treatment partially protects from MPTP toxicity in a progressive macaque model of Parkinson's disease. Exp Neurol 2007;203:415–22.
324. Double KL, Halliday GM, Henderson J, et al. The dopamine receptor agonist lisuride attenuates iron-mediated dopaminergic neurodegeneration. Exp Neurol 2003;184:530–5.
325. JoyceJN, Steve Presgraves S, Renisha L, et al. Neuroprotective effects of the novel D3/D2 receptor agonist and antiparkinson agent, S32504, in vitro against 1-methyl-4-phenylpyridinium (MPP+) and in vivo against 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP): a comparison to ropinirole. Exp Neurol 2003;184:393–407.
326. JoyceJN, Woolsey C, Ryoo H, Borwege S, Diane Hagner D. Low dose pramipexole is neuroprotective in the MPTP mouse model of Parkinson's disease, and downregulates the dopamine transporter via the D3 receptor. BMC Biol 2004;2:22.
327. Iida M, Miyazaki I, Tanaka K, Kabuto H, Iwata-Ichikawa E, Ogawa N. Dopamine D2 receptor-mediated antioxidant and neuroprotective effects of ropinirole, a dopamine agonist. Brain Res 1999;838:51–9.
328. Anderson DW, Neavin T, Smith JA, Schneider JS. Neuroprotective effects of pramipexole in young and aged MPTP-treated mice. Brain Res 2001;905:44–53.
329. Iravani MM, Haddon CO, Cooper JM, Jenner P, Schapira AH. Pramipexole protects against MPTP toxicity in non-human primates. J Neurochem 2006;96:1315–21.
330. Vu TQ, Ling ZD, Ma SY, et al. Pramipexole attenuates the dopaminergic cell loss induced by intraventricular 6-hydroxydopamine. J Neural Transm 2000;107:159–76.
331. Takashimaa H, Tsujihataa M, Kishikawab M, Freed WJ. Bromocriptine protects dopaminergic neurons from levodopa-induced toxicity by stimulating D2 receptors. Exp Neurol 1999;159:98–104.
332. Sawada H, Ibi M, Kihara T, et al. Dopamine D2-type agonists protect mesencephalic neurons from glutamate neurotoxicity: Mechanisms of neuroprotective treatment against oxidative stress. Ann Neurol 1998;44:110–9.
333. Presgraves SP, Borwegea S, Millan MJ, Joyce JN. Involvement of dopamine D2/D3 receptors and BDNF in the neuroprotective effects of S32504 and pramipexole against 1-methyl-4-phenylpyridinium in terminally differentiated SH-SY5Y cells. Exp Neurol 2004;190:157–70.
334. Yu Y, Wang JR, Sun PH, et al. Neuroprotective effects of atypical D1 receptor agonist SKF83959 are mediated via D1 receptor-dependent inhibition of glycogen synthase kinase-3 beta and a receptor-independent anti-oxidative action. J Neurochem 2008;104:946–56.
335. Joyce JN, Millan MJ. Dopamine D3 receptor agonists for protection and repair in Parkinson's disease. Curr Opin Pharmacol 2007;7:100–5.
336. Ramirez AD, Wong SK-F, Menniti FS. Pramipexole inhibits MPTP toxicity in mice by dopamine D3 receptor dependent and independent mechanisms. Eur J Pharmacol 2003;475:29–35.
337. Du F, Li R, Huang Y, Li X, Le W. Dopamine D3 receptor-preferring agonists induce neurotrophic effects on mesencephalic dopamine neurons. Eur J Neurosci 2005;22:2422–30.

Chapter 19
Dopamine Receptor Genetics in Neuropsychiatric Disorders

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Abstract The dopamine system modulates a diverse set of neural functions relevant to neuropsychiatric disorders and is modulated by some drugs used to treat these conditions. As a result, dopamine receptor genes have been a major focus for genetic studies that have analyzed putative associations between polymorphic variants and a wide range of clinical syndromes including alcoholism, substance abuse, schizophrenia, ADHD, anxiety disorders, bipolar and unipolar mood disorders, as well as related traits thought to contribute to these syndromes. In the following chapter, we review the basic genetic organization of the dopamine receptor genes and the evidence for association with these diagnoses. There is considerable inconsistency in the results from these genetic association studies, which is consistent with a complex genetic phenotype, and the neurobiological complexity of behaviors affected in neuropsychiatric illness. Overall, the strongest findings are the associations between variation in the DRD2 gene and alcoholism and the DRD4 gene and ADHD. While these associations do not suggest a major effect on risk in most patients, they do provide important insights into the pathophysiology of these disorders.

Keywords Dopamine · Receptor · Genes · Genetic association · SNP · Alcoholism · ADHD · Schizophrenia · Bipolar disorder · Substance abuse

19.1 Introduction

Over the past two decades, the genetics of dopamine receptors and their role in neuropsychiatric diseases have been studied extensively. The dopamine system is involved in neural functions including locomotion, movement, reward, cognition, and endocrine regulation [1]. Thus, genetic variation in dopamine receptors

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may influence symptoms in, or susceptibility to, various neuropsychiatric diseases. In addition, dopamine receptors are targets for antipsychotic medications that are used to treat delusions and hallucinations in schizophrenia and other psychotic disorders. Important side effects caused by antipsychotics include dopamine system-mediated extrapyramidal motor impairment and prolactin elevation [2]. Conversely, dopamine receptor agonists are psychostimulants that help to alleviate hypokinesia in Parkinson's disease and can cause psychotic symptoms in some cases [3]. Thus, alterations in dopamine receptor function, expression, and localization can have significant implications for both therapeutic efficacy and side effects of several classes of drugs.

There has been an accumulation of evidence to support the notion that variation in dopamine receptor genes can affect susceptibility to a number of neuropsychiatric diseases. In particular, schizophrenia, attention deficit hyperactivity disorder (ADHD), substance abuse, and bipolar disorder have the strongest association. However, even for these disorders, there are conflicting results and no clear evidence of variants exerting a strong effect on susceptibility in the majority of patients. As with other complex diseases and phenotypes, it is probably unrealistic to expect a single genetic variant to account for a large proportion of risk in all patients. With regard to behavioral disorders in particular, there are several additional complications. Symptoms and functional impairments in neuropsychiatric disease are not regulated by single molecular pathways or clearly defined anatomical structures, and neurotransmitter system components act in multiple brain circuits. Thus, the net impact of a given change in dopamine receptor function, expression, or localization cannot predict overall clinical presentation in a deterministic fashion.

A related issue is the heterogeneity of diagnostic groups based largely on clinical criteria like those in the DSM [4], in which patients with similar symptoms do not necessarily share the same genetic etiology. Finally, there are environmental and epigenetic factors that also affect behavior, creating even more variation that cannot be attributed to simple variation in DNA sequence [5]. Nevertheless, the identification of genetic variation that contributes to neuropsychiatric disease susceptibility or progression can generate important insights into pathophysiology, and in conjunction with other kinds of neurobiological studies can enhance our understanding of these complex and heterogeneous diseases. Ultimately, these genetic data will need to be supplemented by mechanistic understanding to determine the role of genetic variation that influences disease predisposition. In this chapter, we will review the polymorphisms present in dopamine receptor genes, survey their association with neuropsychiatric diseases, and discuss the conflicting results in the literature.

19.2 Characteristics of Dopamine Receptors

Five distinct dopamine receptor subtypes, D_1–D_5, have been cloned and characterized [6–9]. These are further subdivided into D_1-like (D_1 and D_5) and D_2-like (D_2, D_3, and D_4) receptors based on their ability to stimulate adenylyl cyclase [10]

(Table 19.1). Dopamine receptors belong to the family of seven transmembrane domain G protein-coupled receptors (Fig. 19.1). D_1-like receptors are able to stimulate adenylyl cyclase and promote the production of cyclic AMP (cAMP) whereas D_2-like receptors inhibit this downstream signaling cascade. Subsequent structural and pharmacological studies confirm this broad functional grouping of dopamine receptor subtypes.

Both the D_1-like and D_2-like receptors show a high degree of DNA and amino acid sequence homology within their transmembrane domains (TMD) [11–15]. D_1 and D_5 receptors share an 80% homology in their TMDs while the D_2 receptor shares a 75% homology with the D_3 receptor and 53% with D_4. The main difference in genomic organization between the two classes of dopamine receptors is

Table 19.1 Molecular characteristics of dopamine receptors

	D_1-like		D_2-like			
	D_1	D_5	D_{2S}	D_{2L}	D_3	D_4
Chromosomal location	5q35.1	4p16.1	11q22-23		3q13.3	11p15.5
Amino acids	446	477	414	443	400	387–515[a]
Exons[b]	2	1	8	8	7	4
Introns[c]	0	0	5	6	5	3

[a] Depends on the number of repeats in exon 3.
[b] Includes coding region, 5′- and 3′-untranslated region.
[c] Within coding region

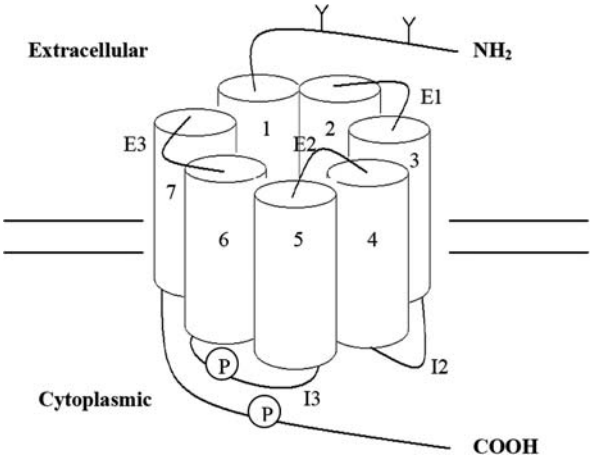

Fig. 19.1 Dopamine receptor structure. D_1-like and D_2-like receptors differ in the number of potential glycosylation sites which are represented on the NH_2-terminus. D_1-like receptors are also characterized by a longer C-terminal tail and a shorter third intracellular loop. Several phosphorylation sites are illustrated on the COOH-terminal as well. E1–E3: extracellular loops; 1–7: transmembrane domains; I2 and I3: intracellular loops

the absence or presence of introns in their coding sequences. D_1-like receptors are encoded by genes lacking introns whereas they are present in D_2, D_3, and D_4 receptor genes. Further analysis revealed that the D_2 receptor-coding region contains six introns, D_3 receptor five introns, and D_4 receptor three [16].

19.2.1 Structural Characteristics of Dopamine Receptors

In terms of structural characteristics of dopamine receptors, the NH_2-terminal stretch of all receptor subtypes has a similar number of amino acid residues but differs in the number of N-glycosylation sites, with D_1 and D_5 having two sites, D_2 four, D_3 three, and D_4 only one. The carboxy-terminus length is about seven times longer for D_1-like receptors than D_2-like. Like other G protein-coupled receptors, the C-terminus is rich in serine and threonine residues which can be phosphorylated by kinases, and contains cysteine residues that are probably involved in the anchorage of the cytoplasmic tail to the membrane [16]. In addition, two cysteine residues are also present in the second and third extracellular loops, enabling the formation of a disulfide bridge for stabilization of the whole receptor structure. One major structural difference between the two groups is that the third intracellular loop is shorter in D_1-like receptors than in the D_2-like. As the third intracellular loop is mainly responsible for G protein coupling and signal transduction, this accounts for the differences in downstream signaling. The shorter third intracellular loop associated with D_1-like receptors typically interacts with the G-stimulatory (G_s) proteins to activate adenylyl cyclase and the production of cAMP. In contrast, the longer loop associated with D_2-like receptors interacts with G-inhibitory (G_i) proteins that inhibit adenylyl cyclase and reduce the production of cAMP [16].

As mentioned previously, D_2-like receptors possess introns in their coding regions. This allows the generation of receptor variants via alternative splicing. Indeed, the D_2 receptor has two main variants, the short isoform D_{2S} and the long isoform D_{2L}, produced by alternative splicing of an 87 bp exon between intron 4 and 5 [12, 17–19]. The D_{2L} isoform has a stretch of 29 amino acid residues in the third cytoplasmic loop that is absent D_{2S} [20]. For D_3 receptors, splice variants appear to generate non-functional proteins [21–23]. No splice variants of the D_4 receptor have been found, but the D_4 receptor gene locus is highly polymorphic, producing great diversity of D_4 receptor isoforms (discussed in more detail below). Although the D_5 receptor gene contains no introns, two pseudogenes sharing 98% identity are found on human chromosomes 1 and 2. With respect to the D_5 receptor, these pseudogenes show 95% amino acid identity but interestingly, they code for a truncated, non-functional form of the receptor [24, 25].

19.2.2 Pharmacological Characteristics of Dopamine Receptors

In addition to structural differences, the D_1-like and D_2-like receptors have differing pharmacological profiles based on variable binding affinity of ligands for the receptor subtypes [16]. Table 19.2 lists several ligands that bind to dopamine receptor

Table 19.2 Pharmacological profile of dopamine receptors on selected agonists and antagonists

	D_1-like		D_2-like		
	D_1	D_5	D_2	D_3	D_4
Agonists					
Apomorphine	1 μM	100 nM	1 nM	10 nM	1 nM
Bromocriptine	100 nM	100 nM	1 nM	1 nM	100 nM
Dopamine	1 μM	100 nM	100 nM	10 nM	10 nM
Antagonists					
(+)-Butaclamol	1 nM	10 nM	1 nM	Undetermined	10 nM
Chlorpromazine	100 nM	100 nM	1 nM	10 nM	10 nM
Clozapine	100 nM	100 nM	100 nM	100 nM	10 nM
Haloperidol	100 nM	100 nM	0.1 nM	10 nM	1 nM
Raclopride	10 μM	Undetermined	1 nM	1 nM	1 μM
SCH-23390	0.1 nM	0.1 nM	1 μM	1 μM	1 μM
Spiperone	100 nM	1 μM	0.1 nM	1 nM	0.1 nM
Sulpiride	10 μM	10 μM	10 nM	10 nM	10 nM

All values are given as inhibition constants (K_i). Estimated orders of magnitude for each value are reported. Modified from Missale et al. [16]

subtypes with different affinities. Not surprisingly, dopamine itself is able to bind to all five receptors but has a higher affinity for D_5 receptors than D_1. Among the D_2-like receptors, dopamine binds D_2 receptors with a lower affinity than D_3 and D_4. Moreover, there are other agonists and antagonists that preferentially bind to D_1-like or D_2-like receptors, permitting distinctions between receptor subtypes to be assessed pharmacologically. For example, the dopamine antagonist sulpiride binds to D_2-like receptors preferentially. However, a clear differentiation between D_1 and D_5 receptors cannot be easily established pharmacologically as they have similar affinity profiles for most dopaminergic drugs. Besides dopamine, (+)-butaclamol is another discriminating dopamine antagonist that has a slightly higher affinity for D_1 receptors than D_5 [9, 14]. On the other hand, the D_2-like receptors are more readily distinguished using drugs with variable affinity for D_2, D_3, or D_4 receptors. As for D_2 receptor isoforms, there is still no compound that is able to clearly discriminate between D_{2S} and D_{2L}. Only a marginal difference in the binding affinities toward the two isoforms has been described with sulpiride and raclopride [26, 27].

19.3 Dopamine Receptor Function and Neuropsychiatric Disease

Dopamine receptors are coupled to a variety of different downstream effectors and hence regulate different activities such as locomotor activity, positive reinforcement or reward, cognition, and endocrine control in the central nervous system. As mentioned before, the most important is the modulation of adenylyl cyclase activity that results in either stimulation or inhibition of cAMP production depending on

the receptor subtype. In most cases, cAMP further regulates the activity of protein kinase A, which is responsible for numerous downstream effects. In addition to cAMP production, dopamine receptors have been shown to modulate the activity of phospholipase C, the release of arachidonic acid, the regulation of calcium and potassium channels, as well as Na^+/H^+ exchangers and Na^+/K^+ ATPases [16]. Recent studies have also demonstrated that the activation of D_{2S} isoform of the D_2 receptors is able to stimulate phospholipase D via coupling with Rho family of G proteins [28]. Phospholipase D is an enzyme responsible for the hydrolysis of phosphatidylcholine to phosphatidic acid and choline, and the end result seems to be associated with an antiproliferative effect [29]. All of these downstream pathways have important implications for neuropsychiatric disease.

19.3.1 D_1 Receptors

The dopamine D_1 receptor (DRD1) gene is mapped to chromosome 5q35.1 and contains two exons separated by a small intron in the 5′ untranslated region (5′ UTR) [30, 31] (Fig. 19.2). The coding region is located within exon 2 and encodes 446 amino acids. Several polymorphisms, most located in the 5′ UTR, have been implicated in neuropsychiatric diseases. In most cases, they are recognized by different restriction enzymes such as Dde I, BstN I, and Hae III. Hence these restriction fragment length polymorphisms (RFLP) allow easy detection using polymerase chain reaction (PCR) methods together with the appropriate restriction enzyme. Screening of the 5′ UTR polymorphisms showed that none of the mutations has an important influence on transcriptional activity [1]. Moreover, two silent mutations, +198 G/A (Leu^{66}) and +1,263 A/G (Ser^{421}), have been reported in the coding region of DRD1 that may play a role in disease susceptibility. Since these variants do not change amino acid sequence, they would not change protein structure, but possibly could affect mRNA abundance or stability.

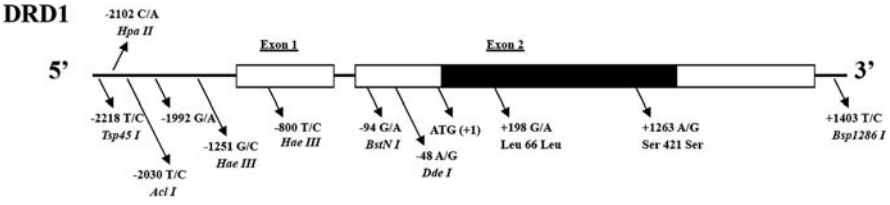

Fig. 19.2 Dopamine D_1 receptor: Schematic representation of polymorphisms (not to scale). Coding regions of exons are marked by *black blocks*, and the 5′ and 3′- UTR are represented by *white blocks*. The first base of the ATG start codon is denoted as +1 and the location of other polymorphisms are given relative to the start codon. Introns are represented as a *straight line* connecting the exons. Restriction enzymes, alteration of amino acids, and other distinct descriptions are given where applicable. (Modified from Kim et al. [42])

Table 19.3 Dopamine D_1 receptor polymorphisms and neuropsychiatric diseases

References	Polymorphisms	Disease phenotype	Number of patients	Ethnicity	Significance/notes
Kojima et al. [34]	–48 A/G	SCZ	148	Japanese	Not significant
Limosin et al. [41]		ALC	72	–	Male-limited association with sensation seeking
Rybakowski et al. [39]		SCZ	138	–	G/G genotype associates with inferior results on WCST
Severino et al. [38]		BP	107	Sardinian	Significant
Dmitrzak-Weglarz et al. [36]		SCZ	407	Polish	Not significant with SCZ but significant with BP
Kim et al. [42]		ALC	535	Korean	Significant association with severity of alcohol-related problems
Ni et al. [35]	–48 A/G –800 T/C +1403 T/C	BP	286	–	Not significant for individual SNPs but significant with haplotype
Del Zompo et al. [37]		BP	170 229	Sardinian Canadian	Not significant for individual SNPs but significant with haplotype
Misener et al. [40]	–48 A/G –800 T/C –1251 G/C +1403 T/C	ADHD	192	–	Haplotype 3 (1.1.1.2) is associated with poor working memory in ADHD
Cichon et al. [33]	–2218 T/C –2102 C/A –2030 T/C –1992 G/A –1251 G/C –800 T/C	SCZ BP	74	German	Not significant with both SCZ and BP for all SNPs

Table 19.3 (continued)

References	Polymorphisms	Disease phenotype	Number of patients	Ethnicity	Significance/notes
Liu et al. [32]	–48 A/G +198 G/A (L66L) +1263 A/G (S421S)	SCZ	131	Asian, African, American, and Caucasian	Not significant
Maron et al. [43]	–94 G/A	PD	127	Caucasian	Significant
Koks et al. [44]	–2102 C/A	MDD	177	Estonian	Significant

Abbreviations: SCZ – schizophrenia; BP – bipolar disorder; ADHD – attention deficit hyperactivity disorder; ALC – alcoholism; PD – panic disorder; MDD – major depressive disorder; SNP – single nucleotide polymorphism; WCST – Wisconsin Card Sorting Test;
– undetermined or not applicable

Overall, studies of individual polymorphisms in DRD1 have produced conflicting genetic linkage or association results in several different diseases (Table 19.3). Of all the single nucleotide polymorphisms (SNPs) reported, the –48 A/G polymorphism has been the most extensively studied. Several case–control studies have reported negative findings in patients with schizophrenia and bipolar disorder [32–37]. In contrast, others have demonstrated significant association with bipolar disorder [36, 38]. Another interesting study conducted by Rybakowsk's group found that the –48 G/G genotype was associated with impairment on the Wisconsin Card Sorting Test (WCST) in schizophrenic patients when compared to controls [39]. This is consistent with executive dysfunction seen in schizophrenia, which is often assessed using the WCST. In addition, some haplotypes assessed with the transmission disequilibrium test (TDT) on the –48 A/G, –800 T/C, and +1,403 T/C polymorphisms have been associated with bipolar disorder in two separate studies [35, 37]. Although there are no association studies of the two synonymous mutations (Leu^{66} and Ser^{421}) with schizophrenia, Liu et al. suggested that they are unlikely to contribute significantly to the schizophrenia [32]. Because of these inconsistent findings, no definitive association can be seen between DRD1 mutations and schizophrenia or bipolar disorder. As for ADHD and alcoholism, a smaller number of studies have investigated relationships with DRD1 polymorphisms. Misener et al. investigated four different polymorphisms (–48 A/G, –800 T/C, –1,251 G/C, and +1,403 T/C) and found a haplotype (haplotype 3 – 1.1.1.2) associated with poor working memory in ADHD [40], while others have found association between –48 A/G and the severity of alcohol-related problems and sensation seeking in alcoholics [41, 42]. Studies have also reported significant association between –94 G/A and –2,102 C/A with panic disorder and major depressive disorder, respectively [43, 44]. Despite these significant association studies with ADHD and alcoholism, replication has been inconsistent, and so firm conclusions about the relationship of variation in the DRD1 gene to these disorders cannot yet be drawn.

19.3.2 D_2 Receptors

The dopamine D_2 receptor is located on chromosome 11q22-23 and consists of eight exons with the coding region spanning exons 2–8 [24, 45] (Fig. 19.3). The gene is over 65,000 bp long and encodes 414 amino acids in D_{2S} and 443 in D_{2L}. Several RFLPs distributed throughout the whole gene are recognized by Taq I, BstN I, Hph I, Nco I, and Hpa II. In particular, three polymorphisms of Taq I, termed Taq I A, B, and D, are the most extensively studied in relation to neuropsychiatric diseases (Table 19.4). Taq I A is located in the 3′ UTR and has been shown to be associated with a reduction in the D_2 receptor density [46, 47]; Taq I B is near the end of intron 1 close to exon 2, and Taq I D is within intron 2. Allele frequencies of the various Taq I polymorphisms differ depending on ancestral background [48]. A number of polymorphisms are located within the coding region, with three of them resulting in a change of amino acid: Val^{96}Ala, Pro^{310}Ser, and Ser^{311}Cys. Amino acid positions 310 and 311 are part of the third cytoplasmic loop where amino acid substitutions

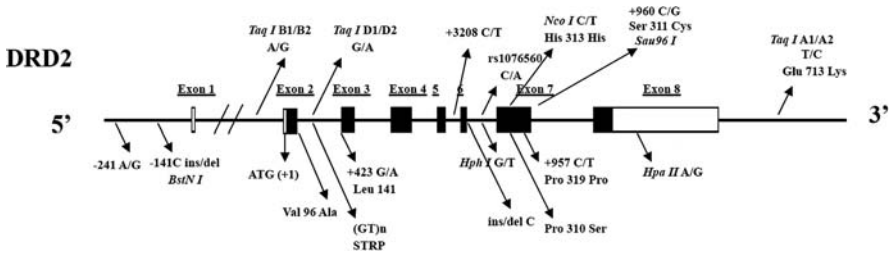

Fig. 19.3 Dopamine D_2 receptor: Schematic representation of polymorphisms (not to scale). Coding regions of exons are marked by *black blocks*, and the 5′ and 3′- UTR are represented by *white blocks*. The first base of the ATG start codon is denoted as +1 and the location of other polymorphisms are given relative to the start codon. Introns are represented as a *straight line* connecting the exons. Restriction enzymes, alteration of amino acids, and other distinct descriptions are given where applicable. (Modified from Kim et al. [42])

can affect the magnitude of cAMP inhibition [1]. All three variants, however, are functionally active in Chinese hamster ovary cells [49]. Although the +957 C/T SNP is a synonymous variant resulting in the same amino acid (Pro^{319}), the T allele has been shown to decrease translation of DRD2 mRNA, DRD2 mRNA stability, and dopamine-induced upregulation of D_2 receptors [50]. In the 5′ promoter region, two mutations –241 A/G and –141C ins/del have been detected and are associated with D_2 receptor density [51].

A large number of studies have investigated the Taq I A polymorphism and its association with alcoholism, with inconsistent results. The first evidence of Taq I A association with alcoholism was reported by Blum et al. [52], and later replicated in several other studies [53–55]. In addition, greater severity of alcohol dependence [56] and early-onset alcoholism [57] have been associated with the Taq I A1 allele. In contrast, a lack of association has been reported in a number of case–control [51, 58–66] and family-based studies [67, 68]. Findings have been similarly inconsistent with the Taq I B polymorphism and alcoholism, with both positive [62, 69] and negative studies [66, 70] present in the literature. Other than the Taq I polymorphism, no association was observed between alcoholism and $Ser^{311}Cys$, $(GT)_n$ short tandem repeat polymorphism (STRP), –141C ins/del, –241 A/G, and other mutations within the coding region [51, 60, 66–68, 71–74]. Linkage disequilibrium studies have not demonstrated associations with haplotypes including the Taq I A polymorphism [60], suggesting that the Taq I polymorphism may exert only a minor effect or interact with genes other than DRD2 to influence alcoholism. The Taq1 A variant appears to alter an amino acid in the ankyrin repeat and kinase domain containing 1 (ANKK1) gene, near the DRD2 locus [75]. It remains unclear whether this significantly influences susceptibility to neuropsychiatric disease, but does represent a potential direction for future research. Pato et al. conducted a meta-analysis of eight studies and found an apparent increase in the relative risk associated with increased severity of alcoholism for the Taq I A1 allele [76]. More recently, a meta-analysis reviewing a total of 40 case–control studies on different ethnic groups indicates a small effect of the Taq I A polymorphism on risk for alcoholism [77] and was further supported by another recent meta-analysis [78].

Table 19.4 Dopamine D_2 receptor polymorphisms and neuropsychiatric diseases

References	Polymorphisms	Disease phenotype	Number of patients	Ethnicity	Significance/notes
Blum et al. [52]	*Taq I* A1/A2	ALC	70	–	Significant with A1 allele
Amadeo et al. [53]		ALC	49	French	Significant
Arinami et al. [54]		ALC	78	Japanese	Significant
Goldman et al. [61]		ALC	119	American-Indians	Not significant
Pato et al. [76]		ALC	–	–	Significant with A1 allele (meta-analysis)
Noble et al. [236]		SMK	172	Caucasian	Significant with A1 allele
Cruz et al. [63]		ALC	38	Mexican	Not significant
Chen et al. [64]		ALC	–	Taiwanese	Not significant
Kono et al. [57]		ALC	100	Japanese	Significant with A1/A1 genotype
Gorwood et al. [105]		BP ALC	122	French	Not significant with both BP and alcoholism
Gorwood et al. [58]		ALC	113	French	Not significant
Lawford et al. [108]		HD	95	Caucasian	Significant with A1 allele
Conner et al. [56]		ALC	106	Australian-Caucasian	Significant with A1 allele (greater severity)
Limosin et al. [237]		ALC	120	French-Caucasian	Significant with A1 allele only in males
Conner et al. [109]		SA	37 11	Caucasian Hispanic	Significant increase in children developing SA with A1 allele
Munafo et al. [77]		ALC	–	–	Significant (meta-analysis)
Smith et al. [78]		ALC	9382	–	Significant association with A1 allele (meta-analysis)
Laurent et al. [81]	+960 C/G (S311C)	SCZ	113	Caucasian	Not significant
Craddock et al. [102]		BP	82	–	Not significant
Chen et al. [80]		SCZ	114	Taiwanese	Not significant
Finckh et al. [72]		ALC	312	German	Not significant
Kaneshima et al. [79]		SCZ	78	Japanese (Okinawan)	Not significant

Table 19.4 (continued)

References	Polymorphisms	Disease phenotype	Number of patients	Ethnicity	Significance/notes
Serretti et al. [107]		BP SCZ MDD DD	1182	Italian	Not significant but may be involved in delusion and disorganization symptomatology
Glatt et al. [84]		SCZ	–	–	Significant with C311 (meta-analysis)
Jonsson et al. [87]		SCZ	173 9152	Swedish	Significant only in males (replicated in meta-analysis)
Levinson et al. [86]		SCZ	–	–	Significant (meta-analysis)
Glatt et al. [85]		SCZ	–	–	Significant with C311 (meta-analysis)
Lawford et al. [89]	+957 C/T (P319P)	SCZ	153	Caucasian	Significant with C/C
Hanninen et al. [88]		SCZ	188	Finnish	Significant
Hoenicka et al. [90]		SCZ	131	Spanish	Significant
Furlong et al. [104]	–141C ins/del	BP	131	East Anglian	Not significant
Li et al. [97]		SCZ	229 trios 151	Chinese Caucasian	Not significant
Breen et al. [93]		SCZ	439	British	Significant
Kirov et al. [103]		BP	122 trios	British-Caucasian	Not significant
Glatt et al. [96]		SCZ	–	–	Not significant (meta-analysis)
Konishi et al. [238]		ALC	200	Mexican-American	Not significant
Xu et al. [110]	*Taq I* B1/B2	HD	486 471	Chinese German	Significant with B1 allele in Chinese and a low risk in Germans

Table 19.4 (continued)

References	Polymorphisms	Disease phenotype	Number of patients	Ethnicity	Significance/notes
Dubertret et al. [82]	*Taq I* A1/A2 *Taq I* B1/B2 *Taq I* D1/D2 –141C ins/del +960 C/G (S311C) $(GT)_n$ STRP	SCZ	103	French	Significant excess of A2 allele of *Taq I* A1/A2 and B2 allele of *Taq I* B1/B2 Not significant with *Taq I* D1/D2, –141C, S311C and $(GT)_n$ STRP Significant haplotype with A2
Smith et al. [111]	*Taq I* A1/A2 *Taq I* B1/B2	SA	–	–	Significant with B1 allele
Blum et al. [69]		ALC	115	White	Significant with A1 and B1 alleles
O'Hara et al. [239]		SA	616 (with controls)	White and Black Americans	Significant in White with A1 and B1 allele Not significant in Black
Dubertret et al. [95]		SCZ	100	French	Significant haplotype with A2/B2
Foley et al. [62]		ALC	161	Caucasian	Significant with *Taq I* B Not significant with *Taq I* A
Ambrosio et al. [98]	*Taq I* A1/A2 +960 C/G (S311C)	SCZ	90 trios	Portuguese	Not significant
Edenberg et al. [67]	*Taq I* A1/A2 $(GT)_n$ STRP	ALC	987 (with controls)	Non-Hispanic Caucasian	Not significant
Ishiguro et al. [55]	*Taq I* A1/A2 –141C ins/del	ALC	209	Japanese	Significant with –141C ins Marginal significant with *Taq I*
Li et al. [106]		BP	118 157	Han Chinese Caucasian	Significant in Chinese but not Caucasian with A1 allele and –141C del

Table 19.4 (continued)

References	Polymorphisms	Disease phenotype	Number of patients	Ethnicity	Significance/notes
Sander et al. [60]		ALC	310	German	Not significant with individual SNPs and A1/–141C ins haplotype
Samochowiec et al. [68]		ALC	100 families	Polish	Significant with A2 allele Not significant with –141C ins/del
Parsons et al. [99]		SCZ	119	Spanish	Significant with *Taq I* A1/A2 (less frequent in SCZ) Not significant with –141C ins/del
Konishi et al. [70]	*Taq I* A1/A2 *Taq I* B1/B2 –141C ins/del	ALC	130	Mexican-American	Significant with –141C ins/del Not significant with *Taq I* A and B
Himei et al. [100]	+960 C/G (S311C) –141C ins/del	SCZ	190	Japanese	Not significant but may be associated with symptoms (C311/–141C del)
Sarkar et al. [101]	+423 G/A (Leu141) +3208 C/T *Nco I* C/T (H313H)	SCZ	14	Caucasian	Not significant with no structural change in all SNPs
Arinami et al. [94]	–141C ins/del –241 A/G	SCZ	260	Japanese	Significant with –141C ins/del Not significant –241A/G
Goldman et al. [65]	+960 C/G (S311C) *Taq I* A1/A2 $(GT)_n$ STRP	SCZ ALC SA	459	American-Indian	Not significant
Vijayan et al. [83]	*Taq I* A1/A2 *Taq I* B1/B2 *Taq I* D1/D2 +960 C/G (S311C) *Nco I* C/T (H313H)	SCZ	213	South Indian	Significant with *Nco I* Not significant with *Taq I* and S311C Significant haplotype with *Taq I* A2 and *Nco I* T allele

Table 19.4 (continued)

References	Polymorphisms	Disease phenotype	Number of patients	Ethnicity	Significance/notes
Kukreti et al. [91]	*Nco I* C/T (H313H) +957 C/T (P319P)	SCZ	101	South Indian	Significant with *Nco I* Not significant with P319
Lu et al. [66]	*Taq I* A1/A2 *Taq I* B1/B2 $(GT)_n$ STRP	ALC	128	Taiwanese	Not significant
Luo et al. [51]	*Taq I* A1/A2 *Taq I* B1/B2 –141C ins/del *Hph I* G/T *Nco I* C/T (H313H) *Hpa II* A/G	ALC	200	Mexican-American	Significant with –141C del Not significant with other individual SNPs Significant haplotype with H1, H2, H4 and H8
Parsian et al. [73]	–141C ins/del –241 A/G +960 C/G (S311C)	ALC	173	White and African-American	Not significant
Gejman et al. [74]	Val 96 Ala Pro 310 Ser +960 C/G (S311C)	SCZ ALC	219	White	Not significant in both SCZ and alcoholism
Gelernter et al. [112]	*Taq I* A1/A2 *Taq I* B1/B2 *Taq I* D1/D2	CD	173	European- and African-American	Not significant
Noble et al. [240]	*Taq I* A1/A2 *Taq I* B1/B2 –141C ins/del *Hph I* G/T +957 C/T (P319P)	ALC	92	Caucasians	Not significant with + 957 C/T and –141C ins/del Significant haplotype with minor alleles of *Taq I* A, B and *Hph I* G/T
Finckh et al. [71]	Intron 6 C ins/del	ALC	270	–	Not significant
Sasabe et al. [241]	rs1076560 C/A	ALC	248	Japanese	Significant with A allele

Abbreviations: SCZ – schizophrenia; BP – bipolar disorder; ALC – alcoholism; SMK – smoking; SA – substance abuse; HD – heroin dependence; MDD – major depressive disorder; DD – delusional disorder; CD – cocaine dependence; SNP – single nucleotide polymorphism; STRP – short tandem repeat polymorphism

– undetermined or not applicable

Neuroleptics that target the dopamine receptors are the mainstay of clinical pharmacological treatment for schizophrenia and bipolar disorder. The dopamine hypothesis of psychosis, in which excess dopamine or dopamine signaling is considered an important etiological factor, has been one of the most well-established theories in the field. Thus, much research has investigated whether mutations in the DRD2 gene confer susceptibility to disorders with prominent psychotic features like schizophrenia and bipolar disorder. Although some studies do not find an association between schizophrenia and the $Ser^{311}Cys$ missense mutation [65, 74, 79–83], several meta-analyses report a significant association with the Cys^{311} allele [84–87]. The synonymous mutation Pro^{319} (+957C/T) was found to be associated with schizophrenia in three European case–control studies [88–90], with a negative finding in a sample of 101 South Indians [91, 92]. This may be related to the ANKK1 protein kinase gene mentioned above, but further research is required to explain the relationship with schizophrenia.

Studies examining association between schizophrenia and other SNPs in the DRD2 gene have also generated inconsistent results (Table 19.4). Association was observed with –141C ins/del [93, 94], Taq I A and B [82, 95], and Nco I C/T [83, 91], whereas other studies have failed to find significant association for various DRD2 polymorphisms [65, 74, 82, 83, 94, 96–101]. Linkage and association studies with bipolar disorder have generally not found associations with the DRD2 gene [102–105]. In a study investigating the Taq I A and –141C ins/del polymorphisms, Li et al. found a significant association with bipolar disorder in a Chinese population but not Caucasian [106], suggesting ethnicity may account for conflicting results. Finally, Serretti et al. examined the missense mutation $Ser^{311}Cys$ and its association with four major psychiatric disorders (bipolar disorder, schizophrenia, major depressive disorder, and delusional disorder) [107], but did not find significant associations.

The association analysis of DRD2 polymorphisms with the abuse of substances other than alcohol has resulted in equivocal findings. The Taq I A1 allele has been shown to be associated with heroin dependence [108] and to increase risk for substance abuse in children of alcoholics [109]. The Taq I B1 allele was also found to predispose to heroin dependence [110] and other substance abuse [111]. In contrast, Goldman et al. examined Taq I A, $Ser^{311}Cys$, and $(GT)_n$ STRP and found no association with substance abuse in a sample of 459 American-Indian subjects [65], while Gelernter et al. revealed a non-significant association between Taq I A, B, D polymorphism and cocaine dependence in an European- and African-American population [112].

19.3.3 D_3 Receptors

The dopamine D_3 receptor (DRD3) is located on chromosome 3q13.3 and contains seven exons separated by six introns (Fig. 19.4). The coding region spans six exons (exons 2–7) that are distributed over 40,000 bp and encodes for a 400-amino acid protein. The most common polymorphism studied in DRD3 is +25 A/G, which is

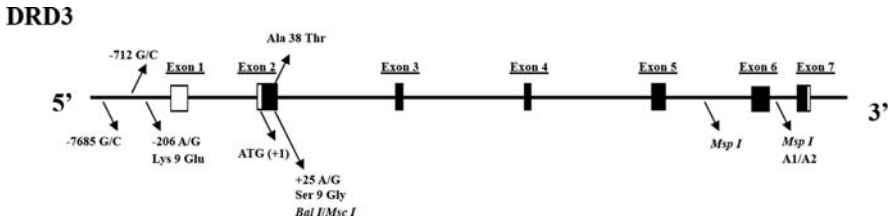

Fig. 19.4 Dopamine D_3 receptor: Schematic representation of polymorphisms (not to scale). Coding regions of exons are marked by *black blocks*, and the 5′ and 3′- UTR are represented by *white blocks*. The first base of the ATG start codon is denoted as +1 and the location of other polymorphisms are given relative to the start codon. Introns are represented as a *straight line* connecting the exons. Restriction enzymes, alteration of amino acids, and other distinct descriptions are given where applicable. (Modified from Kim et al. [42])

a glycine–serine substitution at amino acid position 9, in the N-terminal extracellular domain of the receptor. This mutation creates a Bal I restriction enzyme site and alters the recognition site for Msc I [113]. The SNP –206 A/G located in the 5′ region has also been studied in relation to neuropsychiatric disease. Moreover, polymorphic sites in introns 5 and 6 are identified by the restriction enzyme Msp I and a missense mutation $Ala^{38}Thr$ was also identified in the first transmembrane domain. The functional importance of these polymorphisms has not been examined in detail.

A large number of studies have examined the association between DRD3 $Ser^{9}Gly$ and schizophrenia (114), BP, ADHD, and substance abuse (Table 19.5). Contradictory results have been reported in studies with different ethnic populations and a variety of methods such as case–control, family based, and meta-analysis. The majority of studies report a lack of genetic association with disease [98, 115–128]. With respect to family-based association studies, the haplotype relative risk (HRR) and TDT strategy have been used. Weak association was reported in a case–control study conducted in a Japanese sample [129] while a positive association was found in a mixed sample of 117 North Americans and 97 Italians [130] and in 73 subjects in an East Anglian sample [131]. A meta-analysis of the relationship between $Ser^{9}Gly$ and schizophrenia conducted by the same group produced positive results. However, contradictory results were reported by the same group, who reviewed more than a total of 11,000 individuals and found no significant association between $Ser^{9}Gly$ homozygosity and schizophrenia [132]. Linkage disequilibrium approaches in two separate studies have revealed strong haplotype association with schizophrenia for haplotypes including $Ser^{9}Gly$ and –206 A/G [128, 129].

In bipolar disorder, a possible association with $Ser^{9}Gly$ polymorphism has been detected in a case–control study using a relative risk approach [133]. Although there was no difference in allele or genotype frequencies when compared to normal controls, a significant increase in the frequency of allele 1 was observed in bipolar disorder [133]. A lack of association was seen in a few case–control studies [103, 134, 135]. In ADHD, neither the $Ser^{9}Gly$ nor Msp I polymorphisms were associated

Table 19.5 Dopamine D_3 receptor polymorphisms and neuropsychiatric diseases

References	Polymorphisms	Disease phenotype	Number of patients	Ethnicity	Significance/notes
Nanko et al. [121]	+25 A/G(Ser 9 Gly)	SCZ	91	Japanese	Not significant
Laurent et al. [124]		SCZ	76	French	Not significant
Gorwood et al. [140]		ALC	26	White and Black	Not significant for all three samples
			35	Caucasian	
			72	Scandinavian	
Kennedy et al. [130]		SCZ	117	North American	Significant
			97	Italian	
Parsian et al. [133]		BP	198	Caucasian	Significant increase in the frequency of allele 1
Freimer et al. [242]		CD	124	White and Black	Not significant
Higuchi et al. [137]		ALC	–	Japanese	Not significant
Rietschel et al. [119]		SCZ	146	German	Not significant
Rothschild et al. [120]		SCZ	44 families	–	Not significant
Tanaka et al. [125]		SCZ	100	Japanese	Not significant
Chen et al. [116]		SCZ	178	Han Chinese	Not significant
Parsian et al. [138]		ALC	162	White-Caucasian	Not significant
Duaux et al. [141]		OD	54	French	Significant
Krebs et al. [143]		SA	89	French-Caucasian	Significant
Comings et al. [142]		CD	47	Caucasian	Marginal significant
Kirov et al. [103]		BP	122 trios	British-Caucasian	Not significant
Joober et al. [115]		SCZ	106	Caucasian	Not significant
Kremer et al. [127]		SCZ	129	Palestinian	Not significant
Elvidge et al. [134]		BP	454	British-Caucasian	Not significant
Gorwood et al. [59]		ALC	131	French	Not significant
Virgos et al. [118]		SCZ	540	European-Caucasian	Not significant
Lee et al. [139]		ALC	67	Korean	Not significant

Table 19.5 (continued)

References	Polymorphisms	Disease phenotype	Number of patients	Ethnicity	Significance/notes
Jonsson et al. [117]		SCZ	156	Swedish	Not significant with association study Significant with meta-analysis
Ambrosio et al. [98]		SCZ	90	Portuguese	Not significant
Jonsson et al. [132]		SCZ	11066	–	Not significant (meta-analysis)
Szekeres et al. [243]		SCZ	75	Caucasian	More perseverative errors in WCST with S/S
Rybakowsk et al. [39]		SCZ	138	–	No association with WCST in SCZ
Van Den Bogaert et al. [135]		BP	182	Swedish	Not significant
Dominiquez et al. [123]		SCZ	260	Galician	Not significant
Lorenzo et al. [122]		SCZ	178	Spanish	Not significant
Fathalli et al. [126]		SCZ	408	Caucasian from Canada, Tunisia, and Hungary	Not significant
Ishiguro et al. [129]	+25 A/G(Ser 9 Gly) –206 A/G –712 G/C Ala 38 Thr	SCZ	153	Japanese	Marginal significant with Ser 9 allele Significant haplotype with +25, –206, –712 Not significant with A38T
Staddon et al. [128]	+25 A/G(Ser 9 Gly) –206 A/G –7685 G/C	SCZ	118	Navarra of Basque origin	Not significant with S9G and –206 A/G Significant excess of –7685C allele Significant haplotype with all three SNPs
Sivagnanasundaram et al. [131]	+25 A/G(Ser 9 Gly) –206 A/G	SCZ	73	East Anglia, UK	Significant
Baritaki et al. [244]	–206 A/G	SCZ	114	Greece	Not significant

Table 19.5 (continued)

References	Polymorphisms	Disease phenotype	Number of patients	Ethnicity	Significance/notes
Barr et al. [136]	+25 A/G(Ser 9 Gly) *Msp I*	ADHD	100 families	–	Not significant
Chiaroni et al. [245]		BP	60	–	Homozygosity of S9G associates with manic monopolar form of BP

Abbreviations: SCZ – schizophrenia; BP – bipolar disorder; ADHD – attention deficit hyperactivity disorder; ALC – alcoholism; CD – cocaine dependence; OD – opiate dependence; SNP – single nucleotide polymorphism; WCST – Wisconsin Card Sorting Test.
– undetermined or not applicable

with the disorder in a study of 100 small families [136]. Furthermore, no significant association with alcoholism was seen in several case–control studies involving various ethnic groups [59, 137–140]. In terms of substance abuse, there are reports of association between $Ser^{9}Gly$ and opiate dependence [141], cocaine dependence [142], and other substance dependence in schizophrenic patients [143]. As a whole, the present findings to date do not provide strong support for the role of DRD3 polymorphisms in neuropsychiatric diseases.

19.3.4 D_4 Receptors

The dopamine D_4 receptor (DRD4) gene is located on chromosome 11p15.5 and contains four exons comprising the coding region (Fig. 19.5). The gene is 3,399 bp in size, and encodes a variable number of amino acids depending on the number of repeats present in exon 3. The DRD4 locus is highly polymorphic with a remarkable number of polymorphisms, especially in the 5′ UTR. The most extensively studied polymorphism is the 48 bp tandem repeat (VNTR) in exon 3. It is a hypervariable region located in the third intracellular cytoplasmic loop and consists of 2–11 repeats, typically written as D4.*x*, where *x* is the number of repeats. Allele frequencies vary considerably depending on ethnicity, with D4.2, D4.4, and D4.7 being the most common [144]. The number of repeats has been reported to influence some dimensions of personality such as novelty seeking [145–147] and has an impact on the pharmacological profile [148, 149]. Another 120 bp tandem repeat is found 1.2 kb upstream of the initiation codon and it has been shown to contain transcription factor binding sites [150]. Numerous SNPs are present in the 5′ UTR as well [151]. The promoter for the DRD4 gene, located between position –591 and –123

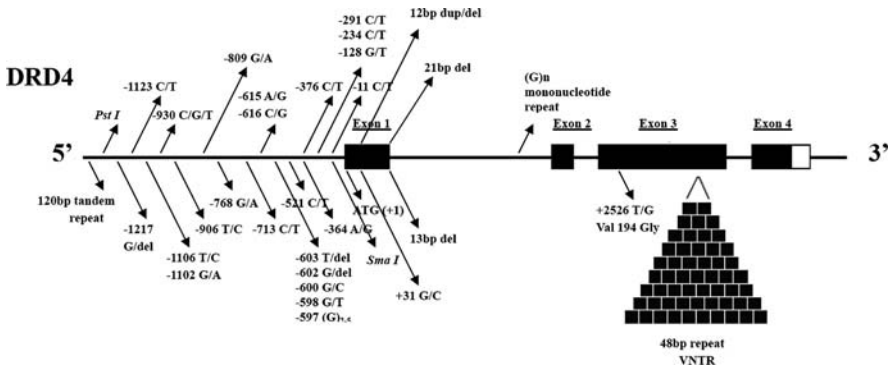

Fig. 19.5 Dopamine D_4 receptor: Schematic representation of polymorphisms (not to scale). Coding regions of exons are marked by *black blocks*, and the 5′ and 3′- UTR are represented by *white blocks*. The first base of the ATG start codon is denoted as +1 and the location of other polymorphisms are given relative to the start codon. Introns are represented as a *straight line* connecting the exons. Restriction enzymes, alteration of amino acids, and other distinct descriptions are given where applicable. (Modified from Kim et al. [42])

relative to the start codon, is responsible for cell type-specific expression of the gene [152] and regulates transcriptional activity [153]. The T allele of the –521C/T polymorphism reduces transcriptional efficiency by 40% compared with the C allele [154]. In the coding region, other than the 48 bp VNTR, exon 1 contains a 12 bp duplication–deletion mutation, a 13 bp deletion that causes a frameshift mutation and a 21 bp deletion. The 12 bp mutation affects amino acids in the N-terminal, extracellular region [155]; the 13 bp frameshift mutation produces a truncated, non-functional form of the protein [1], and the 21 bp deletion removes codons 36–42 in the first transmembrane region [156]. A variable number of repeated G nucleotides are also present in intron 1 but it is not clear if this variant has significant functional impact.

The 48 bp VNTR has been investigated extensively for association with neuropsychiatric diseases, especially ADHD and schizophrenia, as well as variation in personality dimensions, particularly novelty seeking (Table 19.6). Again, conflicting results have been observed in various studies on different ethnic groups and using different methods. No difference in the D4.7 allele frequency or preferential transmission with ADHD was observed in several family-based studies that used the HRR and TDT approach [157–161] nor in case–control studies [158, 162]. Another study reported no association with ADHD in 123 subjects of mixed ethnic groups [163]. Moreover, Mill et al. detected a significant association but no linkage in a sample of British Caucasians using both case–control and family-based samples [164]. An interesting study investigated the relationship between the D4.7 variant and sustained attention in ADHD and found no impairment when compared with controls [165]. Despite the large number of negative findings, strong association between the D4.7 allele and ADHD has been well established in the literature [166–175]. Positive association with a refined phenotype, inattentive-type, and combined type of ADHD was also demonstrated [176, 177]. A recent analysis using neuropsychological tests revealed that the D4.7 allele is associated with significantly more incorrect and impulsive responses [178]. When considering the VNTR data in their entirety, it appears that more repeats (>D4.4) are associated with a higher risk for ADHD, while a small number of repeats may even be protective [179–182]. Other DRD4 polymorphisms have not been investigated as thoroughly for association with ADHD. Nonetheless, several groups have found a lack of association for –521 C/T, –615 A/G, –616 C/G, the 120 bp tandem repeat, and the 12 bp dup/del variant [165, 175, 182, 183]. There are positive findings for –521T [184] and the 120 bp tandem repeat [183, 185] and ADHD. Some haplotypes with the D4.7 polymorphism and the 120 bp duplication have also been associated with ADHD [167, 175]. Moreover, two meta-analyses found a significant association between the D4.7 allele and ADHD [186, 187]. Despite the inconsistencies, the association between ADHD and the 48 bp VNTR in DRD4 is one of the strongest results in neuropsychiatric disease genetics.

The relationship between DRD4 polymorphisms and schizophrenia has also been inconsistent. For the 48 bp VNTR, positive results [188–192] involving most of the different repeats (D4.2–D4.8) are counterbalanced by reports of no association [193–199]. Interestingly, Lung et al. found a similar trend as in ADHD, with a

Table 19.6 Dopamine D_4 receptor polymorphisms and neuropsychiatric diseases

References	Polymorphisms	Disease phenotype	Number of patients	Ethnicity	Significance/notes
Weiss et al. [188]	48 bp VNTR (7R)	SCZ	201	German	Significant
Rowe et al. [177]		ADHD	239	Caucasian, African-American, Hispanic, Asian	Significant with both inattentive-type and combined-type ADHD
Smalley et al. [173]		ADHD	133	Caucasian	Significant
Swanson et al. [176]		ADHD	39	–	Significant association with a refined phenotype of ADHD
Faraone et al. [169]		ADHD	27 triads	–	Significant
Eisenberg et al. [157]		ADHD	48 triads	Jews	Not significant
Hawi et al. [158]		ADHD	78	Irish	Not significant
Kotler et al. [171]		ADHD	98 triads	Israelian	Significant
Tahir et al. [172]		ADHD	104	Turkish	Significant
Faraone et al. [187]		ADHD	–	–	Significant (meta-analysis)
Mill et al. [164]		ADHD	132 85 trios	British-Caucasian	Significant association but no linkage
Rowe et al. [174]		ADHD	187	–	Significant more symptoms with fathers possessing 7R
Grady et al. [167]		ADHD	132	European, Hispanic, African-American, Asian and Native-American	Significant with 7R and haplotype significant associates with 7R
El-Faddagh et al. [166]		ADHD	265	Caucasian	Significant with boys
Langley et al. [178]		ADHD	133	British-Caucasian	Significant more incorrect and impulsive responses with neuropsychological tests
Barkley et al. [163]		ADHD	123	White, Black, and Hispanic	Not significant

Table 19.6 (continued)

References	Polymorphisms	Disease phenotype	Number of patients	Ethnicity	Significance/notes
Brookes et al. [168]		ADHD	776	European-American	Significant
Carrasco et al. [161]		ADHD	51	Chilean	Not significant
Gornick et al. [170]		ADHD	166	Caucasian, African-American, Hispanic, Asian	Significant
Zhang et al. [189]	48 bp VNTR (6R)	SCZ	67	Han Chinese	Significant
Li et al. [106]	48 bp VNTR (1R)	BP	275	Caucasian and Han Chinese	Not significant
McCracken et al. [185]	120 bp TR (5′-UTR)	ADHD	371	Caucasian	Significant with 240 bp allele
Cheuk et al. [160]	48 bp VNTR (4R, 7R)	ADHD	64	Chinese	Not significant
Qian et al. [179]	48 bp VNTR (4-6R)	ADHD	340 202 trios	Han Chinese	Significant
Qian et al. [180]		ADHD	542	Han Chinese	Marginal significant with long repeats (4-6R)
Hong et al. [193]	48 bp VNTR (2R, 7R)	SCZ	124	Chinese and Caucasian	Not significant
Leung et al. [162]		ADHD	32	Han Chinese	Significant with 2R Not significant with 7R
Kaiser et al. [194]	48 bp VNTR (2R, 3R)	SCZ	638	German-Caucasian	Not significant
George et al. [213]	48 bp VNTR (3R, 6R)	ALC	72	Caucasian	Significant
Glatt et al. [201]	48 bp VNTR (2R, 4R, 7R)	SCZ	–	–	Not significant but may have sex-dependent association (meta-analysis)
Bakker et al. [159]		ADHD	236	Dutch	Not significant
Lim et al. [209]	48 bp VNTR (2-11R)	BP	147	English and Caucasian	Not significant
Shaikh et al. [198]		SCZ	191	English and Japanese	Not significant
Kotler et al. [216]		OD	141	Israeli Arabs and Sephardic Jews	Significant
Li et al. [215]		OD	121	Han Chinese	Significant with long repeats (5R–7R)

Table 19.6 (continued)

References	Polymorphisms	Disease phenotype	Number of patients	Ethnicity	Significance/notes
Parsian et al. [138]		ALC	162	White Caucasian	Not significant
Bocchetta et al. [208]		BP	267	Sardinian	Not significant
Franke et al. [214]		OD	396	German	Not significant
Tang et al. [190]		SCZ	510	Chinese	Significant with lacking 2R, 3R and excess of 4R
Lung et al. [200]		SCZ	305	Caucasian and Asian	Not significant with VNTR$\leqq$5R Significant with VNTR$\geqq$6R
Maher et al. [186]		ADHD	571	–	Significant (meta-analysis)
Muglia et al. [210]		BP	154	Caucasian	Significant with 4R but 2R may have protective effect
Tsai et al. [246]		MA	116	Taiwanese	Not significant
Li et al. [181]		ADHD	–	European and Asian	Significant with 5R, 7R Not significant with 2R, 3R 4R may confer a protective effect (meta-analysis)
Hong et al. [247]	12 bp dup/del	SCZ	80	Chinese	Not significant
Arcos-Burgos et al. [175]	48 bp VNTR 120 bp TR (5′-UTR)	ADHD	79 families	South American	Significant with 7R Not significant with 120 bp TR Haplotype significant with 7R and 240 bp TR
Aguirre et al. [191]		SCZ	149	Mexican	Significant with 3R, 5R, 6R, 8R Not significant with 120 bp TR Haplotype significant with 3R, 5R, 6R, 8R

Table 19.6 (continued)

References	Polymorphisms	Disease phenotype	Number of patients	Ethnicity	Significance/notes
Kohn et al. [196]	48 bp VNTR 12 bp dup/del	SCZ	98	Israeli Jews	Not significant
Serretti et al. [248]		SCZ BP UP	651	Italian	Not significant
Barr et al. [195]	48 bp VNTR (4R, 7R) $(G)_n$ repeat (9R)	SCZ	Kindred	Swedish	Not significant
Okuyama et al. [154]	–521 C/T	SCZ	252	Japanese	Marginal significant with C-allele
Jonsson et al. [205]		SCZ	132	Swedish	Not significant
Rybakowsk et al. [39]		SCZ	138	–	Not significant with WCST in SCZ
Guan et al. [184]		ADHD	401	Han Chinese	Significant with T-allele
Ishiguro et al. [212]	–521 C/T 48 bp VNTR	ALC	185	Japanese	Not significant
Lung et al. [192]		SCZ	630	Chinese	Significant with long (>4R) VNTR Not significant with –521
Munafo et al. [218]		PT	–	–	Significant with –521 Not significant with 48 bp VNTR (meta-analysis)
Szilagyi et al. [217]	120 bp TR (5′-UTR) –521 C/T 48 bp VNTR	HD	73	Caucasian	Significant with –521 Not significant with 120 bp TR and 48 bp VNTR
Jonsson et al. [207]	48 bp VNTR –521 C/T 12 bp dup/del	SCZ	5696	–	Significant with –521 Not significant with 48 bp VNTR and 12 bp duplication (meta-analysis)

Table 19.6 (continued)

References	Polymorphisms	Disease phenotype	Number of patients	Ethnicity	Significance/notes
Seeman et al. [249]	+2526 T/G (V194G)	SCZ	92	African and Caucasian	Not significant
Bhaduri et al. [182]	120 bp TR (5′-UTR) 12 bp dup/del 48 bp VNTR (6R, 7R)	ADHD	50	Indians	Significant with 6R and 7R Not significant with 120 bp TR and 12 bp duplication
Kereszturi et al. [183]	120 bp TR (5′-UTR) –616 C/G –615 A/G –521 C/T	ADHD	173	Caucasian	Significant with 120 bp TR (1 repeat) Not significant with –616, –615 and –521
Ambrosio et al. [197]	–616 C/G –521 C/T 48 bp VNTR	SCZ	90 trios	Portuguese	Not significant
Bellgrove et al. [165]	48 bp VNTR (7R) –616 C/G	ADHD	54	European	Not significant
Petronis et al. [199]	48 bp VNTR (7R) $(G)_n$ repeat *Sma I* RFLP 12 bp dup/del	SCZ	50	European	Not significant with all SNPs but a trend toward excess of 7R
Mitsuyasu et al. [202]	–768 G/A –616 C/G –521 C/T –376 C/T –291 C/T 48 bp VNTR	SCZ	208	Japanese	Not significant
Golimbet et al. [203]	–809 G/A –616 G/C –521 C/T	SCZ	240	Russian	Not significant

Table 19.6 (continued)

References	Polymorphisms	Disease phenotype	Number of patients	Ethnicity	Significance/notes
Xing et al. [204]	–616 G/C –603 T/del –602 G/del –600 G/C –521 C/T –376 C/T 120 bp TR (5′-UTR)	SCZ	210	Chinese	Significant with 120 bp TR Not significant with –616, –603, –602, –600, –521, –376 Haplotype significant with 120 bp TR, –616, –602, –521, –376
Chang et al. [211]	*Sma I* RFLP 12 bp dup/del 13 bp del $(G)_n$ repeat + 2526 T/G (V194G) 48 bp VNTR	ALC	62	Taiwanese	Not significant with individual SNPs or as haplotypes
Paterson et al. [206]	*Pst I*	SCZ	41	Caucasian	Not significant
Nothen et al. [250]	13 bp del	SCZ BP	236	German	Not significant
Mitsuyasu et al. [151]	120 bp TR (5′-UTR) –1217 G/del –1123 C/T –1106 T/C –1102 G/A –930 C/G/T –906 T/C –809 G/A –768 G/A –713 C/T	SCZ	216	Japanese	Not significant with individual SNPs and weak association with haplotypes

Table 19.6 (continued)

References	Polymorphisms	Disease phenotype	Number of patients	Ethnicity	Significance/notes
	–616 C/G				
	–615 A/G				
	–603 T/del				
	–600 G/C				
	–598 G/T				
	–597 $(G)_{2–5}$				
	–521 C/T				
	–376 C/T				
	–364 A/G				
	–291 C/T				
	–234 C/A				
	–128 G/T				
	–11 C/T				
	+31 G/C				
	12 bp dup/del				
	21 bp del				
	13 bp del				
	48 bp VNTR				

Abbreviations: SCZ – schizophrenia; BP – bipolar disorder; ADHD – attention deficit hyperactivity disorder; ALC – alcoholism; HD – heroin dependence; OD – opiate dependence; UP – unipolar disorder; PT – personality traits; SNP – single nucleotide polymorphism; VNTR – variable number of tandem repeats; TR – tandem repeats; MA – methamphetamine abuse.
– undetermined or not applicable

greater number of repeats in the VNTR (>5) associated with increased disease susceptibility [200]. A meta-analysis conducted by Glatt et al. concluded that there was no association for the 48 bp VNTR and schizophrenia, although sex-dependent association may be present [201]. The considerable ethnic variation in this polymorphism complicates the interpretation of inconsistencies between studies based on different ethnic groups. Other SNPs in the 5′ UTR have been studied in several groups with their relation to schizophrenia, but the most comprehensive examined 28 polymorphisms tested for association with schizophrenia in a sample of 216 Japanese patients [151]. No significant association was observed with any individual SNP, but there was weak association for some particular haplotypes. This is consistent with the negative or weak findings of other studies that also investigated the same polymorphisms [154, 197, 202–206]. One meta-analysis reviewed several DRD4 polymorphisms and its relationship with schizophrenia in a total of 5,696 individuals [207]; a significant association was found with the –521 C/T allele but not for the 48 bp VNTR nor the 12 bp mutation in exon 1.

There are few studies investigating the association of DRD4 polymorphisms with bipolar disorder or substance abuse. Two groups found no association between the 48 bp VNTR variant and bipolar disorder [208, 209], whereas one study detected an association with D4.4 in 154 patients of Caucasian origin [210]. A rare D4.1 variant and the 13 bp deletion in exon 1 were tested as well; no association was found [106, 206]. For alcoholism, there are several studies reporting no association [138, 211, 212], and only one positive finding [213]. Case–control and family-based studies of opioid dependence include one case–control study of 396 German probands showing no association [214] and several studies reporting association between long repeats of the 48 bp VNTR and –521 C/T and opioid dependence [215–217]. The relationship between novelty seeking and the DRD4 gene has received considerable publicity, and there is an interesting meta-analysis of the association between 48 bp VNTR and –521 C/T polymorphisms with approach-related personality traits [218]. No overall effect was found for the 48 bp VNTR, but –521 C/T was shown to be significantly associated with traits such as novelty seeking and impulsivity.

19.3.5 D_5 Receptors

The dopamine D_5 receptor (DRD5) has not been investigated as much as some of the other subtypes discussed above. The DRD5 gene is located on chromosome 4p16.1 and contains only one exon with the coding region embedded (Fig. 19.6). The gene is 2,031 bp long and encodes 477 amino acids. Not many polymorphisms have been discovered in the DRD5 gene, but a dinucleotide repeat microsatellite polymorphism $(CT/GT/GA)_n$, located 18.5 kb from the 5′ end of the gene is highly polymorphic with at least 12 possible alleles [219]. In particular, the 148 bp allele has been extensively studied in relation to neuropsychiatric disease (Table 19.7). Within the 5′ UTR, a $(TC)_n$ dinucleotide repeat located within the promoter region has also been studied in schizophrenia and bipolar disorder [220]. However, there is no evidence that this repeat affects transcription activity or function. In addition,

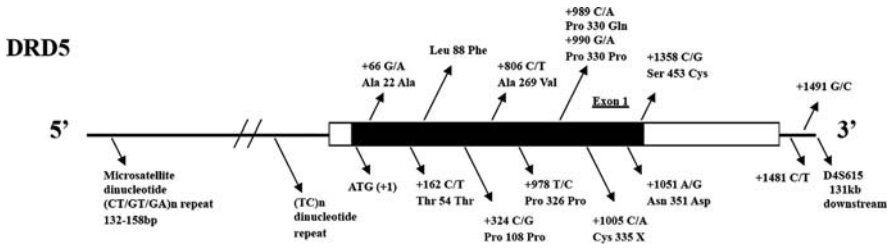

Fig. 19.6 Dopamine D_5 receptor: Schematic representation of polymorphisms (not to scale). Coding regions of exons are marked by *black blocks*, and the 5′ and 3′- UTR are represented by *white blocks*. The first base of the ATG start codon is denoted as +1 and the location of other polymorphisms are given relative to the start codon. Introns are represented as a *straight line* connecting the exons. Restriction enzymes, alteration of amino acids, and other distinct descriptions are given where applicable. (Modified from Kim et al. [42])

Sobell et al. described nine different SNPs in the coding region, including five missense and four silent mutations [221]. Of the five missense mutations, $Ala^{269}Val$ in the third extracellular loop, $Pro^{330}Gln$ in the third cytoplasmic loop, $Asn^{351}Asp$ in the seventh transmembrane domain, and $Ser^{453}Cys$ in the C-terminus result in protein sequence changes and may have important functional consequences. A nonsense mutation Cys^{335} results in a truncated protein. Moreover, two polymorphisms (+1,481 C/T and +1,491 G/C) present in the 3′ end have been investigated [220]. Interestingly, one recently discovered novel SNP, D4S615, located far from the coding region, may be involved in schizophrenia [222].

As mentioned before, the most common allele of the microsatellite polymorphism is the 148 bp allele and a number of different studies have examined association with schizophrenia, bipolar disorder, ADHD, and substance abuse. As with other genetic association studies of dopamine receptor genes, contradictory results have been reported with both case–control and family-based studies. No association with ADHD was observed in some studies [159, 172, 223, 224], while others have demonstrated association [225–227]. A meta-analysis of European subjects revealed increased risk effect for ADHD with the 148 bp allele vs. a protective effect of the 136 bp allele [181]. This was further supported by another meta-analysis which also demonstrated a significant association between the 148 bp allele and ADHD [186].

No association with the 148 bp allele and schizophrenia was seen in two different pedigrees [228] and in a case–control Caucasian study [224], whereas a positive finding was reported in a larger Scottish sample ($n = 428$) [222]. Sobell et al. also noted non-significant case–control results with schizophrenia for polymorphisms in the coding region [221], suggesting that the resulting changes in protein sequence do not affect susceptibility to schizophrenia. A lack of association and no difference in allele frequencies with both schizophrenia and bipolar disorder were found in a family linkage study [220]. To further support this notion, no association was seen in a family-based and a case–control study on the $(CT/GT/GA)_n$ dinucleotide repeat with bipolar disorder [103, 222]. In addition, the D4S615 microsatellite polymorphism has been shown to be associated with

Table 19.7 Dopamine D_5 receptor polymorphisms and neuropsychiatric diseases

References	Polymorphisms	Disease phenotype	Number of patients	Ethnicity	Significance/notes
Vanyukov et al. [251]	$(CT/GT/GA)_n$ repeat 148 bp	SA	42	European-American	Significant with more robust differences in females
Daly et al. [225]		ADHD	118	Irish	Significant
Barr et al. [223]		ADHD	116	Caucasian	Not significant
Maher et al. [186]		ADHD	571	–	Significant (meta-analysis)
Lowe et al. [227]		ADHD	–	Large combined homogenous international	Significant with inattentive and combined clinical subtypes
Manor et al. [226]		ADHD	164	Israelian	Marginal significant
Bakker et al. [159]		ADHD	236	Dutch	Not significant
Li et al. [181]	$(CT/GT/GA)_n$ repeat 136, 146 and148 bp	ADHD	–	European	Significant effect of 148 bp from meta-analysis Not significant with 136 and 146 bp
Muir et al. [222]	$(CT/GT/GA)_n$ repeat 148 bp D4S615	SCZ BP	428	Scottish	Significant with SCZ for 148 bp and D4S615 but not with LD Not significant with BP
Asherson et al. [220]	$(TC)_n$ repeat +978 T/C +1481 C/T +1491 G/C	SCZ BP	227	Caucasian from UK	Not significant
Kalsi et al. [228]	$(CT/GT/GA)_n$ repeat 134–156 bp	SCZ	11 12 (pedigrees)	Icelandic English	Not significant but maybe of etiological importance
Kirov et al. [103]		BP	122 trios	British-Caucasian	Not significant
Tahir et al. [172]		ADHD	111	Turkish	Not significant
Mill et al. [224]	$(CT/GT/GA)_n$ repeat 148 bp D4S615	ADHD SCZ	188	Caucasian	Significant with D4S615 Not significant with 148 bp

Table 19.7 (continued)

References	Polymorphisms	Disease phenotype	Number of patients	Ethnicity	Significance/notes
Sobell et al. [221]	Cys 335 X Asn 351 Asp Ala 269 Val Ser 453 Cys Pro 330 Gln 4 other silent SNPs	SCZ	78	Caucasian, African-American, Asian, Native-American	Not significant with all SNPs

Abbreviations: SCZ – schizophrenia; BP – bipolar disorder; ADHD – attention deficit hyperactivity disorder; SA – substance abuse; SNP – single nucleotide polymorphism; LD – linkage disequilibrium.
– undetermined or not applicable

schizophrenia in two separate studies, suggesting that the downstream region of DRD5 may link to molecular mechanisms involved in schizophrenia [222, 224]. A possible interpretation of these results given by the group was that the D4S615 polymorphisms show independent linkage disequilibrium with an allele at 4p16 that increases susceptibility to schizophrenia [222]. Further evidence is required to confirm this hypothesis and the task of unraveling this putative mechanism remains a challenge.

19.4 Conclusion

The relationship between dopamine receptor polymorphisms and neuropsychiatric diseases has been studied extensively, but inconsistent results have emerged. Although there are some replicated associations between specific polymorphisms and some disorders, these examples are tempered by non-significant results from other studies. Overall, the associations between (1) alcoholism and the DRD2 Taq I A polymorphism, and (2) DRD4 variation, ADHD, and related traits of impulsivity and novelty seeking have the strongest support. A number of confounding factors may account for these inconsistencies. Case–control studies are susceptible to false associations due to population stratification, with some SNP frequencies varying considerably among different ethnic groups. Family-based association tests avoid this problem, but at the cost of smaller sample sizes due to the difficulty of recruiting multiple family members in addition to the proband [229]. In addition, neuropsychiatric diseases are both genetically and biologically complex. Polygenic disease models involve many susceptibility and protective genes that may interact, meaning that each candidate gene may exert only a small effect, may interact with other genetic variants, or may be operating only in some cases or families. As a result, single studies are often underpowered to detect these small signals amidst considerable noise. In this sense, haplotype analysis can be helpful, but the analysis of concurrent or combinatorial variation in many genes and SNPs remains a challenge, especially as whole-genome association data from large collaborative samples begin to emerge.

The genetic study of neuropsychiatric disease is complicated further by the lack of defining pathological signatures to establish diagnosis. This leads to problems of etiological heterogeneity, ascertainment bias, and phenocopies that no amount of analytical refinement can address. While genetic studies of complex diseases such as cancer, autoimmune disorders, cardiovascular, and metabolic disease are also affected by the complexity of polygenetic interactions, at least diagnosis can be established with objective biochemical or pathological markers. Perhaps the most difficult confounding factor is the multidetermined nature of behavior and the related issue of non-deterministic relationships between brain structure and function. With our current and limited understanding of the emergent properties of neuron, network, and circuit assemblies in the brain, it may not be possible to detect simple relationships between DNA variation and the higher level psychological functions involved in neuropsychiatric disease.

In conclusion, the source of inconsistency in individual genetic association and linkage studies cannot be easily explained, though variations in ethnic grouping, phenotype assignment, sampling bias, and etiological heterogeneity are certainly not helpful. Addressing these confounds will be a major challenge for the genetic analysis of any complex disease in the future [77]. Improved genetic analysis methods that incorporate information on gene–gene interactions will be useful, but gene–environment interactions and epigenetic mechanisms are also likely to be important, and a way of integrating data on all of these components is needed. In this review, we focused on genetic association studies of dopamine receptor polymorphisms and major neuropsychiatric disorders, but did not seek to cover all normal and disease phenotypes that have been investigated. These other disorders include Parkinson's disease, Tourette syndrome, Huntington's chorea, Alzheimer's disease, and novelty seeking, for example. Furthermore, dopamine receptor gene variation has also been associated with differences in the efficacy and side effects of dopaminergic drugs [1]. Studies have demonstrated the association between DRD2 –241A/G polymorphism and risperidone response in schizophrenic patients [230], whereas the Taq I A allele has been reported to increase risk for hyperprolactinemia-related side effects with nemonapride [231]. In addition, one of the most important side effects in the long-term treatment of schizophrenia with typical antipsychotics is tardive dyskinesia (TD). Different dopamine receptor variants have been investigated, with DRD3 being the most extensively studied in relation to the risk of TD. A strong association was first reported with the $Ser^{9}Gly$ variant [232] and confirmed by a recent meta-analysis [233], although some studies do not support the original finding [234, 235]. However, the pharmacogenetics of dopamine receptor genes is itself a large area and beyond the scope of this review.

References

1. Wong AH, Buckle CE, Van Tol HH. Polymorphisms in dopamine receptors: what do they tell us? Eur J Pharmacol 2000;410:183–203.
2. Lader M. Some adverse effects of antipsychotics: prevention and treatment. J Clin Psychiatry 1999;60(Suppl 12):18–21.
3. Emilien G, Maloteaux JM, Geurts M, Hoogenberg K, Cragg S. Dopamine receptors – physiological understanding to therapeutic intervention potential. Pharmacol Ther 1999;84: 133–56.
4. American Psychiatric association. Diagnostics and Statistical Manual of Mental Disorders. 4th Edition, text revised. Washington, DC: American Psychiatric Association, 2000.
5. Wong AH, Gottesman, II, Petronis A. Phenotypic differences in genetically identical organisms: the epigenetic perspective. Hum Mol Genet 2005;14 (Spec No. 1):R11–8.
6. Sokoloff P, Giros B, Martres MP, Bouthenet ML, Schwartz JC. Molecular cloning and characterization of a novel dopamine receptor (D3) as a target for neuroleptics. Nature 1990;347:146–51.
7. Van Tol HH, Bunzow JR, Guan HC, et al. Cloning of the gene for a human dopamine D4 receptor with high affinity for the antipsychotic clozapine. Nature 1991;350:610–4.
8. Sunahara RK, Guan HC, O'Dowd BF, et al. Cloning of the gene for a human dopamine D5 receptor with higher affinity for dopamine than D1. Nature 1991;350:614–9.

9. Tiberi M, Jarvie KR, Silvia C, et al. Cloning, molecular characterization, and chromosomal assignment of a gene encoding a second D1 dopamine receptor subtype: differential expression pattern in rat brain compared with the D1A receptor. Proc Natl Acad Sci USA 1991;88:7491–5.
10. Kebabian JW, Calne DB. Multiple receptors for dopamine. Nature 1979;277:93–6.
11. Civelli O, Bunzow JR, Grandy DK. Molecular diversity of the dopamine receptors. Annu Rev Pharmacol Toxicol 1993;33:281–307.
12. Gingrich JA, Caron MG. Recent advances in the molecular biology of dopamine receptors. Annu Rev Neurosci 1993;16:299–321.
13. Jackson DM, Westlind-Danielsson A. Dopamine receptors: molecular biology, biochemistry and behavioural aspects. Pharmacol Ther 1994;64:291–370.
14. Seeman P, Van Tol HH. Dopamine receptor pharmacology. Trends Pharmacol Sci 1994; 15:264–70.
15. Sokoloff P, Schwartz JC. Novel dopamine receptors half a decade later. Trends Pharmacol Sci 1995;16:270–5.
16. Missale C, Nash SR, Robinson SW, Jaber M, Caron MG. Dopamine receptors: from structure to function. Physiol Rev 1998;78:189–225.
17. Dal Toso R, Sommer B, Ewert M, et al. The dopamine D2 receptor: two molecular forms generated by alternative splicing. EMBO J 1989;8:4025–34.
18. Giros B, Sokoloff P, Martres MP, Riou JF, Emorine LJ, Schwartz JC. Alternative splicing directs the expression of two D2 dopamine receptor isoforms. Nature 1989;342: 923–6.
19. Monsma FJ, Jr., McVittie LD, Gerfen CR, Mahan LC, Sibley DR. Multiple D2 dopamine receptors produced by alternative RNA splicing. Nature 1989;342:926–9.
20. Vallone D, Picetti R, Borrelli E. Structure and function of dopamine receptors. Neurosci Biobehav Rev 2000;24:125–32.
21. Fishburn CS, Belleli D, David C, Carmon S, Fuchs S. A novel short isoform of the D3 dopamine receptor generated by alternative splicing in the third cytoplasmic loop. J Biol Chem 1993;268:5872–8.
22. Giros B, Martres MP, Pilon C, Sokoloff P, Schwartz JC. Shorter variants of the D3 dopamine receptor produced through various patterns of alternative splicing. Biochem Biophys Res Commun 1991;176:1584–92.
23. Snyder LA, Roberts JL, Sealfon SC. Alternative transcripts of the rat and human dopamine D3 receptor. Biochem Biophys Res Commun 1991;180:1031–5.
24. Grandy DK, Marchionni MA, Makam H, et al. Cloning of the cDNA and gene for a human D2 dopamine receptor. Proc Natl Acad Sci USA 1989;86:9762–6.
25. Weinshank RL, Adham N, Macchi M, Olsen MA, Branchek TA, Hartig PR. Molecular cloning and characterization of a high affinity dopamine receptor (D1 beta) and its pseudogene. J Biol Chem 1991;266:22427–35.
26. Castro SW, Strange PG. Differences in the ligand binding properties of the short and long versions of the D2 dopamine receptor. J Neurochem 1993;60:372–5.
27. Malmberg A, Jackson DM, Eriksson A, Mohell N. Unique binding characteristics of antipsychotic agents interacting with human dopamine D2A, D2B, and D3 receptors. Mol Pharmacol 1993;43:749–54.
28. Senogles SE. The D2s dopamine receptor stimulates phospholipase D activity: a novel signaling pathway for dopamine. Mol Pharmacol 2000;58:455–62.
29. Senogles SE. D2s dopamine receptor mediates phospholipase D and antiproliferation. Mol Cell Endocrinol 2003;209:61–9.
30. Grandy DK, Zhou QY, Allen L, et al. A human D1 dopamine receptor gene is located on chromosome 5 at q35.1 and identifies an EcoRI RFLP. Am J Hum Genet 1990;47: 828–34.
31. Zhou QY, Li C, Civelli O. Characterization of gene organization and promoter region of the rat dopamine D1 receptor gene. J Neurochem 1992;59:1875–83.

32. Liu Q, Sobell JL, Heston LL, Sommer SS. Screening the dopamine D1 receptor gene in 131 schizophrenics and eight alcoholics: identification of polymorphisms but lack of functionally significant sequence changes. Am J Med Genet 1995;60:165–71.
33. Cichon S, Nothen MM, Stober G, et al. Systematic screening for mutations in the 5′-regulatory region of the human dopamine D1 receptor (DRD1) gene in patients with schizophrenia and bipolar affective disorder. Am J Med Genet 1996;67:424–8.
34. Kojima H, Ohmori O, Shinkai T, Terao T, Suzuki T, Abe K. Dopamine D1 receptor gene polymorphism and schizophrenia in Japan. Am J Med Genet 1999;88:116–9.
35. Ni X, Trakalo JM, Mundo E, et al. Linkage disequilibrium between dopamine D1 receptor gene (DRD1) and bipolar disorder. Biol Psychiatry 2002;52:1144–50.
36. Dmitrzak-Weglarz M, Rybakowski JK, Slopien A, et al. Dopamine receptor D1 gene -48A/G polymorphism is associated with bipolar illness but not with schizophrenia in a Polish population. Neuropsychobiology 2006;53:46–50.
37. Del Zompo M, De Luca V, Severino G, et al. Haplotype association study between DRD1 gene and bipolar type I affective disorder in two samples from Canada and Sardinia. Am J Med Genet B Neuropsychiatr Genet 2007;144:237–41.
38. Severino G, Congiu D, Serreli C, et al. A48G polymorphism in the D1 receptor genes associated with bipolar I disorder. Am J Med Genet B Neuropsychiatr Genet 2005; 134:37–8.
39. Rybakowski JK, Borkowska A, Czerski PM, Kapelski P, Dmitrzak-Weglarz M, Hauser J. An association study of dopamine receptors polymorphisms and the Wisconsin Card Sorting Test in schizophrenia. J Neural Transm 2005;112:1575–82.
40. Misener VL, Luca P, Azeke O, et al. Linkage of the dopamine receptor D1 gene to attention-deficit/hyperactivity disorder. Mol Psychiatry 2004;9:500–9.
41. Limosin F, Loze JY, Rouillon F, Ades J, Gorwood P. Association between dopamine receptor D1 gene DdeI polymorphism and sensation seeking in alcohol-dependent men. Alcohol Clin Exp Res 2003;27:1226–8.
42. Kim DJ, Park BL, Yoon S, et al. 5′ UTR polymorphism of dopamine receptor D1 (DRD1) associated with severity and temperament of alcoholism. Biochem Biophys Res Commun 2007;357:1135–41.
43. Maron E, Nikopensius T, Koks S, et al. Association study of 90 candidate gene polymorphisms in panic disorder. Psychiatr Genet 2005;15:17–24.
44. Koks S, Nikopensius T, Koido K, et al. Analysis of SNP profiles in patients with major depressive disorder. Int J Neuropsychopharmacol 2006;9:167–74.
45. Grandy DK, Litt M, Allen L, et al. The human dopamine D2 receptor gene is located on chromosome 11 at q22-q23 and identifies a TaqI RFLP. Am J Hum Genet 1989;45:778–85.
46. Pohjalainen T, Rinne JO, Nagren K, et al. The A1 allele of the human D2 dopamine receptor gene predicts low D2 receptor availability in healthy volunteers. Mol Psychiatry 1998;3: 256–60.
47. Thompson J, Thomas N, Singleton A, et al. D2 dopamine receptor gene (DRD2) Taq1 A polymorphism: reduced dopamine D2 receptor binding in the human striatum associated with the A1 allele. Pharmacogenetics 1997;7:479–84.
48. Barr CL, Kidd KK. Population frequencies of the A1 allele at the dopamine D2 receptor locus. Biol Psychiatry 1993;34:204–9.
49. Cravchik A, Sibley DR, Gejman PV. Functional analysis of the human D2 dopamine receptor missense variants. J Biol Chem 1996;271:26013–7.
50. Duan J, Wainwright MS, Comeron JM, et al. Synonymous mutations in the human dopamine receptor D2 (DRD2) affect mRNA stability and synthesis of the receptor. Hum Mol Genet 2003;12:205–16.
51. Luo HR, Hou ZF, Wu J, Zhang YP, Wan YJ. Evolution of the DRD2 gene haplotype and its association with alcoholism in Mexican Americans. Alcohol 2005;36:117–25.
52. Blum K, Noble EP, Sheridan PJ, et al. Allelic association of human dopamine D2 receptor gene in alcoholism. JAMA 1990;263:2055–60.

53. Amadeo S, Abbar M, Fourcade ML, et al. D2 dopamine receptor gene and alcoholism. J Psychiatr Res 1993;27:173–9.
54. Arinami T, Itokawa M, Komiyama T, et al. Association between severity of alcoholism and the A1 allele of the dopamine D2 receptor gene TaqI A RFLP in Japanese. Biol Psychiatry 1993;33:108–14.
55. Ishiguro H, Arinami T, Saito T, et al. Association study between the -141C Ins/Del and TaqI A polymorphisms of the dopamine D2 receptor gene and alcoholism. Alcohol Clin Exp Res 1998;22:845–8.
56. Connor JP, Young RM, Lawford BR, Ritchie TL, Noble EP. D(2) dopamine receptor (DRD2) polymorphism is associated with severity of alcohol dependence. Eur Psychiatry 2002;17:17–23.
57. Kono Y, Yoneda H, Sakai T, et al. Association between early-onset alcoholism and the dopamine D2 receptor gene. Am J Med Genet 1997;74:179–82.
58. Gorwood P, Batel P, Gouya L, Courtois F, Feingold J, Ades J. Reappraisal of the association between the DRD2 gene, alcoholism and addiction. Eur Psychiatry 2000;15:90–6.
59. Gorwood P, Limosin F, Batel P, Duaux E, Gouya L, Ades J. The genetics of addiction: alcohol-dependence and D3 dopamine receptor gene. Pathol Biol (Paris) 2001;49:710–7.
60. Sander T, Ladehoff M, Samochowiec J, Finckh U, Rommelspacher H, Schmidt LG. Lack of an allelic association between polymorphisms of the dopamine D2 receptor gene and alcohol dependence in the German population. Alcohol Clin Exp Res 1999;23:578–81.
61. Goldman D, Brown GL, Albaugh B, et al. DRD2 dopamine receptor genotype, linkage disequilibrium, and alcoholism in American Indians and other populations. Alcohol Clin Exp Res 1993;17:199–204.
62. Foley PF, Loh EW, Innes DJ, et al. Association studies of neurotransmitter gene polymorphisms in alcoholic Caucasians. Ann NY Acad Sci 2004;1025:39–46.
63. Cruz C, Camarena B, Mejia JM, et al. The dopamine D2 receptor gene TaqI A1 polymorphism and alcoholism in a Mexican population. Arch Med Res 1995;26:421–6.
64. Chen CH, Chien SH, Hwu HG. Lack of association between TaqI A1 allele of dopamine D2 receptor gene and alcohol-use disorders in atayal natives of Taiwan. Am J Med Genet 1996;67:488–90.
65. Goldman D, Urbanek M, Guenther D, Robin R, Long JC. A functionally deficient DRD2 variant [Ser311Cys] is not linked to alcoholism and substance abuse. Alcohol 1998;16: 47–52.
66. Lu RB, Ko HC, Chang FM, et al. No association between alcoholism and multiple polymorphisms at the dopamine D2 receptor gene (DRD2) in three distinct Taiwanese populations. Biol Psychiatry 1996;39:419–29.
67. Edenberg HJ, Foroud T, Koller DL, et al. A family-based analysis of the association of the dopamine D2 receptor (DRD2) with alcoholism. Alcohol Clin Exp Res 1998;22:505–12.
68. Samochowiec J, Kucharska-Mazur J, Grzywacz A, et al. Family-based and case-control study of DRD2, DAT, 5HTT, COMT genes polymorphisms in alcohol dependence. Neurosci Lett 2006;410:1–5.
69. Blum K, Noble EP, Sheridan PJ, et al. Genetic predisposition in alcoholism: association of the D2 dopamine receptor TaqI B1 RFLP with severe alcoholics. Alcohol 1993;10: 59–67.
70. Konishi T, Calvillo M, Leng AS, Lin KM, Wan YJ. Polymorphisms of the dopamine D2 receptor, serotonin transporter, and GABA(A) receptor beta(3) subunit genes and alcoholism in Mexican-Americans. Alcohol 2004;32:45–52.
71. Finckh U, Rommelspacher H, Schmidt LG, Rolfs A. Allele-specific PCR for simultaneous amplification of both alleles of a deletion polymorphism in intron 6 of the human dopamine 2 receptor gene (DRD2). DNA Seq 1996;6:87–94.
72. Finckh U, von Widdern O, Giraldo-Velasquez M, et al. No association of the structural dopamine D2 receptor (DRD2) variant 311Cys with alcoholism. Alcohol Clin Exp Res 1996;20:528–32.

73. Parsian A, Cloninger CR, Zhang ZH. Functional variant in the DRD2 receptor promoter region and subtypes of alcoholism. Am J Med Genet 2000;96:407–11.
74. Gejman PV, Ram A, Gelernter J, et al. No structural mutation in the dopamine D2 receptor gene in alcoholism or schizophrenia. Analysis using denaturing gradient gel electrophoresis. JAMA 1994;271:204–8.
75. Neville MJ, Johnstone EC, Walton RT. Identification and characterization of ANKK1: a novel kinase gene closely linked to DRD2 on chromosome band 11q23.1. Hum Mutat 2004;23:540–5.
76. Pato CN, Macciardi F, Pato MT, Verga M, Kennedy JL. Review of the putative association of dopamine D2 receptor and alcoholism: a meta-analysis. Am J Med Genet 1993;48: 78–82.
77. Munafo MR, Matheson IJ, Flint J. Association of the DRD2 gene Taq1A polymorphism and alcoholism: a meta-analysis of case-control studies and evidence of publication bias. Mol Psychiatry 2007;12:454–61.
78. Smith L, Watson M, Gates S, Ball D, Foxcroft D. Meta-analysis of the association of the Taq1A polymorphism with the risk of alcohol dependency: a HuGE gene-disease association review. Am J Epidemiol 2008;167:125–38.
79. Kaneshima M, Higa T, Nakamoto H, Nagamine M. An association study between the Cys311 variant of dopamine D2 receptor gene and schizophrenia in the Okinawan population. Psychiatry Clin Neurosci 1997;51:379–81.
80. Chen CH, Chien SH, Hwu HG. No association of dopamine D2 receptor molecular variant Cys311 and schizophrenia in Chinese patients. Am J Med Genet 1996;67:418–20.
81. Laurent C, Bodeau-Pean S, Campion D, et al. No major role for the dopamine D2 receptor Ser–>Cys311 mutation in schizophrenia. Psychiatr Genet 1994;4:229–30.
82. Dubertret C, Gouya L, Hanoun N, et al. The 3′ region of the DRD2 gene is involved in genetic susceptibility to schizophrenia. Schizophr Res 2004;67:75–85.
83. Vijayan NN, Bhaskaran S, Koshy LV, et al. Association of dopamine receptor polymorphisms with schizophrenia and antipsychotic response in a South Indian population. Behav Brain Funct 2007;3:34.
84. Glatt SJ, Faraone SV, Tsuang MT. Meta-analysis identifies an association between the dopamine D2 receptor gene and schizophrenia. Mol Psychiatry 2003;8:911–5.
85. Glatt SJ, Jonsson EG. The Cys allele of the DRD2 Ser311Cys polymorphism has a dominant effect on risk for schizophrenia: evidence from fixed- and random-effects meta-analyses. Am J Med Genet B Neuropsychiatr Genet 2006;141:149–54.
86. Levinson DF. Meta-analysis in psychiatric genetics. Curr Psychiatry Rep 2005;7:143–51.
87. Jonsson EG, Sillen A, Vares M, Ekholm B, Terenius L, Sedvall GC. Dopamine D2 receptor gene Ser311Cys variant and schizophrenia: association study and meta-analysis. Am J Med Genet B Neuropsychiatr Genet 2003;119B:28–34.
88. Hanninen K, Katila H, Kampman O, et al. Association between the C957T polymorphism of the dopamine D2 receptor gene and schizophrenia. Neurosci Lett 2006;407:195–8.
89. Lawford BR, Young RM, Swagell CD, et al. The C/C genotype of the C957T polymorphism of the dopamine D2 receptor is associated with schizophrenia. Schizophr Res 2005; 73:31–7.
90. Hoenicka J, Aragues M, Rodriguez-Jimenez R, et al. C957T DRD2 polymorphism is associated with schizophrenia in Spanish patients. Acta Psychiatr Scand 2006;114:435–8.
91. Kukreti R, Tripathi S, Bhatnagar P, et al. Association of DRD2 gene variant with schizophrenia. Neurosci Lett 2006;392:68–71.
92. Meta-Analysis of All Published Schizophrenia-Association Studies (Case-Control Only) rs6277. Schizophrenia Research Forum, 2005–2008. (Accessed July 2, 2008, at http://www.schizophreniaforum.org/res/sczgene/meta.asp?geneID=93)
93. Breen G, Brown J, Maude S, et al. -141 C del/ins polymorphism of the dopamine receptor 2 gene is associated with schizophrenia in a British population. Am J Med Genet 1999;88: 407–10.

94. Arinami T, Gao M, Hamaguchi H, Toru M. A functional polymorphism in the promoter region of the dopamine D2 receptor gene is associated with schizophrenia. Hum Mol Genet 1997;6:577–82.
95. Dubertret C, Gorwood P, Gouya L, Deybach JC, Ades J. Association and excess of transmission of a DRD2 haplotype in a sample of French schizophrenic patients. Schizophr Res 2001;49:203–12.
96. Glatt SJ, Faraone SV, Tsuang MT. DRD2 -141C insertion/deletion polymorphism is not associated with schizophrenia: results of a meta-analysis. Am J Med Genet B Neuropsychiatr Genet 2004;128:21–3.
97. Li T, Arranz M, Aitchison KJ, et al. Case-control, haplotype relative risk and transmission disequilibrium analysis of a dopamine D2 receptor functional promoter polymorphism in schizophrenia. Schizophr Res 1998;32:87–92.
98. Ambrosio AM, Kennedy JL, Macciardi F, et al. Family association study between DRD2 and DRD3 gene polymorphisms and schizophrenia in a Portuguese population. Psychiatry Res 2004;125:185–91.
99. Parsons MJ, Mata I, Beperet M, et al. A dopamine D2 receptor gene-related polymorphism is associated with schizophrenia in a Spanish population isolate. Psychiatr Genet 2007;17: 159–63.
100. Himei A, Koh J, Sakai J, Inada Y, Akabame K, Yoneda H. The influence on the schizophrenic symptoms by the DRD2Ser/Cys311 and -141C Ins/Del polymorphisms. Psychiatry Clin Neurosci 2002;56:97–102.
101. Sarkar G, Kapelner S, Grandy DK, et al. Direct sequencing of the dopamine D2 receptor (DRD2) in schizophrenics reveals three polymorphisms but no structural change in the receptor. Genomics 1991;11:8–14.
102. Craddock N, Roberts Q, Williams N, McGuffin P, Owen MJ. Association study of bipolar disorder using a functional polymorphism (Ser311–>Cys) in the dopamine D2 receptor gene. Psychiatr Genet 1995;5:63–5.
103. Kirov G, Jones I, McCandless F, Craddock N, Owen MJ. Family-based association studies of bipolar disorder with candidate genes involved in dopamine neurotransmission: DBH, DAT1, COMT, DRD2, DRD3 and DRD5. Mol Psychiatry 1999;4:558–65.
104. Furlong RA, Coleman TA, Ho L, et al. No association of a functional polymorphism in the dopamine D2 receptor promoter region with bipolar or unipolar affective disorders. Am J Med Genet 1998;81:385–7.
105. Gorwood P, Bellivier F, Ades J, Leboyer M. The DRD2 gene and the risk for alcohol dependence in bipolar patients. Eur Psychiatry 2000;15:103–8.
106. Li T, Liu X, Sham PC, et al. Association analysis between dopamine receptor genes and bipolar affective disorder. Psychiatry Res 1999;86:193–201.
107. Serretti A, Lattuada E, Lorenzi C, Lilli R, Smeraldi E. Dopamine receptor D2 Ser/Cys 311 variant is associated with delusion and disorganization symptomatology in major psychoses. Mol Psychiatry 2000;5:270–4.
108. Lawford BR, Young RM, Noble EP, et al. The D(2) dopamine receptor A(1) allele and opioid dependence: association with heroin use and response to methadone treatment. Am J Med Genet 2000;96:592–8.
109. Conner BT, Noble EP, Berman SM, et al. DRD2 genotypes and substance use in adolescent children of alcoholics. Drug Alcohol Depend 2005;79:379–87.
110. Xu K, Lichtermann D, Lipsky RH, et al. Association of specific haplotypes of D2 dopamine receptor gene with vulnerability to heroin dependence in 2 distinct populations. Arch Gen Psychiatry 2004;61:597–606.
111. Smith SS, O'Hara BF, Persico AM, et al. Genetic vulnerability to drug abuse. The D2 dopamine receptor Taq I B1 restriction fragment length polymorphism appears more frequently in polysubstance abusers. Arch Gen Psychiatry 1992;49:723–7.
112. Gelernter J, Kranzler H, Satel SL. No association between D2 dopamine receptor (DRD2) alleles or haplotypes and cocaine dependence or severity of cocaine dependence in European- and African-Americans. Biol Psychiatry 1999;45:340–5.

113. Crocq MA, Mant R, Asherson P, et al. Association between schizophrenia and homozygosity at the dopamine D3 receptor gene. J Med Genet 1992;29:858–60.
114. Gene Overview of All Published Schizophrenia-Association Studies for DRD3. Schizophrenic Research Forum, 2005–2008. (Accessed July 2, 2008, at http://www.schizophreniaforum.org/res/sczgene/geneoverview.asp?geneid=224)
115. Joober R, Toulouse A, Benkelfat C, et al. DRD3 and DAT1 genes in schizophrenia: an association study. J Psychiatr Res 2000;34:285–91.
116. Chen CH, Liu MY, Wei FC, Koong FJ, Hwu HG, Hsiao KJ. Further evidence of no association between Ser9Gly polymorphism of dopamine D3 receptor gene and schizophrenia. Am J Med Genet 1997;74:40–3.
117. Jonsson EG, Flyckt L, Burgert E, et al. Dopamine D3 receptor gene Ser9Gly variant and schizophrenia: association study and meta-analysis. Psychiatr Genet 2003;13:1–12.
118. Virgos C, Martorell L, Valero J, et al. Association study of schizophrenia with polymorphisms at six candidate genes. Schizophr Res 2001;49:65–71.
119. Rietschel M, Nothen MM, Albus M, et al. Dopamine D3 receptor Gly9/Ser9 polymorphism and schizophrenia: no increased frequency of homozygosity in German familial cases. Schizophr Res 1996;20:181–6.
120. Rothschild LG, Badner J, Cravchik A, Gershon ES, Gejman PV. No association detected between a D3 receptor gene-expressed variant and schizophrenia. Am J Med Genet 1996;67:232–4.
121. Nanko S, Sasaki T, Fukuda R, et al. A study of the association between schizophrenia and the dopamine D3 receptor gene. Hum Genet 1993;92:336–8.
122. Lorenzo CV, Baca-Garcia E, Hernandez MD, et al. No association between the Ser9Gly polymorphism of the dopamine D3 receptor gene and schizophrenia in a Spanish sample. Am J Med Genet B Neuropsychiatr Genet 2007;144:344–6.
123. Dominguez E, Loza MI, Padin F, et al. Extensive linkage disequilibrium mapping at HTR2A and DRD3 for schizophrenia susceptibility genes in the Galician population. Schizophr Res 2007;90:123–9.
124. Laurent C, Savoye C, Samolyk D, et al. Homozygosity at the dopamine D3 receptor locus is not associated with schizophrenia. J Med Genet 1994;31:260.
125. Tanaka T, Igarashi S, Onodera O, et al. Association study between schizophrenia and dopamine D3 receptor gene polymorphism. Am J Med Genet 1996;67:366–8.
126. Fathalli F, Rouleau GA, Xiong L, et al. No association between the DRD3 Ser9Gly polymorphism and schizophrenia. Schizophr Res 2008;98:98–104.
127. Kremer I, Rietschel M, Dobrusin M, et al. No association between the dopamine D3 receptor Bal I polymorphism and schizophrenia in a family-based study of a Palestinian Arab population. Am J Med Genet 2000;96:778–80.
128. Staddon S, Arranz MJ, Mancama D, et al. Association between dopamine D3 receptor gene polymorphisms and schizophrenia in an isolate population. Schizophr Res 2005;73:49–54.
129. Ishiguro H, Okuyama Y, Toru M, Arinami T. Mutation and association analysis of the 5′ region of the dopamine D3 receptor gene in schizophrenia patients: identification of the Ala38Thr polymorphism and suggested association between DRD3 haplotypes and schizophrenia. Mol Psychiatry 2000;5:433–8.
130. Kennedy JL, Billett EA, Macciardi FM, et al. Association study of dopamine D3 receptor gene and schizophrenia. Am J Med Genet 1995;60:558–62.
131. Sivagnanasundaram S, Morris AG, Gaitonde EJ, McKenna PJ, Mollon JD, Hunt DM. A cluster of single nucleotide polymorphisms in the 5′-leader of the human dopamine D3 receptor gene (DRD3) and its relationship to schizophrenia. Neurosci Lett 2000;279:13–6.
132. Jonsson EG, Kaiser R, Brockmoller J, Nimgaonkar VL, Crocq MA. Meta-analysis of the dopamine D3 receptor gene (DRD3) Ser9Gly variant and schizophrenia. Psychiatr Genet 2004;14:9–12.
133. Parsian A, Chakraverty S, Todd RD. Possible association between the dopamine D3 receptor gene and bipolar affective disorder. Am J Med Genet 1995;60:234–7.

134. Elvidge G, Jones I, McCandless F, Asherson P, Owen MJ, Craddock N. Allelic variation of a BalI polymorphism in the DRD3 gene does not influence susceptibility to bipolar disorder: results of analysis and meta-analysis. Am J Med Genet 2001;105:307–11.
135. Van Den Bogaert A, Sleegers K, De Zutter S, et al. No allelic association or interaction of three known functional polymorphisms with bipolar disorder in a northern Swedish isolated population. Psychiatr Genet 2006;16:209–12.
136. Barr CL, Wigg KG, Wu J, et al. Linkage study of two polymorphisms at the dopamine D3 receptor gene and attention-deficit hyperactivity disorder. Am J Med Genet 2000;96:114–7.
137. Higuchi S, Muramatsu T, Matsushita S, Murayama M. No evidence of association between structural polymorphism at the dopamine D3 receptor locus and alcoholism in the Japanese. Am J Med Genet 1996;67:412–4.
138. Parsian A, Chakraverty S, Fisher L, Cloninger CR. No association between polymorphisms in the human dopamine D3 and D4 receptors genes and alcoholism. Am J Med Genet 1997;74:281–5.
139. Lee MS, Ryu SH. No association between the dopamine D3 receptor gene and Korean alcohol dependence. Psychiatr Genet 2002;12:173–6.
140. Gorwood P, Martres MP, Ades J, et al. Lack of association between alcohol-dependence and D3 dopamine receptor gene in three independent samples. Am J Med Genet 1995;60: 529–31.
141. Duaux E, Gorwood P, Griffon N, et al. Homozygosity at the dopamine D3 receptor gene is associated with opiate dependence. Mol Psychiatry 1998;3:333–6.
142. Comings DE, Gonzalez N, Wu S, et al. Homozygosity at the dopamine DRD3 receptor gene in cocaine dependence. Mol Psychiatry 1999;4:484–7.
143. Krebs MO, Sautel F, Bourdel MC, et al. Dopamine D3 receptor gene variants and substance abuse in schizophrenia. Mol Psychiatry 1998;3:337–41.
144. Chang FM, Kidd JR, Livak KJ, Pakstis AJ, Kidd KK. The world-wide distribution of allele frequencies at the human dopamine D4 receptor locus. Hum Genet 1996;98:91–101.
145. Ebstein RP, Novick O, Umansky R, et al. Dopamine D4 receptor (D4DR) exon III polymorphism associated with the human personality trait of Novelty Seeking. Nat Genet 1996;12:78–80.
146. Benjamin J, Li L, Patterson C, Greenberg BD, Murphy DL, Hamer DH. Population and familial association between the D4 dopamine receptor gene and measures of Novelty Seeking. Nat Genet 1996;12:81–4.
147. Kluger AN, Siegfried Z, Ebstein RP. A meta-analysis of the association between DRD4 polymorphism and novelty seeking. Mol Psychiatry 2002;7:712–7.
148. Asghari V, Schoots O, van Kats S, et al. Dopamine D4 receptor repeat: analysis of different native and mutant forms of the human and rat genes. Mol Pharmacol 1994;46:364–73.
149. Jovanovic V, Guan HC, Van Tol HH. Comparative pharmacological and functional analysis of the human dopamine D4.2 and D4.10 receptor variants. Pharmacogenetics 1999; 9:561–8.
150. Seaman MI, Fisher JB, Chang F, Kidd KK. Tandem duplication polymorphism upstream of the dopamine D4 receptor gene (DRD4). Am J Med Genet 1999;88:705–9.
151. Mitsuyasu H, Kawasaki H, Ninomiya H, et al. Genetic structure of the dopamine receptor D4 gene (DRD4) and lack of association with schizophrenia in Japanese patients. J Psychiatr Res 2007;41:763–75.
152. Mitsuyasu H, Ozawa H, Takeda Y, Fukumaki Y. Novel polymorphisms in the upstream region of the human dopamine D4 receptor (DRD4) gene. J Hum Genet 1999;44:416–8.
153. Kamakura S, Iwaki A, Matsumoto M, Fukumaki Y. Cloning and characterization of the 5′-flanking region of the human dopamine D4 receptor gene. Biochem Biophys Res Commun 1997;235:321–6.
154. Okuyama Y, Ishiguro H, Toru M, Arinami T. A genetic polymorphism in the promoter region of DRD4 associated with expression and schizophrenia. Biochem Biophys Res Commun 1999;258:292–5.

155. Catalano M, Nobile M, Novelli E, Nothen MM, Smeraldi E. Distribution of a novel mutation in the first exon of the human dopamine D4 receptor gene in psychotic patients. Biol Psychiatry 1993;34:459–64.
156. Cichon S, Nothen MM, Catalano M, et al. Identification of two novel polymorphisms and a rare deletion variant in the human dopamine D4 receptor gene. Psychiatr Genet 1995;5: 97–103.
157. Eisenberg J, Zohar A, Mei-Tal G, et al. A haplotype relative risk study of the dopamine D4 receptor (DRD4) exon III repeat polymorphism and attention deficit hyperactivity disorder (ADHD). Am J Med Genet 2000;96:258–61.
158. Hawi Z, McCarron M, Kirley A, Daly G, Fitzgerald M, Gill M. No association of the dopamine DRD4 receptor (DRD4) gene polymorphism with attention deficit hyperactivity disorder (ADHD) in the Irish population. Am J Med Genet 2000;96:268–72.
159. Bakker SC, van der Meulen EM, Oteman N, et al. DAT1, DRD4, and DRD5 polymorphisms are not associated with ADHD in Dutch families. Am J Med Genet B Neuropsychiatr Genet 2005;132:50–2.
160. Cheuk DK, Li SY, Wong V. Exon 3 polymorphisms of dopamine D4 receptor (DRD4) gene and attention deficit hyperactivity disorder in Chinese children. Am J Med Genet B Neuropsychiatr Genet 2006;141:907–11.
161. Carrasco X, Rothhammer P, Moraga M, et al. Genotypic interaction between DRD4 and DAT1 loci is a high risk factor for attention-deficit/hyperactivity disorder in Chilean families. Am J Med Genet B Neuropsychiatr Genet 2006;141:51–4.
162. Leung PW, Lee CC, Hung SF, et al. Dopamine receptor D4 (DRD4) gene in Han Chinese children with attention-deficit/hyperactivity disorder (ADHD): increased prevalence of the 2-repeat allele. Am J Med Genet B Neuropsychiatr Genet 2005;133:54–6.
163. Barkley RA, Smith KM, Fischer M, Navia B. An examination of the behavioral and neuropsychological correlates of three ADHD candidate gene polymorphisms (DRD4 7+, DBH TaqI A2, and DAT1 40 bp VNTR) in hyperactive and normal children followed to adulthood. Am J Med Genet B Neuropsychiatr Genet 2006;141:487–98.
164. Mill J, Curran S, Kent L, et al. Attention deficit hyperactivity disorder (ADHD) and the dopamine D4 receptor gene: evidence of association but no linkage in a UK sample. Mol Psychiatry 2001;6:440–4.
165. Bellgrove MA, Hawi Z, Lowe N, Kirley A, Robertson IH, Gill M. DRD4 gene variants and sustained attention in attention deficit hyperactivity disorder (ADHD): effects of associated alleles at the VNTR and -521 SNP. Am J Med Genet B Neuropsychiatr Genet 2005;136: 81–6.
166. El-Faddagh M, Laucht M, Maras A, Vohringer L, Schmidt MH. Association of dopamine D4 receptor (DRD4) gene with attention-deficit/hyperactivity disorder (ADHD) in a high-risk community sample: a longitudinal study from birth to 11 years of age. J Neural Transm 2004;111:883–9.
167. Grady DL, Chi HC, Ding YC, et al. High prevalence of rare dopamine receptor D4 alleles in children diagnosed with attention-deficit hyperactivity disorder. Mol Psychiatry 2003;8: 536–45.
168. Brookes K, Xu X, Chen W, et al. The analysis of 51 genes in DSM-IV combined type attention deficit hyperactivity disorder: association signals in DRD4, DAT1 and 16 other genes. Mol Psychiatry 2006;11:934–53.
169. Faraone SV, Biederman J, Weiffenbach B, et al. Dopamine D4 gene 7-repeat allele and attention deficit hyperactivity disorder. Am J Psychiatry 1999;156:768–70.
170. Gornick MC, Addington A, Shaw P, et al. Association of the dopamine receptor D4 (DRD4) gene 7-repeat allele with children with attention-deficit/hyperactivity disorder (ADHD): an update. Am J Med Genet B Neuropsychiatr Genet 2007;144:379–82.
171. Kotler M, Manor I, Sever Y, et al. Failure to replicate an excess of the long dopamine D4 exon III repeat polymorphism in ADHD in a family-based study. Am J Med Genet 2000;96: 278–81.

172. Tahir E, Yazgan Y, Cirakoglu B, Ozbay F, Waldman I, Asherson PJ. Association and linkage of DRD4 and DRD5 with attention deficit hyperactivity disorder (ADHD) in a sample of Turkish children. Mol Psychiatry 2000;5:396–404.
173. Smalley SL, Bailey JN, Palmer CG, et al. Evidence that the dopamine D4 receptor is a susceptibility gene in attention deficit hyperactivity disorder. Mol Psychiatry 1998;3: 427–30.
174. Rowe DC, Stever C, Chase D, Sherman S, Abramowitz A, Waldman ID. Two dopamine genes related to reports of childhood retrospective inattention and conduct disorder symptoms. Mol Psychiatry 2001;6:429–33.
175. Arcos-Burgos M, Castellanos FX, Konecki D, et al. Pedigree disequilibrium test (PDT) replicates association and linkage between DRD4 and ADHD in multigenerational and extended pedigrees from a genetic isolate. Mol Psychiatry 2004;9:252–9.
176. Swanson JM, Sunohara GA, Kennedy JL, et al. Association of the dopamine receptor D4 (DRD4) gene with a refined phenotype of attention deficit hyperactivity disorder (ADHD): a family-based approach. Mol Psychiatry 1998;3:38–41.
177. Rowe DC, Stever C, Giedinghagen LN, et al. Dopamine DRD4 receptor polymorphism and attention deficit hyperactivity disorder. Mol Psychiatry 1998;3:419–26.
178. Langley K, Marshall L, van den Bree M, et al. Association of the dopamine D4 receptor gene 7-repeat allele with neuropsychological test performance of children with ADHD. Am J Psychiatry 2004;161:133–8.
179. Qian Q, Wang Y, Li J, Yang L, Wang B, Zhou R. Association studies of dopamine D4 receptor gene and dopamine transporter gene polymorphisms in Han Chinese patients with attention deficit hyperactivity disorder. Beijing Da Xue Xue Bao 2003;35:412–8.
180. Qian Q, Wang Y, Zhou R, Yang L, Faraone SV. Family-based and case-control association studies of DRD4 and DAT1 polymorphisms in Chinese attention deficit hyperactivity disorder patients suggest long repeats contribute to genetic risk for the disorder. Am J Med Genet B Neuropsychiatr Genet 2004;128:84–9.
181. Li D, Sham PC, Owen MJ, He L. Meta-analysis shows significant association between dopamine system genes and attention deficit hyperactivity disorder (ADHD). Hum Mol Genet 2006;15:2276–84.
182. Bhaduri N, Das M, Sinha S, et al. Association of dopamine D4 receptor (DRD4) polymorphisms with attention deficit hyperactivity disorder in Indian population. Am J Med Genet B Neuropsychiatr Genet 2006;141:61–6.
183. Kereszturi E, Kiraly O, Csapo Z, et al. Association between the 120-bp duplication of the dopamine D4 receptor gene and attention deficit hyperactivity disorder: genetic and molecular analyses. Am J Med Genet B Neuropsychiatr Genet 2007;144:231–6.
184. Guan LL, Wang YF, Li J, Wang B, Yang L, Qian QJ. Association analysis of dopamine D4 receptor gene polymorphism and attention deficit hyperactivity disorder with/without disruptive behavior disorder. Beijing Da Xue Xue Bao 2007;39:233–6.
185. McCracken JT, Smalley SL, McGough JJ, et al. Evidence for linkage of a tandem duplication polymorphism upstream of the dopamine D4 receptor gene (DRD4) with attention deficit hyperactivity disorder (ADHD). Mol Psychiatry 2000;5:531–6.
186. Maher BS, Marazita ML, Ferrell RE, Vanyukov MM. Dopamine system genes and attention deficit hyperactivity disorder: a meta-analysis. Psychiatr Genet 2002;12:207–15.
187. Faraone SV, Doyle AE, Mick E, Biederman J. Meta-analysis of the association between the 7-repeat allele of the dopamine D(4) receptor gene and attention deficit hyperactivity disorder. Am J Psychiatry 2001;158:1052–7.
188. Weiss J, Magert HJ, Cieslak A, Forssmann WG. Association between different psychotic disorders and the DRD4 polymorphism, but no differences in the main ligand binding region of the DRD4 receptor protein compared to controls. Eur J Med Res 1996;1:439–45.
189. Zhang ML, Yuan GZ, Yao JJ, et al. Associations between six functional genes and schizophrenia. Zhonghua Yi Xue Yi Chuan Xue Za Zhi 2003;20:69–71.

190. Tang Y, Wang Y, Cai Z. Schizophrenia and dopamine D4 gene polymorphism in Chinese population: association analysis. Zhonghua Yi Xue Za Zhi 2001;81:995–8.
191. Aguirre AJ, Apiquian R, Fresan A, Cruz-Fuentes C. Association analysis of exon III and exon I polymorphisms of the dopamine D4 receptor locus in Mexican psychotic patients. Psychiatry Res 2007;153:209–15.
192. Lung FW, Chen N, Shu BC. Dopamine D4 receptor gene and the -521C>T polymorphism of the upstream region of the dopamine D4 receptor gene in schizophrenia. Psychiatr Genet 2006;16:139–43.
193. Hong CJ, Lee YL, Sim CB, Hwu HG. Dopamine D4 receptor variants in Chinese sporadic and familial schizophrenics. Am J Med Genet 1997;74:412–5.
194. Kaiser R, Konneker M, Henneken M, et al. Dopamine D4 receptor 48-bp repeat polymorphism: no association with response to antipsychotic treatment, but association with catatonic schizophrenia. Mol Psychiatry 2000;5:418–24.
195. Barr CL, Kennedy JL, Lichter JB, et al. Alleles at the dopamine D4 receptor locus do not contribute to the genetic susceptibility to schizophrenia in a large Swedish kindred. Am J Med Genet 1993;48:218–22.
196. Kohn Y, Ebstein RP, Heresco-Levy U, et al. Dopamine D4 receptor gene polymorphisms: relation to ethnicity, no association with schizophrenia and response to clozapine in Israeli subjects. Eur Neuropsychopharmacol 1997;7:39–43.
197. Ambrosio AM, Kennedy JL, Macciardi F, et al. No evidence of association or linkage disequilibrium between polymorphisms in the 5′ upstream and coding regions of the dopamine D4 receptor gene and schizophrenia in a Portuguese population. Am J Med Genet B Neuropsychiatr Genet 2004;125:20–4.
198. Shaikh S, Gill M, Owen M, et al. Failure to find linkage between a functional polymorphism in the dopamine D4 receptor gene and schizophrenia. Am J Med Genet 1994;54: 8–11.
199. Petronis A, Macciardi F, Athanassiades A, et al. Association study between the dopamine D4 receptor gene and schizophrenia. Am J Med Genet 1995;60:452–5.
200. Lung FW, Tzeng DS, Shu BC. Ethnic heterogeneity in allele variation in the DRD4 gene in schizophrenia. Schizophr Res 2002;57:239–45.
201. Glatt SJ, Faraone SV, Tsuang MT. Schizophrenia is not associated with DRD4 48-base-pair-repeat length or individual alleles: results of a meta-analysis. Biol Psychiatry 2003;54: 629–35.
202. Mitsuyasu H, Hirata N, Sakai Y, et al. Association analysis of polymorphisms in the upstream region of the human dopamine D4 receptor gene (DRD4) with schizophrenia and personality traits. J Hum Genet 2001;46:26–31.
203. Golimbet VE, Gritsenko IK, Alfimova MV, et al. Dopamine receptor DRD4 gene polymorphism and its association with schizophrenia spectrum disorders and personality traits of patients. Zh Nevrol Psikhiatr Im S S Korsakova 2005;105:42–7.
204. Xing QH, Wu SN, Lin ZG, et al. Association analysis of polymorphisms in the upstream region of the human dopamine D4 receptor gene in schizophrenia. Schizophr Res 2003;65: 9–14.
205. Jonsson EG, Ivo R, Forslund K, et al. No association between a promoter dopamine D(4) receptor gene variant and schizophrenia. Am J Med Genet 2001;105:525–8.
206. Paterson AD, Ying DJ, Petronis A, et al. A PstI restriction fragment length polymorphism in the 5′ untranslated region of DRD4 is not associated with schizophrenia. Psychiatr Genet 1996;6:191–3.
207. Jonsson EG, Sedvall GC, Nothen MM, Cichon S. Dopamine D4 receptor gene (DRD4) variants and schizophrenia: meta-analyses. Schizophr Res 2003;61:111–9.
208. Bocchetta A, Piccardi MP, Palmas MA, Chillotti C, Oi A, Del Zompo M. Family-based association study between bipolar disorder and DRD2, DRD4, DAT, and SERT in Sardinia. Am J Med Genet 1999;88:522–6.

209. Lim LC, Nothen MM, Korner J, et al. No evidence of association between dopamine D4 receptor variants and bipolar affective disorder. Am J Med Genet 1994;54:259–63.
210. Muglia P, Petronis A, Mundo E, Lander S, Cate T, Kennedy JL. Dopamine D4 receptor and tyrosine hydroxylase genes in bipolar disorder: evidence for a role of DRD4. Mol Psychiatry 2002;7:860–6.
211. Chang FM, Ko HC, Lu RB, Pakstis AJ, Kidd KK. The dopamine D4 receptor gene (DRD4) is not associated with alcoholism in three Taiwanese populations: six polymorphisms tested separately and as haplotypes. Biol Psychiatry 1997;41:394–405.
212. Ishiguro H, Saito T, Shibuya H, Arinami T. Association study between genetic polymorphisms in the 14-3-3 eta chain and dopamine D4 receptor genes and alcoholism. Alcohol Clin Exp Res 2000;24:343–7.
213. George SR, Cheng R, Nguyen T, Israel Y, O'Dowd BF. Polymorphisms of the D4 dopamine receptor alleles in chronic alcoholism. Biochem Biophys Res Commun 1993;196:107–14.
214. Franke P, Nothen MM, Wang T, et al. DRD4 exon III VNTR polymorphism-susceptibility factor for heroin dependence? Results of a case-control and a family-based association approach. Mol Psychiatry 2000;5:101–4.
215. Li T, Xu K, Deng H, et al. Association analysis of the dopamine D4 gene exon III VNTR and heroin abuse in Chinese subjects. Mol Psychiatry 1997;2:413–6.
216. Kotler M, Cohen H, Segman R, et al. Excess dopamine D4 receptor (D4DR) exon III seven repeat allele in opioid-dependent subjects. Mol Psychiatry 1997;2:251–4.
217. Szilagyi A, Boor K, Szekely A, et al. Combined effect of promoter polymorphisms in the dopamine D4 receptor and the serotonin transporter genes in heroin dependence. Neuropsychopharmacol Hung 2005;7:28–33.
218. Munafo MR, Yalcin B, Willis-Owen SA, Flint J. Association of the dopamine D4 receptor (DRD4) gene and approach-related personality traits: meta-analysis and new data. Biol Psychiatry 2008;63:197–206.
219. Sherrington R, Mankoo B, Attwood J, et al. Cloning of the human dopamine D5 receptor gene and identification of a highly polymorphic microsatellite for the DRD5 locus that shows tight linkage to the chromosome 4p reference marker RAF1P1. Genomics 1993;18:423–5.
220. Asherson P, Mant R, Williams N, et al. A study of chromosome 4p markers and dopamine D5 receptor gene in schizophrenia and bipolar disorder. Mol Psychiatry 1998;3:310–20.
221. Sobell JL, Lind TJ, Sigurdson DC, et al. The D5 dopamine receptor gene in schizophrenia: identification of a nonsense change and multiple missense changes but lack of association with disease. Hum Mol Genet 1995;4:507–14.
222. Muir WJ, Thomson ML, McKeon P, et al. Markers close to the dopamine D5 receptor gene (DRD5) show significant association with schizophrenia but not bipolar disorder. Am J Med Genet 2001;105:152–8.
223. Barr CL, Wigg KG, Feng Y, et al. Attention-deficit hyperactivity disorder and the gene for the dopamine D5 receptor. Mol Psychiatry 2000;5:548–51.
224. Mill J, Curran S, Richards S, Taylor E, Asherson P. Polymorphisms in the dopamine D5 receptor (DRD5) gene and ADHD. Am J Med Genet B Neuropsychiatr Genet 2004;125: 38–42.
225. Daly G, Hawi Z, Fitzgerald M, Gill M. Mapping susceptibility loci in attention deficit hyperactivity disorder: preferential transmission of parental alleles at DAT1, DBH and DRD5 to affected children. Mol Psychiatry 1999;4:192–6.
226. Manor I, Corbex M, Eisenberg J, et al. Association of the dopamine D5 receptor with attention deficit hyperactivity disorder (ADHD) and scores on a continuous performance test (TOVA). Am J Med Genet B Neuropsychiatr Genet 2004;127:73–7.
227. Lowe N, Kirley A, Hawi Z, et al. Joint analysis of the DRD5 marker concludes association with attention-deficit/hyperactivity disorder confined to the predominantly inattentive and combined subtypes. Am J Hum Genet 2004;74:348–56.
228. Kalsi G, Sherrington R, Mankoo BS, et al. Linkage study of the D5 dopamine receptor gene (DRD5) in multiplex Icelandic and English schizophrenia pedigrees. Am J Psychiatry 1996;153:107–9.

229. Gorwood P, Genetic association studies in behavioral neuroscience. In: Cruzio W, Gerlai R, eds., Molecular Genetic Techniques for Behavioural Neuroscience. Amsterdam: Elsevier, 1999:113–21.
230. Xing Q, Qian X, Li H, et al. The relationship between the therapeutic response to risperidone and the dopamine D2 receptor polymorphism in Chinese schizophrenia patients. Int J Neuropsychopharmacol 2007;10:631–7.
231. Mihara K, Kondo T, Suzuki A, et al. Prolactin response to nemonapride, a selective antagonist for D2 like dopamine receptors, in schizophrenic patients in relation to Taq1A polymorphism of DRD2 gene. Psychopharmacology (Berl) 2000;149:246–50.
232. Steen VM, Lovlie R, MacEwan T, McCreadie RG. Dopamine D3-receptor gene variant and susceptibility to tardive dyskinesia in schizophrenic patients. Mol Psychiatry 1997;2: 139–45.
233. Bakker PR, van Harten PN, van Os J. Antipsychotic-induced tardive dyskinesia and the Ser9Gly polymorphism in the DRD3 gene: a meta analysis. Schizophr Res 2006;83:185–92.
234. Chong SA, Tan EC, Tan CH, Mythily, Chan YH. Polymorphisms of dopamine receptors and tardive dyskinesia among Chinese patients with schizophrenia. Am J Med Genet B Neuropsychiatr Genet 2003;116B:51–4.
235. Lattuada E, Cavallaro R, Serretti A, Lorenzi C, Smeraldi E. Tardive dyskinesia and DRD2, DRD3, DRD4, 5-HT2A variants in schizophrenia: an association study with repeated assessment. Int J Neuropsychopharmacol 2004;7:489–93.
236. Noble EP, St Jeor ST, Ritchie T, et al. D2 dopamine receptor gene and cigarette smoking: a reward gene? Med Hypotheses 1994;42:257–60.
237. Limosin F, Gorwood P, Loze JY, et al. Male limited association of the dopamine receptor D2 gene TaqI a polymorphism and alcohol dependence. Am J Med Genet 2002;112:343–6.
238. Konishi T, Luo HR, Calvillo M, Mayo MS, Lin KM, Wan YJ. ADH1B*1, ADH1C*2, DRD2 (-141C Ins), and 5-HTTLPR are associated with alcoholism in Mexican American men living in Los Angeles. Alcohol Clin Exp Res 2004;28:1145–52.
239. O'Hara BF, Smith SS, Bird G, et al. Dopamine D2 receptor RFLPs, haplotypes and their association with substance use in black and Caucasian research volunteers. Hum Hered 1993;43:209–18.
240. Noble EP, Zhang X, Ritchie TL, Sparkes RS. Haplotypes at the DRD2 locus and severe alcoholism. Am J Med Genet 2000;96:622–31.
241. Sasabe T, Furukawa A, Matsusita S, Higuchi S, Ishiura S. Association analysis of the dopamine receptor D2 (DRD2) SNP rs1076560 in alcoholic patients. Neurosci Lett 2007;412:139–42.
242. Freimer M, Kranzler H, Satel S, et al. No association between D3 dopamine receptor (DRD3) alleles and cocaine dependence. Addict Biol 1996;1:281–7.
243. Szekeres G, Keri S, Juhasz A, et al. Role of dopamine D3 receptor (DRD3) and dopamine transporter (DAT) polymorphism in cognitive dysfunctions and therapeutic response to atypical antipsychotics in patients with schizophrenia. Am J Med Genet B Neuropsychiatr Genet 2004;124:1–5.
244. Baritaki S, Rizos E, Zafiropoulos A, et al. Association between schizophrenia and DRD3 or HTR2 receptor gene variants. Eur J Hum Genet 2004;12:535–41.
245. Chiaroni P, Azorin JM, Dassa D, et al. Possible involvement of the dopamine D3 receptor locus in subtypes of bipolar affective disorder. Psychiatr Genet 2000;10:43–9.
246. Tsai SJ, Cheng CY, Shu LR, et al. No association for D2 and D4 dopamine receptor polymorphisms and methamphetamine abuse in Chinese males. Psychiatr Genet 2002;12:29–33.
247. Hong CJ, Chiu HJ, Chang YS, Sim CB. Twelve-nucleotide repeat polymorphism of D4 dopamine receptor gene in Chinese familial schizophrenic patients. Biol Psychiatry 1998;43:432–5.
248. Serretti A, Lilli R, Di Bella D, et al. Dopamine receptor D4 gene is not associated with major psychoses. Am J Med Genet 1999;88:486–91.

249. Seeman P, Ulpian C, Chouinard G, et al. Dopamine D4 receptor variant, D4GLYCINE194, in Africans, but not in Caucasians: no association with schizophrenia. Am J Med Genet 1994;54:384–90.
250. Nothen MM, Cichon S, Hemmer S, et al. Human dopamine D4 receptor gene: frequent occurrence of a null allele and observation of homozygosity. Hum Mol Genet 1994;3: 2207–12.
251. Vanyukov MM, Moss HB, Gioio AE, Hughes HB, Kaplan BB, Tarter RE. An association between a microsatellite polymorphism at the DRD5 gene and the liability to substance abuse: pilot study. Behav Genet 1998;28:75–82.

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