Current Topics in Behavioral Neurosciences 60

Isabelle Boileau
Ginetta Collo *Editors*

# Therapeutic Applications of Dopamine D3 Receptor Function

## New Insight After 30 Years Of Research

Springer

# Current Topics in Behavioral Neurosciences

Volume 60

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Editors

# Therapeutic Applications of Dopamine D3 Receptor Function

## New Insight After 30 Years Of Research

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# Preface

The Current Topics in Behavioral Neurosciences volume: Therapeutic Applications of Dopamine D3 Receptor Function reviews the state of the knowledge on the dopamine D3 receptor and its role in human behavior and disease (i.e., neuropsychiatric illnesses including schizophrenia, mood disorders, Parkinson's disease, restless legs syndrome, addictions, and substance use disorders). We present an 11-chapter volume from leading experts across multidisciplinary areas (imaging, biobehavioral testing and clinical trials, preclinical models/molecular pharmacology) converging on the therapeutic implications and potential of the D3 receptor.

The D3 dopamine receptor is a member of the D2-like family of G protein-coupled dopamine receptors. It was cloned and characterized more than 30 years ago. A key feature of the D3 dopamine receptor system, which has attracted considerable attention, is its anatomical localization remarkably restricted to the limbic circuitry. This has spurred the hypothesis that D3 dopamine receptor involvement could contribute to the pathophysiology of neuropsychiatric disorders or to some features of neuropsychiatric disorders, including but not limited to psychosis, addictions and substance abuse, mood and movement disorders.

This volume opens up with a history of the D3 dopamine receptor from cloning to clinical trials (Pierre Sokoloff and Bernard Le Foll) broadening our understanding of D3 dopamine receptor function in neuropsychiatry illness and introducing the idea that targeting D3 dopamine receptors may be a viable approach. This introductory chapter is followed by a cross-species review of D3 dopamine receptor biodistribution (Eugenia Gurevich). Federica Bono et al. provide a review of the literature regarding the heterodimerization of D1 and D3 dopamine receptors in rodents, which represent a potential drug target for the treatment of dyskinetic behaviors induced by long-term levodopa treatment in Parkinson's disease patients. The involvement of the D3 dopamine receptor in mesocorticolimbic circuit structural plasticity, through the activation of mammalian target of rapamycin complex 1 (mTORC1) pathway, is reviewed by Emilio Merlo Pich et al. proposing a new working hypothesis on the role of D3 dopamine receptors in treatment-resistant

depression. Jimbin Xu provides new important insight on the role of the D3 dopamine receptor in the progression of Parkinson's disease. Mathieu Favier et al. summarize recent findings from their rodent lesion model which suggests a critical role of dopamine D3 receptors in parkinsonian apathy and highlight this receptor as a promising target for treating motivational deficits. Sebastiano Torrisi et al. review the literature on the role of dopamine D3 receptors in cognition, proposing that drugs targeting D3 dopamine receptors are potential cognitive enhancers for several neuropsychiatric disorders. Amy Hauck Newman et al. describe the effects of new dopamine D3 receptor antagonists/partial agonists on preclinical models of self-administration of drugs such as opiates and psychostimulants. Kevin Butler et al. review the preclinical and clinical literature on the role of the D3 dopamine receptors in nicotine dependence. Stefan Clemens presents an updated summary of the current treatments of restless legs syndrome characterized by a D3 dopamine receptor targeting. Finally, Sheida Koosari et al. introduce the readers to positron emission tomography (PET) imaging in human pharmacology research and describe the available D3 dopamine receptor radiotracers and their kinetics, emphasizing the use of [$^{11}$C]-(+)-PHNO in substance abuse, obesity, aging, traumatic brain injury, schizophrenia, Parkinson's disease, and dementia.

In summary, we aim for this volume to inspire future work on the D3 dopamine receptor through a series of thought-provoking reviews and discussions.

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# Contents

**A Historical Perspective on the Dopamine D3 Receptor** . . . . . 1
Pierre Sokoloff and Bernard Le Foll

**Location, Location, Location: The Expression of D3 Dopamine Receptors in the Nervous System** . . . . . 29
Eugenia V. Gurevich

**Recent Advances in Dopamine D3 Receptor Heterodimers: Focus on Dopamine D3 and D1 Receptor–Receptor Interaction and Striatal Function** . . . . . 47
Federica Bono, Veronica Mutti, Zaira Tomasoni, Giulia Sbrini, Cristina Missale, and Chiara Fiorentini

**Involvement of DA D3 Receptors in Structural Neuroplasticity of Selected Limbic Brain Circuits: Possible Role in Treatment-Resistant Depression** . . . . . 73
Emilio Merlo Pich, Laura Cavalleri, Caterina Toma, and Ginetta Collo

**Dopamine D3 Receptor in Parkinson Disease: A Prognosis Biomarker and an Intervention Target** . . . . . 89
Jinbin Xu

**Dopamine D3 Receptors: A Potential Target to Treat Motivational Deficits in Parkinson's Disease** . . . . . 109
Mathieu Favier, Carole Carcenac, Marc Savasta, and Sebastien Carnicella

**Dopamine D3 Receptor, Cognition and Cognitive Dysfunctions in Neuropsychiatric Disorders: From the Bench to the Bedside** . . . . . 133
Sebastiano Alfio Torrisi, Federica Geraci, Gabriella Contarini, Salomone Salomone, Filippo Drago, and Gian Marco Leggio

**Current Perspectives on Selective Dopamine $D_3$ Receptor Antagonists/Partial Agonists as Pharmacotherapeutics for Opioid and Psychostimulant Use Disorders** . . . . . 157
Amy Hauck Newman, Zheng-Xiong Xi, and Christian Heidbreder

**The Role of Dopamine D3 Receptors in Tobacco Use Disorder: A Synthesis of the Preclinical and Clinical Literature** . . . . . 203
Kevin Butler, Bernard Le Foll, and Patricia Di Ciano

**D3 Receptors and Restless Legs Syndrome** . . . . . 229
Stefan Clemens

**D3 Receptors and PET Imaging** . . . . . 251
Sheida Koohsari, Yanghong Yang, and David Matuskey

# A Historical Perspective on the Dopamine D3 Receptor


**Pierre Sokoloff and Bernard Le Foll**

## Contents

1 Molecular Cloning and Characterization of the $D_3$ Receptor ..... 3
2 Expression and Distribution of the D3 Receptors in the Brain ..... 5
3 The Role of the D3 Receptor in Behavioral Sensitization ..... 9
4 Hunting for D3 Receptor-Selective Compounds ..... 11
5 Functional Role of D3 Receptors Elucidated in Preclinical Models with Selective Drugs ..... 15
5.1 Motor Function ..... 15
5.2 Addiction ..... 15
5.3 Schizophrenia ..... 16
5.4 Cognition ..... 17
6 D3 Receptors in the Therapeutic Area ..... 17
7 Conclusions ..... 20
References ..... 21

**Abstract** Before 1990, the multiplicity of dopamine receptors beyond D1 and D2 had remained a controversial concept, despite its substantial clinical implications, at a time when it was widely accepted that dopamine interacted with only two receptor

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subtypes, termed D1 and D2, differing one from the other by their pharmacological specificity and opposite effects on adenylyl cyclase. It was also generally admitted that the therapeutic efficacy of antipsychotics resulted from blockade of D2 receptors. Thanks to molecular biology techniques, the D3 receptor could be characterized as a distinct molecular entity having a restricted anatomical gene expression and different signaling, which could imply peculiar functions in controlling cognitive and emotional behaviors. Due to the structural similarities of D2 and D3 receptors, the search for D3-selective compounds proved to be difficult, but nevertheless led to the identification of fairly potent and in vitro and in vivo selective compounds. The latter permitted to confirm a role of D3 receptors in motor functions, addiction, cognition, and schizophrenia, which paved the way for the development of new drugs for the treatment of psychiatric disorders.

**Keywords** Addiction-Schizophrenia · Anatomical distribution · Clinical trials · D3-selective agents · Molecular cloning · Signaling

At the dawn of the 1980s, it was widely accepted that dopamine (DA) affected its target neuronal and endocrine cells via interaction with only two receptor subtypes, termed D1 and D2, differing one from the other by their pharmacological specificity for DA agonists and antagonists, and their opposite effect on adenylyl cyclase (Garau et al. 1978; Kebabian and Calne 1979). It was also generally admitted that the potent blockade of D2 receptors was responsible for therapeutic efficacy of antipsychotics. However, the idea was raised by Schwartz and colleagues that antipsychotic agents may interact to a variable extent with more than a single DA receptor subtype, i.e., that the dual categorization of DA receptors was incomplete. This conviction was based mainly upon the observation that a series of "atypical antipsychotics," although inactive at D1 receptors, were able to distinguish subclasses of D2 receptors in binding studies in the brain but not anterior pituitary, and in behavioral studies (Martres et al. 1984). Also, the existence in the brain, but not pituitary, of a dopamine receptor subtype rather insensitive to modulation by guanine nucleotides was put forward (Sokoloff et al. 1984). However, no highly discriminative agent could be identified, so the idea that more than a single molecular entity, the D2 receptor, was responsible for the various actions of antipsychotics remained controversial, in spite of its substantial clinical implications.

The concept of multiplicity of DA receptor subtypes has radically evolved from the late 1980s with the introduction into this field of molecular biology techniques, which have firstly confirmed that D1 and D2 receptors were distinct molecular entities (Bunzow et al. 1988; Dearry et al. 1990; Sunahara et al. 1990; Zhou et al. 1990) and, secondly unraveled the existence of two isoforms of the D2 receptor generated by alternative mRNA splicing (Giros et al. 1989; Monsma et al. 1989). Much less expected were the subsequent discoveries of D3 (Sokoloff et al. 1990), D4 (Van Tol et al. 1991) and D5 (Sunahara et al. 1991) receptors, named according to the accepted nomenclature, although an alternative naming ($D_{1A}$, $D_{2A}$, $D_{2B}$, $D_{2C}$, and $D_{1B}$) was also used at a time, based on the gene organization, functional and

pharmacological similarities, respectively between $D_1$ and $D_5$, and between D2, D3, and D4 receptors.

This introductory review relates seminal findings on the D3 receptor that have initiated investigations on the role of this receptor in the control of various behaviors and paved the way for the development of new drugs for the treatment of psychiatric disorders. More comprehensive reviews on recent findings can be found in the present collective work and recent articles (Kiss et al. 2021; Lanza and Bishop 2021; Manvich et al. 2019).

## 1 Molecular Cloning and Characterization of the $D_3$ Receptor

The cloning of the $D_2$ receptor gene in 1988 (Sunahara et al. 1990) facilitated the search for new genes displaying sequence similarity with it. While many researchers were focused on cloning the D1 receptor gene (Dearry et al. 1990; Sunahara et al. 1990; Zhou et al. 1990), the proponents of $D_2$ receptor heterogeneity were seeking for genes homologous to this receptor gene.

The cloning of cDNA encoding the $D_3$ receptor (Sokoloff et al. 1990) was achieved by combining screening of cDNA and genomic phage libraries with a probe derived from the $D_2$ receptor cDNA (Giros et al. 1989) and reverse transcription and polymerase chain reaction (RT-PCR). Sequence analysis of a positive genomic clone revealed an open reading encoding an unknown protein with strong homology with the two first transmembrane domains of the $D_2$ receptor, terminating at the exact position of the beginning of the first intron of the $D_2$ receptor. Then a degenerated oligonucleotide from the sequence encoding the seventh transmembrane domain of the $D_2$ receptor and a primer derived from the first genomic clone were used in RT-PCR to get a cDNA, of which a fragment was used to screen again the genomic library and get the 3′-end of the cDNA. The sequence analysis of the complete cDNA revealed an open reading frame of 446 codons, i.e., 446 amino-acids containing seven putative transmembrane domains characteristic of all members of the G protein-coupled receptors superfamily. Like the $D_2$ receptor gene, but unlike most other members of this superfamily, the $D_3$ receptor gene contains 5 introns, two of which at the same position as in the $D_2$ receptor gene. Notably, the sequence corresponding to the third intracellular loop is interrupted by only one intron, thus, excluding the possibility of alternative splicing at this level, as it occurs in the $D_2$ receptor gene (Giros et al. 1989). The homology between the rat $D_2$ and $D_3$ receptors is 52% overall but as high as 75% if only the transmembrane domains are considered. Functionally significant amino-acid residues could be noted in the D3 receptor: Asp110, responsible for salt-linking ammonium groups of monoamines; Ser 193 and Ser 196 for hydrogen bonding of the two hydroxyl groups of catechols, and Cys 103 and Cys 181 that may participate in the formation of an extracellular disulfide bridge.

When expressed in CHO cells, both D2 and D3 receptors could be clearly distinguished from each other by the affinities of DA agonists and antagonists. DA itself displayed a 20 times higher affinity for the D3 compared to the D2 receptor. The affinity of DA was not significantly modified by the addition of a guanyl nucleotide, which was reminiscent of the high affinity component of dopamine binding not modulated by guanyl nucleotides previously identified in crude brain membranes (Sokoloff et al. 1984). Among dopamine agonists, most of which are or have been used to treat symptoms of Parkinson's disease (PD), apomorphine and bromocriptine displayed similar potencies at both receptors, whereas TL 99, pergolide, and quinpirole – considered to be putative autoreceptor-selective agents (Clark and White 1987; Wolf and Roth 1987) – were much more potent at D3 than at D2 receptors.

Most antipsychotics also displayed nanomolar potencies at the D3 receptor, which therefore was found to be a potential target for therapeutic agents used to treat psychotic symptoms. However, their affinity varied depending on the receptor. For instance, haloperidol, spiperone, thioproperazine, and prochlorperazine were 10 to 20 times more potent at D2 than at D3 receptors, whereas (−)sulpiride, clozapine, thioridazine, amisulpride, or raclopride were only two to three times more potent at D2 than at D3 receptor. This major feature could be anticipated to have important therapeutic implications, because antipsychotics of the first series were considered at that time as "typical antipsychotics," i.e., compounds eliciting PD-like motor side effects, whereas those of the second series were considered as "atypical antipsychotics," less associated with extrapyramidal symptoms (Carlsson 1978). Therefore, these different clinical profiles might reflect the ability of neuroleptics to differentially block D2 and D3 receptors in the brain, a statement which should be considered with caution given that antipsychotics interact with a variety of non-dopamine receptors that might also influence their clinical profile. The only antagonists exhibiting limited but significantly higher potency at D3 than at D2 receptors were UH 232 and AJ 76, two putative autoreceptor-selective agents (Svensson et al. 1986a).

The initial investigation of the receptor signaling (Sokoloff et al. 1990) revealed important differences between D2 and D3 receptors. When expressed in CHO cells, D2 receptor stimulation inhibited forskolin-induced cyclic adenosyl-monophosphate (cAMP) accumulation, an effect which did not occur after D3 receptor stimulation. Combined with the lack of modulation of dopamine binding by a guanyl nucleotide, this finding questioned the functionality of D3 receptors, although the lack of functional consequences of D3 receptor stimulation as observed at that time could be also explained by the use of an inadequate recipient cell line. It was after almost 4 years and various attempts using different recipient cells (Ahlgren-Beckendorf and Levant 2004) that first reports on efficient D3 receptor functional coupling appeared. Stimulation of D3 receptor was found to inhibit cAMP accumulation, but to a much lesser extent than that of D2 receptor (Chio et al. 1994) but it was later found that D3 receptor potently and selectively inhibits adenylyl cyclase type V (Robinson and Caron 1997). Efficacious $\alpha_i/\alpha_o$-dependent responses of D3 receptor to DA agonists were also reported on extracellular cell acidification and mitogenesis (Chio et al.

1994), as well as on DA release in a neuron-derived cell line (Tang et al. 1994). It was found later that the D3 receptor pathways were diverse and include both G protein-dependent and independent mechanisms, which can account for the complex cellular responses reported above (see Ahlgren-Beckendorf and Levant (2004) for a review).

The anatomical distribution of D3 receptor mRNA, as assessed by Northern blotting, semi-quantitative PCR and in situ hybridization revealed a markedly distinct relative abundance within brain regions, as compared to D2 receptors. Highest D3 receptor mRNA levels were found, in descending order, in the olfactory tubercle-islands of Calleja complex, hypothalamus, striatum and substantia nigra. Within the striatal complex, highest levels were found in the "limbic" areas, e.g., ventral and ventromedial parts of the putamen, shell of nucleus accumbens, suggesting a particular role for the D3 receptor in controlling cognitive and emotional behavior (see next section). No D3 receptor expression was detected in the pituitary, even with the highly sensitive RT-PCR techniques, whereas D2 receptor expression was found highly abundant in this area, supporting the notion that the guanyl nucleotide-insensitive binding site previously identified in striatal but not anterior pituitary membranes (Sokoloff et al. 1984) may correspond to D3 receptors. D3 receptors also constitute autoreceptors, i.e., receptors expressed by DA neurons: mRNA could be detected by RT-PCR and decreased by 65–69% after DA neuron degeneration induced by infusion of 6-hydroxydopamine into the medial forebrain bundle, which concentrates DA neuron fibers originating from the substantia nigra and ventral tegmental area. Furthermore, D3 receptor transcripts were detected in the kidney, an organ in which dopamine in low doses exerts vasodilatory actions (Ferro 2003); later the presence of D3 receptor protein in proximal tubules of kidney was confirmed by immunohistochemistry (Nürnberger et al. 2004).

These studies established the characterization of a novel DA receptor, of which the existence may have been anticipated from previous binding studies. The major features of this new receptor is its affinity for antiparkinsonian and antipsychotic drugs, which might exert, at least part of their clinical effects by binding to the D3 receptor, and its particular expression as a postsynaptic receptor in the limbic parts of the striatal complex, and as an autoreceptor on DA neurons. However, it could not be ascertained at that time whether antipsychotics block the D3 receptor at therapeutic dosage, nor if blockade of D3 receptor, or both D2 and D3 receptors are necessary for the therapeutic efficacy of these drugs.

## 2 Expression and Distribution of the D3 Receptors in the Brain

Initially, the expression of D3 receptors was determined by in situ hybridization with cRNA antisense or oligonucleotide probes in rat (Bouthenet et al. 1991b; Diaz et al. 1995; Le Moine and Bloch 1996; Sokoloff et al. 1990) and human (Mengod et al.

1992) brains. Then the selective radioligands 7-[$^{3}$H]hydroxy-N,N-di-n-propyl-2-aminotetralin ([$^{3}$H]7-OH-DPAT) (Lévesque et al. 1992) and [$^{125}$I]*R*-(+)-*trans*-7-Hydroxy-2-(*N*-*n*-propyl-*N*-3′-iodo-2′-propenyl)aminotetralin ([$^{125}$I]7-OH-PIPAT) (Burris et al. 1994) were used in receptor autoradiography studies. Later, anti-D3 receptor antibodies were developed for immunohistochemistry studies, which should be considered with caution, because their sensitivity and selectivity have rarely been validated by using either D3 receptor-knockout mice (Diaz et al. 2000) or with multiple monoclonal antibodies targeting different D3 receptor epitopes (Wolstencroft et al. 2007). More recently, [$^{11}$C](+)-4-propyl-9-hydroxynaphthoxazine [[$^{11}$C](+)-PHNO] has been developed as a radioligand for quantifying D3 receptors in the brain by Positron Emission Tomography (PET). Initially, (+)-PHNO was identified as a D2-like agonist for the treatment of PD (Martin et al. 1984), which was later found to display moderate selectivity for the D3 receptor (Freedman et al. 1994)]. In particular, (+)-PHNO has limited selectivity for D3 receptors with respect to $D2_{high}$ affinity state, so that [$^{11}$C](+)-PHNO labels both D2 and D3 receptors in brain (Willeit et al. 2006). However, taking advantage of the high proportion of D3 relative to D2 receptors in the substantia nigra and conversely the high proportion of D2 relative to D3 receptors in the dorsal striatum, D2 and D3 binding potentials of [$^{11}$C](+)-PHNO could be separately quantitated (Rabiner et al. 2009; Searle et al. 2013; Smart et al. 2020).

These various techniques have converged to offer a clear, and somewhat surprising picture of the distribution of D3 receptors in brain areas, especially by comparison with D2 receptors. *Firstly*, D3 receptors are less abundant than D2 receptors. D3 receptor transcript levels are several orders of magnitude lower than D2 receptor transcript in brain regions, as assessed by Northern blotting (Bouthenet et al. 1991b). When the receptor bindings are considered, in the caudate nucleus/putamen the D2/D3 ratio is ~6 in primate brain (Willeit et al. 2006) and ~100 in rat brain (Lévesque et al. 1992).

*Secondly*, the distribution of D3 receptors is much restricted than that of D2 receptors when considering the whole brain (see Fig. 1a, b for a comparison of transcripts distribution), or within a same brain region. For instance, D2 receptors are evenly distributed in medium spiny neurons of the nucleus accumbens, whereas D3 receptors are restricted in the ventromedial part of the shell (Fig. 1c–f; (Bouthenet et al. 1991b; Diaz et al. 1995; Le Moine and Bloch 1996). In some instances, D2 and D3 receptors are complementary within subregions: D2 receptors are expressed in the lateral division of the bed nucleus of the stria terminalis, whereas D3 receptors are expressed in the medial division (Bouthenet et al. 1991b). In the mammillary nucleus, D2 receptors are expressed in the lateral part, whereas D3 receptors are expressed in the medial part (Bouthenet et al. 1991b). Moreover, at the cellular level, there is a partial segregation of the phenotypes of D2 and D3 receptor-expressing neurons. D2 receptors are mainly expressed in enkephalin neurons projecting to the substantia nigra with relays in the pallidum, whereas D3 receptors are mainly expressed in substance P/dynorphin/neurotensin neurons also expressing D1 receptors, projecting directly to the ventral tegmental area (Diaz et al. 1995; Le Moine and Bloch 1996).

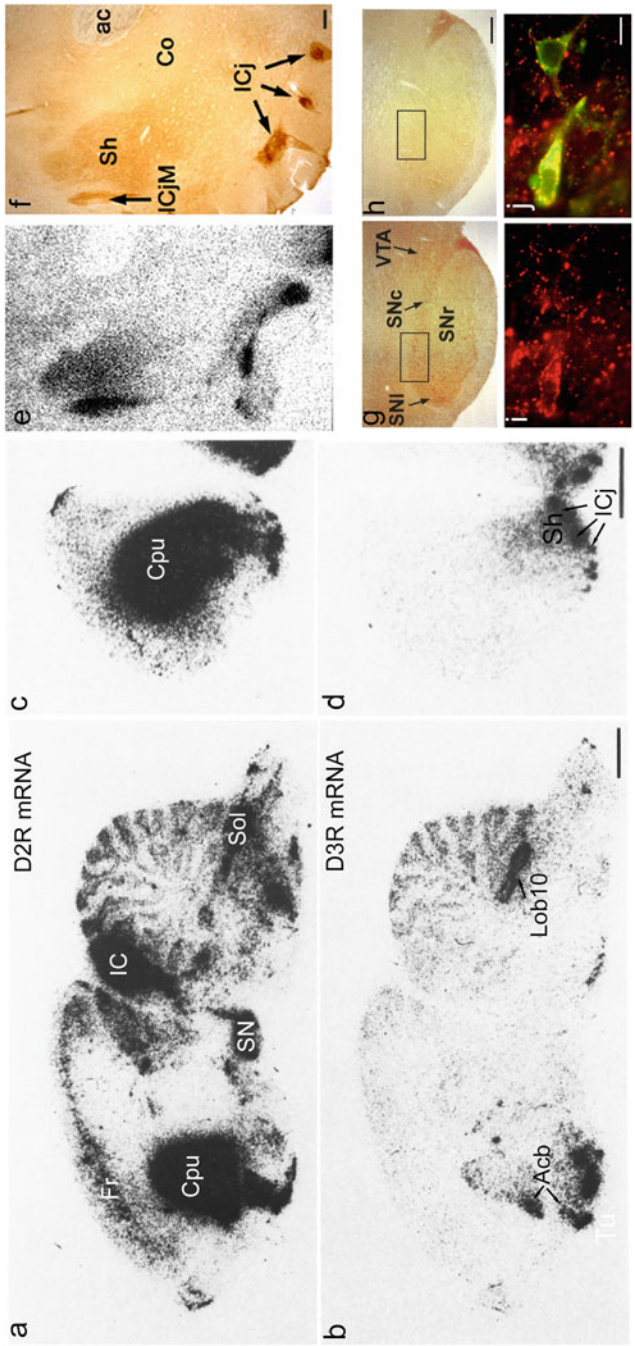


**Fig. 1** Distribution of D3 receptor transcripts and comparison with D2 receptor transcripts (**a**–**c**), D3 receptor binding (**e**) and immunoreactivity (**f**–**j**) in the rat brain. (**a**–**d**) In situ hybridization with [$^{32}$P]-labeled cRNA antisense probes of D2 (**a**, **c**) or D3 (**b**, **d**) receptor on sagittal (**a**, **b**) and coronal (**c**, **d**) sections. (**e**) [$^{125}$I]7-OH-PIPAT binding autoradiography on a coronal section at the level of nucleus accumbens. (**f**) D3 receptor immunoreactivity at the same level as in e. No signal was detected when the antibody was presaturated with the immunogen or in D3 receptor-knockout mice (*not shown*, see Diaz (2000)). (**g**) (**h**) D3 receptor immunoreactivity on a coronal section at the level of substantia nigra/ventral tegmental area, without (**g**) or with (**h**) preincubation with the immunogen. (**i**, **j**) Microphotographs of D3 receptor (**i**) and D3 receptor + tyrosine hydroxylase immunoreactivities of neurons taken in the rectangle in **g**, **h**. *ac* anterior commissura, *Acb* nucleus accumbens, *Co* nucleus accumbens core, *CPu* caudate-putamen, *Fr* frontal cortex, *ICj* islands of Calleja, *ICjM* island of Calleja major, *Lob 10* cerebellar lobule 10, *Sh* nucleus accumbens shell, *SN* substantia nigra, *SNc* substantia nigra pars compacta, *SNl* substantia nigra pars lateralis, *SNr* substantia nigra pars reticulata, *Sol* nucleus of the solitary tract, *Tu* olfactory tubercle. Scale bars: 2 mm (**a**–**c**); 0.5 mm (e, f); 25 μm (**i**, **j**)

*Thirdly*, D3 receptors constitute both presynaptic heteroreceptors, i.e., receptors expressed by non-dopaminergic terminals and autoreceptors, i.e., receptors expressed by DA neurons. In the substantia nigra and ventral tegmental area of the rat, most of D3 receptor immunoreactivity (Fig. 1g, h) is located on GABAergic afferent terminals arising from the shell of nucleus accumbens (Diaz et al. 2000). This abundant expression, if it also occurs in the human brain, may account for the D3 receptor selectivity of [$^{11}$C](+)-PHNO uptake in PET experiments. However, in these regions, D3 receptor immunoreactivity also appears at the plasma membrane with a characteristic punctate distribution, not associated with synaptic boutons, in all positive tyrosine hydroxylase-positive neurons, i.e., DA neurons (Fig. 1i, j; (Diaz et al. 2000). These D3 autoreceptors could control DA neuron activities in agreement with the elevated DA extracellular levels in projection areas of these neurons found in D3 receptor-knockout mice (Koeltzow et al. 1998).

*Fourthly*, D3 receptors are expressed in rather unexpected areas, such as lobules 9 and 10 of the cerebellar cortex (Diaz et al. 1995). Here, D3 receptor mRNA and binding sites are located on the Purkinje cell perikarya of the granular layer and in the molecular layer, respectively. The presence of high levels of D2-like binding sites had been previously detected in this area (Martres et al. 1985). Interestingly, a DA pathway from the ventral tegmental area to the cerebellum has been discovered (Ikai et al. 1992), with single neurons projecting to both cerebral and cerebellar cortices by way of axon collaterals (Ikai et al. 1994).

What could be inferred on D3 receptor function(s) from the distribution of D3 receptors in brain? The largest D3 receptor densities occur in granule cells of the islands of Calleja and in γ-aminobutyric acid (GABA)ergic medium-sized spiny neurons of the rostral and ventromedial shell of nucleus accumbens (Fig. 1a–f). The output neurons from the nucleus accumbens receive their dopaminergic innervations from the ventral tegmental area and reach the entorhinal and prefrontal cortices after relays in the ventral pallidum and mediodorsal thalamus. In turn, the shell of nucleus accumbens receives projections from the cerebral cortex (infralimbic, ventral, agranular, insular, and piriform areas), hippocampus and bed nucleus of the stria terminalis and amygdala (the two latter regions also express D3 receptors (Bouthenet et al. 1991a; Khaled et al. 2014), and also projects to the ventral tegmental area from which dopaminergic afferents originate (Pennartz et al. 1994; Zahm and Brog 1992). These various specific connections of the shell of nucleus accumbens, a part of the "extended amygdala" (Heimer et al. 1995), suggest that this area is involved in a series of feedback or feed-forward loops, involving notably the prefrontal cortex and ventral tegmental area and subserving control of emotional cognition, motivation, and reward. Hence, it may be anticipated that D3 receptors participate in the regulation of these functions, notably by regulating DA neuron activities, through either modulation of descending accumbal GABAergic afferents (Avalos-Fuentes et al. 2013; Cruz-Trujillo et al. 2013) or autoreceptor effects on DA neurons.

## 3 The Role of the D3 Receptor in Behavioral Sensitization

Behavioral sensitization is a process by which repeated stimulation of a receptor results in progressive enhancement of the response to this stimulation (Robinson and Becker 1986). It notably occurs after repeated administrations of DA indirect agonists, such as drugs of abuse, like amphetamine and cocaine, and levodopa used in the treatment of PD. In the latter example, the disease-related loss of DA neurons resulting in rigidity and tremors is initially compensated by treatment with levodopa. However, in the long term, levodopa elicits unwanted movements, e.g., dyskinesia and chorea, as well as psychological disturbances such as hallucinations, both suggestive of excessive responses to DA. Behavioral sensitization can also be observed in animal models of PD, such as rats with unilateral lesions of the nigrostriatal pathway by the neurotoxin 6-hydroxydopamine (6-OHDA) (Marshall and Ungerstedt 1977), in which the rotational behavior elicited by DA agonists can be enhanced upon repeated administrations (Juncos et al. 1989). The mechanisms responsible for these changes have remained unclear.

In 6-OHDA-lesioned rats lacking DA innervation, D3 receptor mRNA and binding in nucleus accumbens was strongly decreased in the lesioned side, whereas repeated receptor blockade by an antipsychotic or administration of reserpine, a DA-depleting agent, had no effects on D3 receptor expression (Lévesque et al. 1995). This phenomenon is paradoxical because it has been generally observed that receptor blockade or interruption of the neurotransmission induces receptor overexpression and supersensitivity. Indeed, D2 receptor mRNA and binding were increased in the 6-hydroxydopamine-lesioned side (Lévesque et al. 1995). Because the effects of 6-OHDA on D3 receptor expression could be reproduced by baclofen, a type B-GABA agonist that ablates DA neuron activity or by colchicine, an inhibitor of axonal transport, it was concluded that D3 receptor expression depends on an anterograde factor present in DA neurons and released in an activity-dependent manner (Lévesque et al. 1995).

Surprisingly, when 6-OHDA-lesioned rats were repeatedly treated with levodopa, expressions of D3 receptor mRNA and binding in the lesioned side progressively increased in the nucleus accumbens, where D3 receptor is normally expressed, but also appeared in the core of nucleus accumbens and dorsal striatum, where D3 receptor is normally absent (Bordet et al. 1997). This ectopic expression is reproduced by the D1-like agonist SKF 38393 and antagonized by the D1 receptor antagonist SCH23390, and therefore dependent on D1 (or D5) receptor stimulation. No such changes were observed for D1 and D2 receptor expressions. Concurrently with D3 receptor expression, rotational behavior was increased upon repeated levodopa administrations and this excessive behavior was antagonized by nafadotride, a partially selective D3 receptor antagonist, at low dosage, and may be dependent on imbalance between the expressions of dynorphin and substance P (Bordet et al. 1997).

Similar findings were obtained in a non-primate model of PD (Bezard et al. 2003), the MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) model, which more

accurately mimics human PD symptoms, including akinesia and rigidity (Burns et al. 1983) and levodopa-induced dyskinesia (LID), indistinguishable in both repertoire and severity in patients with PD (Bezard et al. 2001). In MPTP-intoxicated monkeys, D3 receptor expression was strongly lowered (−68%) in the caudate nucleus, which was compensated by treatment with levodopa in MPTP-intoxicated monkeys without LID. Nonetheless, D3 receptor binding was much higher in MPTP-intoxicated monkeys with LID than in nondyskinetic monkeys in the putamen and internal segment of globus pallidus. Moreover, D3 receptor binding in the putamen correlated with the occurrence and severity of LID ($r^2 = 0.50$, $P < 0.05$, $n = 8$) and LID were attenuated by the selective partial D3 receptor agonist BP897, without impairment of motor functions. Furthermore, D3 receptor binding, as assessed by PET with [$^{11}$C](+)-PHNO, was reduced in drug-naive patients with PD (Boileau et al. 2009), and increased in PD patients with LID, with respect to patients without LID (Payer et al. 2016), although this latter finding could have been confounded by reduction of the DA levels, which was more severe in patients with LID (Stoessl 2016).

Brain-derived neurotrophic factor (BDNF) was examined as the potential regulating factor of D3 receptor expression after DA neuron deafferentation and repeated DA receptor stimulation, because it is expressed in DA neurons projecting to the nucleus accumbens shell and D3 receptor and TrkB, the receptor for BDNF, mRNAs colocalize in this region (Guillin et al. 2001). D3 receptor expression was found largely lower in BDNF-knockout mice and BDNF infusion in the nucleus accumbens compensated for the loss of D3 receptor expression after DA neuron deafferentation. Moreover, the ectopic D3 receptor expression and increased rotational behavior appearing after repeated levodopa administrations in 6-OHDA rats was blocked by infusion of IgG-TrkB, a selective BDNF antagonist. Levodopa also increased TrkB gene expression in the 6-OHDA-lesioned, but not the contralateral side. It was also found that cortical BDNF plays an important role in this process because cortical ablation abolished increased rotational behavior and BDNF expression in frontal and cingulate cortex was upregulated by repeated levodopa administrations in the 6-OHDA lesioned side.

Taken together, these observations indicate that D3 receptor induction in the striatum is triggered by the combination of a D1 (or D5) receptor stimulation-dependent elevation of BDNF in cortico-striatal neurons, together with a denervation-dependent upregulation of striatal TrkB expression. The results suggest a wider role for BNDF in the regulation of D3 receptor responsiveness in other conditions implicating DA and BDNF, and brain regions with anatomical and functional relationships with regions expressing D3 receptors, such as the hippocampus that modulates stress responses and is important in emotional cognition and memory, or the amygdala that processes contextual cue-associated drug of abuse taking. For instance, in major depressive disorders (MDD), BDNF expression is decreased in postmortem hippocampal regions taken from suicide victims or patients with MDD, but increased after long-term use of antidepressant drugs (Autry and Monteggia 2012). D3 receptors are elevated in the nucleus accumbens of rats chronically treated by various antidepressant drugs and electroconvulsive shocks (Lammers et al. 2000). In paradigms related to drug addiction, cocaine-conditioned

mice have increased levels of BDNF transcripts and D3 receptors in the nucleus accumbens (Le Foll et al. 2002), increased D3 receptor expression accompanies locomotor sensitization to nicotine (Le Foll et al. 2003a), and even a single administration of drugs (cocaine, methamphetamine, morphine) induces a transient increase of BDNF mRNA in the cortex and a long-lasting elevation of D3 receptors in the nucleus accumbens (Le Foll et al. 2005a).

## 4 Hunting for D3 Receptor-Selective Compounds

Several agonists were found to display D3 receptor binding selectivity, including quinpirole [12] and 7-OH-DPAT (Lévesque et al. 1992). Namely, the latter compound was used as a radioligand, which can specifically label D3 receptors in in vitro studies; however, only in special experimental conditions, e.g., in the absence of sodium. The use of 7-OH-DPAT as an allegedly "selective" agonist in behavioral experiments was totally misleading because this compound hardly distinguishes D3 and $D2_{high}$ receptors (see Lévesque (1996) for a discussion). A functional in vitro test identified PD128,907 ([(R-(+)-trans-3,4a,10b-tetrahydro-4-propyl-2H,5H-[1] benzopyrano[4,3-b]-1,4-oxazin-9-ol)]) as the most D3-selective agonist (Sautel et al. 1995a), but its in vivo selectivity has rarely been ascertained by using selective antagonists (Collins et al. 2005) or D3 receptor-knockout mice (Witkin et al. 2008). Whereas none of the classically designed DA agonists has proved to be highly selective for D3 receptors, more recently designed biased (Xu et al. 2017) or bitopic (Adhikari et al. 2021; Battiti et al. 2020) agonists seem to fulfill selectivity criteria.

Among antagonists, (+)-AJ76 and [+]-UH232, two DA antagonists with preference for DA autoreceptors (Svensson et al. 1986b), and nafadotride (Sautel et al. 1995b) were the only compounds initially found to display preference, yet modest (3–7 times) for D3 receptor binding with respect to D2. The first compound with significant D3 receptor selectivity was BP897, which displays 70-fold higher affinity for D3 receptor as compared to D2 (Pilla et al. 1999). The chemical structure of BP897 contains several structural elements, which were found common with those of all D3-preferring or D3-selective compounds that were identified later (Fig. 2a). The pharmacophore (Fig. 2b) fits with the X-ray structure of the D3 receptor (Chien et al. 2010), which identifies an orthosteric binding site that binds DA or its mimics, and a secondary binding site that permits high affinity and selectivity (Fig. 2c). The "east side" orthosteric pharmacophore of the molecules contains a mandatory protonable nitrogen and an aromatic ring supposed to mimic the aminoethyl and the phenyl group of DA, respectively. These two chemical moieties are the most buried parts of the ligand, in a cavity highly conserved among Type I G protein-coupled receptors, namely formed by Ser-192, Ser-193, Val-111, Phe-197, Phe-346, and Asp-110 (Fig. 2c), the latter forming a salt bridge with the protonable nitrogen. The "west side" of the molecules bind to a secondary binding site in a less conserved cavity (Fig. 2c), which may explain why binding to this secondary site confers selectivity.

Most of the D3-prefering or D3-selective antagonists that have been developed, some of them up to clinical trials, were identified before the availability of X-ray structures of the D3 receptor, which nevertheless could help improve the design of new compounds. The structures and D3-selectivity of selected compounds are reported in Fig. 2a and Table 1. SB-277011 and NGB 2904 are highly selective in vitro and have been extensively used in behavioral studies. For the other compounds, it is interesting to compare D3 receptor binding selectivity as assessed in vitro in transfected cells and in vivo by PET (Table 1). BP897 has been used in one PET study in baboons, but merely served as a probe to characterize [$^{11}$C]-PHNO binding in several brain regions, which led to the identification of globus pallidus as a D3-rich region (Narendran et al. 2006a). ABT-925 displays a modest D3-selectivity in in vitro binding experiments (25-fold) and a more limited selectivity of 4.2 in PET experiments (Graff-Guerrero et al. 2010), calculated as the $EC_{50}$ ratio and assuming that [$^{11}$C]-PHNO binding in substantia nigra and caudate represent true D3 and D2 receptor binding, respectively, which is in line with various other studies with this radioligand. GSK598809 has a similar D3 receptor binding selectivity in in vitro and PET experiments (100 vs. 75-fold).

Cariprazine has a limited selectivity in in vitro experiments and a more limited selectivity in PET experiments after acute administration. However, the selectivity increased to three- to sixfold after subchronic treatment, likely due to accumulation of cariprazine's metabolites, desmethyl- and didesmethyl-cariprazine, which display higher D3 receptor potency (~twofold) and D3 receptor selectivity (25–46-fold, see Table 1 and (European Medicines Agency 2017)), and become prominent (82% of total active species (European Medicines Agency 2017)) after chronic treatment. Yet, after chronic treatment, the D3-selectivity of cariprazine seems sufficient to ensure a higher D3 vs D2 receptor occupancy at low doses (Girgis et al. 2016). The higher D3 receptor selectivity and potency of the metabolites may explain why among other explanations (see Sokoloff and Le Foll (2017) for a discussion), $D_3$ receptor occupancy by cariprazine is sustained and increased with subchronic dosing, while currently available antipsychotic agents seem to bind to $D_3$ receptors in expected proportions after acute dosing (Girgis et al. 2015), but not after subchronic dosing (Graff-Guerrero et al. 2009; Mizrahi et al. 2011). Admittedly, the measurement of D3 receptor occupancy after chronic treatment by antipsychotic drugs is confounded by additional events, which could be either D3 receptor upregulation or DA depletion in D3-rich brain regions (substantia nigra, ventral tegmental area) (Sokoloff and Le Foll 2017). If it were the case, the real D3-selectivity of cariprazine on chronic treatment would be underestimated.

F17464 has a high selective D3 receptor occupancy in PET experiments after acute administration, in agreement with its in vitro binding selectivity. However, whether its selectivity is conserved after chronic treatment remains to be determined. Finally, BP1.4979, a recently published compound, displays another interesting feature. It has a moderate D3 receptor selectivity in PET experiments compared to high binding selectivity in vitro, but its selectivity increases after chronic treatment, which could not be explained by the occurrence of an active, more D3-selective metabolite.

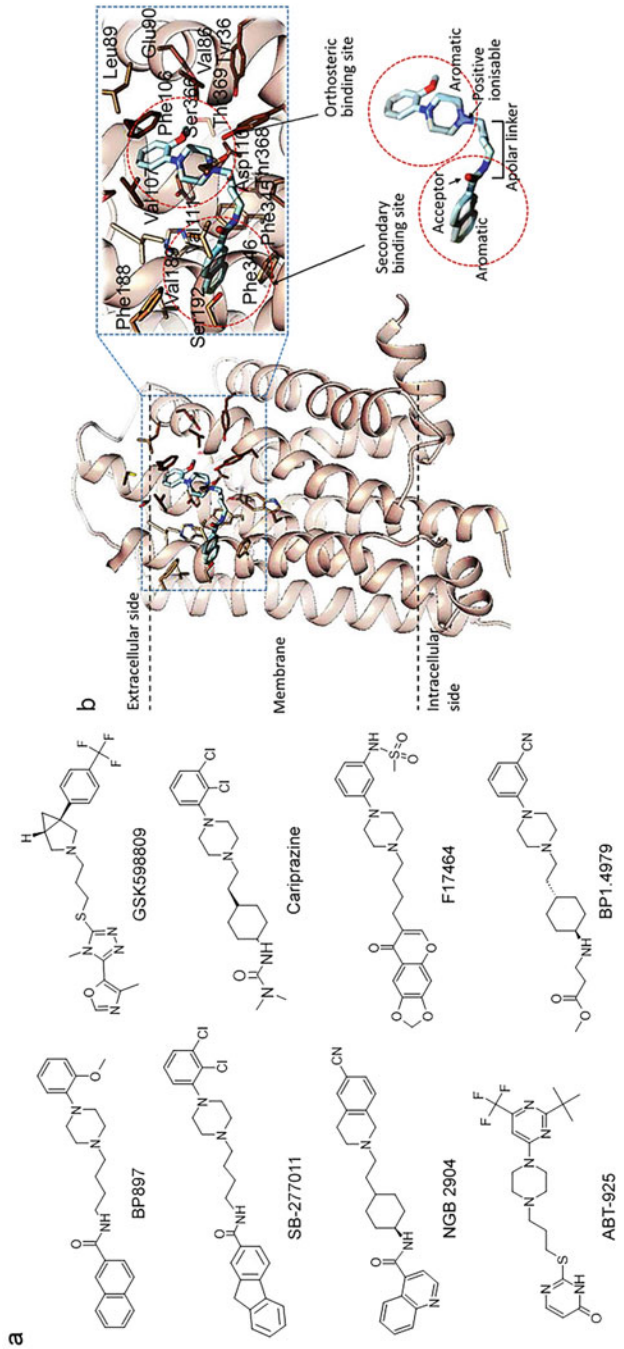


**Fig. 2** Structure of D3-preferring or D-selective compounds (**a**) and docking of BP897 into the D3 receptor (**b**). The common structural features of D3-compounds define the pharmacophore, drawn with BP897 in **b** and deduced from common structural features of the other compounds. The D3 receptor-BP897 complex showing the orthosteric and secondary binding sites is shown in **b**, by courtesy of Didier Rognan (Laboratory of Innovative Therapeutics, Illkirch, France)

**Table 1** In vitro and in vivo selectivity of D3-preferring and D3-selective compounds, with respect to D2 receptor

| Name or code | In vitro D3/D2 binding selectivity ratio[a] | Intrinsic activity at D3 receptors[b] | D2 and D3 receptor occupancies measured by PET | References[c] |
|---|---|---|---|---|
| BP 897 | 70 | Partial agonist (55%) | BP897 (0.25 mg/kg i.v.) reduced [$^{11}$C]-PHNO and [$^{11}$C]raclopride by 57% and 29% in globus pallidus, respectively | (Pilla et al. 1999) (Narendran et al. 2006b) |
| SB-277011 | 100 | Antagonist | Not determined | (Stemp et al. 2000) |
| NGB 2904 | 155 | Antagonist | Not determined | (Robarge et al. 2001) |
| ABT-925 | 25 | Antagonist | D3 occupancy reached 75% at after single dosing at 600 mg ($EC_{50}$ were 2.4 and 10.3 μg/mL in substantia nigra and caudate, respectively) | (Unger et al. 2002) (Graff-Guerrero et al. 2010) |
| GSK598809 | 100 | Antagonist | 75-fold lower $EC_{50}$ for D3 receptor compared to D2. | (Micheli et al. 2010) (Searle et al. 2013) |
| Cariprazine<br>DCAR[d]<br>DDCAR[e] | 6, 8[f]<br>25, 46[f]<br>21,34[f] | Partial agonist (30%) | Three- to sixfold lower $EC_{50}$ for D3 receptor compared to D2 after a 2-week daily dosing | (Kiss et al. 2006) (European Medicines Agency 2017) (Girgis et al. 2016) |
| F17464 | 76 | Antagonist | After single dosing at 30 mg, F17464 occupies D3 and D2 receptor at 6–9 h post-dosing, by 89–98% and <18%, respectively. $EC_{50}$ for D3 occupancy was estimated at 20 ng/mL. Due to low occupancy, $EC_{50}$ for D2 occupancy could not be determined | (Sokoloff et al. 2016) (Slifstein et al. 2020) |
| BP1.4979 | 192 | Partial agonist (32%) | At 12 h post-administration, a single dose of 10 mg achieved D3 and D2 receptors occupancies of 66% and 8%, respectively | (Di Ciano et al. 2019) |

[a] Determined by using recombinant cells expressing either human D2 or D3 receptors
[b] Percentage of maximal agonist response is given in parentheses
[c] First reference on pharmacological characterization of the compound, followed by reference to PET study, if exists
[d] Desmethyl-cariprazine
[e] Didesmethyl-cariprazine
[f] The two values were calculated with respect to D2L and D2S, respectively. Except for cariprazine, all other in vitro selectivity ratios were calculated with respect to D2L

These findings show that in vivo selectivity of D3-preferring and D3-selective compounds measured in PET experiments roughly agrees with their in vitro binding selectivity after acute administration, yet it is slightly lower for some compounds, especially those with limited in vitro selectivity. Additionally, the selectivity of D3-preferring or D3-selective compounds can be increased after chronic treatment, which does not occur with classic antipsychotic drugs that are D2-preferring. This implies that large and selective D3 receptor occupancy can be achieved with some compounds in a selected range of doses.

## 5 Functional Role of D3 Receptors Elucidated in Preclinical Models with Selective Drugs

### *5.1 Motor Function*

In contrast to D2 antagonists, that clearly produce impairment of motor function and cataplexy, D3 antagonists do not appear to interfere negatively with motor function and do not impair locomotor activity in preclinical models (Reavill et al. 2000). It should be noted that some investigators reported under some conditions increase of locomotor activity following D3 blockade or D3 gene deletion; those effects have not been consistent across studies and overall there does not seem to be much of an impact of D3 blockade on motor function in normal animals (see Kiss et al. (2021) for more detailed discussion). It can have, nevertheless, an impact on motor function in DA neuron-depleted animals as reported above. Also blockade of D3 receptor inhibits drug cue-induced hyperlocomotion, in paradigms using cocaine (Le Foll et al. 2002), nicotine (Le Foll et al. 2003b), or morphine (Lv et al. 2019)

### *5.2 Addiction*

Due to its localization in reward areas, there has been rapidly great interest at exploring the role of D3 in animal models of addictions (Caine and Koob 1993). Initial comparison of various D2/D3 antagonists revealed that their potencies to decrease cocaine self-administration were correlated with their functional potency at the D3, but not at the D2 (Caine et al. 1997). However, the first study testing a D3 selective ligand (the partial agonist BP 897 with 70-fold selectivity for D3 over D2) indicated no impact on cocaine self-administration under a Fixed ratio 1 (FR1) schedule of reinforcement in rats (Pilla et al. 1999). This lack of effect of D3 antagonism on direct reinforcement (measured with low schedule of reinforcement) has been shown across multiple drugs of abuse, such as nicotine (Andreoli et al. 2003) and methamphetamine (Higley et al. 2011). Alcohol seems to be an exception, with alcohol intake being decreased even in condition of low ratio requirements

(Vengeliene et al. 2006). Interestingly, experiments testing the impact of D3 antagonism with high ratio requirement (either Fixed or Progressive ratio of reinforcement) found an attenuation of drug intake across drugs of abuse (Gilbert and Meyer 2003; Higley et al. 2011; Xi et al. 2005). In addition, across various models testing the reactivity to drug-associated cues, the D3 has been shown to be critically involved. The first clear evidence was reported by Pilla et al. testing the BP 897 partial agonist on second order schedule of reinforcement (Pilla et al. 1999). Under this schedule of reinforcement, drug seeking is maintained by regular presentation of drug-associated cues. D3 blockade also decreased reactivity to cocaine- (Le Foll et al. 2002) and to nicotine-associated cues (Le Foll et al. 2003b). D3 receptors were shown to influence both acquisition and expression of conditioned place preference (Ashby et al. 2003; Vorel et al. 2002; Le Foll et al. 2005b). Context-induced reinstatement of drug seeking is also decreased by D3 blockade (Sabioni et al. 2016). Overall, those experiments established clearly that in rodents, reactivity to drug-associated cues could be reduced by D3 blockade. In addition, other experiments have established that other triggers for reinstatement such as stress and drug priming could also be attenuated by D3 blockade (see Galaj et al. (2020); Sokoloff and Le Foll (2017) for reviews).

## *5.3 Schizophrenia*

Dopamine D3 ligands have also been tested in various animal models of schizophrenia. The first highly selective D3 antagonist, SB-277011-A had no effect on stimulant-induced hyperlocomotion and did not affect prepulse inhibition deficits in apomorphine- or quinpirole-treated rats (two D2/D3 agonists) (Reavill et al. 2000). However, this D3 antagonist reversed the prepulse inhibition deficit observed in isolation-reared rats (Reavill et al. 2000). Various D3 selective ligands also have shown some ability to decrease MK-801 induced locomotion, a model of schizophrenia. Those drugs typically are able to decrease MK-801 induced locomotion at doses that are lower than the doses known to affect locomotion (see Sokoloff and Le Foll (2017). But the findings have not been consistent across drugs. For example, the ratio of $ED_{50}$ measured for inhibition of spontaneous locomotion vs MK-801 induced locomotion was around 2 for SB-277011A, while it was around 36 for F17464, a highly potent D3 receptor antagonist and 5-HT1A agonist (Cosi et al. 2021), with moderate affinity for the D2 receptor. F17464 was also able to reverse the MK-801-induced social interaction deficits in mice (Sokoloff and Le Foll 2017), measured using a "resident-intruder" behavioral model (Dixon et al. 1994; Mohn et al. 1999). Those findings suggest that D3 blockade can produce some improvement of positive and negative symptoms of schizophrenia, however, the participation of D2 in those responses is still debated.

### *5.4 Cognition*

In contrast to D2 antagonists that produce impairment of cognition, D3 antagonists have pro-cognitive effects (see Nakajima et al. (2013) for a review). On the other hand, D3 agonists typically result in impairment of cognitive function. Those effects have so far mostly been shown in rodent models and could be mediated by increase of acetylcholine and/or dopamine in cortical area (Nakajima et al. 2013).

## 6 D3 Receptors in the Therapeutic Area

Some D3-preferring or D3-selective compounds have reached clinical development in various indications, including schizophrenia, smoking cessation, bipolar disorders, major depressive disorder, and restless legs syndrome (Table 2). The study with BP897 in acute schizophrenia had a negative outcome, i.e., no significant changes of the PANSS (Positive and Negative Schizophrenia Scale) at the end of treatment period, but this study was inconclusive because a fraction of patients, in which BP897 plasma concentration was assessed, showed much lower exposure than active exposures in rat and monkey models. It was concluded that the dose of BP897 may have been too low to produce an effect. The study with ABT-927 was inconclusive too: the clinical outcome was negative at the two doses tested, but a subsequent PET study (Table 1) showed that at the highest tested dose (150 mg), D3 receptor occupancy, estimated to be lower than 50% (Graff-Guerrero et al. 2010; Redden et al. 2011), may have been too low to be efficacious. GSK598809 was evaluated in abstinent treatment-seeking patients with tobacco smoking addiction for its ability to reduce craving (Mugnaini et al. 2013). One of the clinical endpoints was the difference in average response times to color-name words related to cigarette smoking (smoking reaction time) and neutral control words (neutral reaction time) and was considered an index of the degree to which smoking-related content disrupts ongoing cognitive processes. GSK598809 significantly but transiently reduced the score related to this endpoint, but it was noted that GSK-598809-treated participants increased their cigarette consumption and puffs number after the abstinent period. The dose of GSK598809 used was carefully selected through an elegant translational pharmacokinetics/pharmacodynamics study, which included measurement of active doses and plasma concentrations in a rat model (nicotine-conditioned place preference) and measurement of in vivo D3 receptor occupancy in rats, baboons, and humans, which was estimated to peak at 89%. It was suggested that higher D3 receptor occupancy may have produced more durable effects. No further development of BB897, ABT-925, and GSK598809 has been reported, so it is likely that these compounds have been discontinued. BP1.4979 is a newly developed compound, which has been engaged in two clinical trials (Table 2); however, publicly available information regarding its efficacy in clinical trials is lacking.

**Table 2** Summary of clinical trials with D3-preferring/selective antagonists and partial agonists

| Drug | Patient population and study design | Study outcome | Reference |
|---|---|---|---|
| BP897 | 77 patients with acute schizophrenia<br>Randomized 2:1 to BP897 10 mg b.i.d. or placebo and treated for 4 weeks | No significant change on the PANSS at 4 weeks in the intent-to-treat population. Plasma concentrations of BP897, available in some patients were much lower than active concentrations in rats and monkey models | (Lecrubier 2003) |
| ABT-925 | Acute schizophrenia<br>155 patients randomized 1:1:1 to ABT-925 50 mg o.a.d., 150 mg o.a.d. or placebo and treated for 6 weeks | No significant improvements on the PANSS total score nor on secondary endpoints. Subsequent PET study showed that the dose used achieved insufficient D3 receptor occupancy | (Redden et al. 2011) |
| GSK598809 | 40 treatment-seeking subjects with smoking addiction, randomized 1:1 to GSK598809 75 mg single dose and placebo, in cross-over design | A single dose, estimated to achieve 89% maximal D3 receptor occupancy, transiently reduced subjective craving in abstinent smokers. After the abstinence period, GSK-treated patients increased cigarette consumption and puffs, compared to placebo-treated | (Mugnaini et al. 2013) |
| Cariprazine[a] | Various studies including a total of 2,952 patients with acute or chronic schizophrenia, randomized for fixed or flexible doses of cariprazine 1.5–6 mg o.a.d. or placebo | In phase III studies, significant improvement both in the short and long term, relapse prevention studies, at each investigated dose levels compared to placebo | (European Medicines Agency 2017) |
| | 461 patients with schizophrenia with predominant negative symptoms, randomized 1:1 to cariprazine (target dose: 4.5 mg o.a.d., dose range: 3–6 mg o.a.d.) or risperidone (target dose: 4.5 mg o.a.d., dose range: 3–6 mg o.a.d.) and treated for 26 weeks | Cariprazine performed better (−1.4 point of difference) than risperidone on the PANSS score for negative symptoms and the PSP score for self-care, socially useful activities, personal and social relationships, and disturbing and aggressive behavior area | (Németh et al. 2017) |

(continued)

Cariprazine has had an impressive clinical development, with proof-of-concept studies performed in acute and chronic schizophrenia, including forms with dominance of negative symptoms, mania and depression in bipolar I disorder and major depressive disorder (Table 2). Over a range of doses of 0.75–12 mg per day, cariprazine showed efficacy, at least at one dose, in all indications. Cariprazine is marketed in Europe, the USA, and other countries for the treatment of schizophrenia, bipolar depression, and bipolar disorder. The improvement over risperidone by

**Table 2** (continued)

| Drug | Patient population and study design | Study outcome | Reference |
|---|---|---|---|
| | 312 patients with acute mania of bipolar I disorder, randomized 1:1 to cariprazine at flexible dose of 3–12 mg or placebo and treated for 3 weeks | Cariprazine at 1.5 mg significantly improved the Young Mania rating scale (YMRS) total score over placebo. Suicidal ideation was less in the cariprazine 1.5-mg/day group than in the placebo group | (Sachs et al. 2015) |
| | 571 patients with bipolar I depression randomized 1:1:1:1 to cariprazine 0.75 mg, 1.5 mg, 3 mg or placebo for 8 weeks | Cariprazine at 1.5 mg significantly improved the MADRS total score over placebo | (Durgam et al. 2016b) |
| | 819 patients with major depressive disorder and inadequate response to antidepressants randomized 1:1:1 to cariprazine 1–2 mg, 2–4.5 mg or placebo as adjunctive treatment for 8 weeks | Cariprazine at 2–4.5 mg significantly improved the MADRS total score over placebo. No suicide-related adverse events were reported | (Durgam et al. 2016a) |
| F17464 | 134 patients with acute schizophrenia, randomized 1:1 to F17464 20 mg b.i.d. or placebo and treated for 6 weeks | Significant improvements at 6 weeks of the PANSS total score, positive PANSS subscale, Marder positive factor score and PANSS resolution criteria. Adverse effects were limited, with no weight gain, extrapyramidal symptoms except rare akathisia | (Bitter et al. 2019) |
| BP1.4979 | 219 subjects with tobacco smoking addiction randomized 1:1:1:1 to BP1.4979 3 mg, 10 mg, 15 mg of placebo and treated for 3 months | Undisclosed | (Bioprojet (Aubin HJ) 2015) |
| | Anticipated recruitment of 91 patients with restless legs syndrome, randomized to BP1.4979 15 mg or placebo and treated for 2 weeks | Terminated study after inclusion of 29 patients (study facing recruitment difficulties related to stringent eligibility criteria) | (Bioprojet (Ghorayeb I) 2020) |

[a] Only major studies are reported

cariprazine in schizophrenia with predominant negative symptoms may seem modest (−1.4 points on a scale ranging from 7 to 49) but it should be emphasized that cariprazine is the only antipsychotic drug that has proven superiority with respect to a classic antipsychotic in this condition. The question of the contribution of D3 receptor blockade in the efficacy of cariprazine cannot be definitively answered. The preferential occupancy of D3 over D2 receptors observed after chronic treatment suggests that D3 receptor blockade may at least contribute to the efficacy of low doses demonstrated in negative symptoms of schizophrenia, bipolar I depression, and major depressive disorders, which is in line with a preclinical study showing that the anxiolytic- and antidepressant-like actions of cariprazine are dependent on the D3 receptor-mediated mechanism (Duric et al. 2017). However, taking the above considerations that cariprazine may have higher D3-selectivity than it could be assessed by PET, it cannot be excluded that D3 receptor blockade may also be responsible, at least in part, for the actions of cariprazine on other symptoms.

F17464 was evaluated in acute schizophrenia (Bitter et al. 2019) and showed modest but significant effects over placebo on symptoms of schizophrenia, evaluated on different symptomatic scales assessing global symptomatology and specific positive, but not negative symptoms, which was expected in this study population with acute exacerbation. The safety profile was favorable. F17464 has demonstrated high and selective D3 receptor occupancy after acute administration of a dose of 30 mg, the highest tested dose (Slifstein et al. 2020). In the clinical trial, F17464 was administered at a dose of 20 mg twice a day. How plasma levels and D2 receptor occupancy accumulate upon repeated administrations is unknown. Therefore, it is not possible to ascertain that the selectivity for D3 receptor occupancy has been conserved in the clinical trial, and thus to define the contribution of D3 receptor blockade to the beneficial effects of the compound in schizophrenia.

## 7 Conclusions

Although there were premises of its existence, the discovery of the D3 receptor in 1990 was largely unexpected at a time when the dual classification of DA receptors in D1 and D2 subtypes was widely accepted. The reasons why the D3 receptor was previously unanticipated are its lower abundance and more restricted distribution, compared with those of the prototypical DA receptors and the absence of discriminant pharmacological agents. Nevertheless, other new DA receptor subtypes with similar restricted distribution, e.g., D4 and D5 receptors were subsequently discovered (Sunahara et al. 1991; Van Tol et al. 1991). It proved to be difficult to assign functional roles to D4 and D5 receptors, although subtype-selective agents exist (Jardemark et al. 2002; Mohr et al. 2006). In contrast, the D3 receptor has been robustly involved, in preclinical models, in exacerbated motor responses to DA agonists in DA neuron-depleted animals, exacerbated motor or motivational responses to drug-associated cues and schizophrenia-like behaviors. These roles

have received some support from clinical studies, however to a very limited extent so far.

The functions of D3 receptor, which can be delineated in the aforementioned models, are seemingly related to the general concept of "sensitization," which manifests by increased dopaminergic responses. Sensitization is associated with treatment-related side effects of Parkinson's disease, e.g. LID (Klawans 1973), addiction (Kalivas and Stewart 1991; Robinson and Becker 1986), and schizophrenia (Laruelle 2000) and has been linked in animals to increased D3 receptor expression in some paradigms (Bezard et al. 2003; Bordet et al. 1997; Le Foll et al. 2002). Hence, the D3 receptor may mediate excessive and pathological DA neurotransmission, which may be mitigated by D3-selective blockers.

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# Location, Location, Location: The Expression of D3 Dopamine Receptors in the Nervous System


**Eugenia V. Gurevich**

## Contents

1 Introduction ..... 30
2 The D3 Receptor Expression in the Mature Rodent Brain ..... 31
3 The D3 Receptor Expression in the Mature Human Brain ..... 32
4 The D3 Receptor Expression in the Developing Brain ..... 38
5 Conclusion ..... 41
References ..... 41

**Abstract** When the rat D3 dopamine receptor (D3R) was cloned and the distribution of its mRNA examined in 1990–1991, it attracted attention due to its peculiar distribution in the brain quite different from that of its closest relative, the D2 receptor. In the rat brain, the D3R mRNA is enriched in the limbic striatum as opposed to the D2 receptor, which is highly expressed in the motor striatal areas. Later studies in the primate and human brain confirmed relative enrichment of the D3R in the limbic striatum but also demonstrated higher abundance of the D3R in the primate as compared to the rodent brain. Additionally, in the rodent brain, the D3R in the dorsal striatum appears to be co-expressed with the D1 dopamine receptor-bearing striatal neurons giving rise to the direct output striatal pathway, although the picture is less clear with respect to the nucleus accumbens. In contrast, in the primate striatum, the D3R co-localizes with the D2 receptor throughout the basal ganglia as well as in extrastriatal brain areas. The relative abundance of the D3R in the limbic striatum, its output structures, secondary targets, and some of the other connected limbic territories may underpin its role in reward, drug dependence, and impulse control. Selective expression of D3R in the brain proliferative areas may point to its important role in the neural development as well as in neurodevelopmental abnormalities associated with schizophrenia and other developmental brain disorders.

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## 1 Introduction

Dopamine (DA) acts via five subtypes of G protein-coupled DA receptors. The DA receptor family is small comprising only 5 receptor subtypes. Two of the receptors belong to the D1-like subfamily (D1 and D5) and three – to the D2-like subfamily (D2, D3, D4). D1-like receptors couple to Gs/Golf and induce the cAMP accumulation, whereas D2-like receptors couple to Gi/Go and inhibit cAMP production. The D1 subtype is the main D1-like receptor expressed at a very high level in the striatum. The D2 receptor (D2R) is another major DA receptor subtype highly abundant throughout the striatum: in the caudate nucleus, putamen, and nucleus accumbens. A peculiar feature of the striatal expression of the DA receptors is the segregation of the main subtypes, D1 and D2, to different populations of the medium spiny striatal output neurons (MSN). The D1 receptors are largely localized on MSN 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 (Aubert et al. 2000; Gerfen 2000; Yung et al. 1995). The D2R is located on a different population of MSN than those expressing D1 receptors with less than 5% overlap. D2R is found on neurons expressing enkephalin and projecting to the globus pallidus external (indirect pathway) (Aubert et al. 2000; Le Moine and Bloch 1995; Nadjar et al. 2006). It is strongly believed that a coordinated activity of the direct and indirect output pathways is required for the proper control of voluntary movements by the basal ganglia circuitry (Alexander and Crutcher 1990; Klaus et al. 2019; Grillner and Robertson 2016). D2 receptors are also localized presynaptically on nigrostriatal dopaminergic terminals and on the substantia nigra neurons (Gurevich et al. 1999; Gurevich and Joyce 1999; Sesack et al. 1994; Levey et al. 1993).

The DA D3 receptor (D3R) is another member of the D2-like subfamily. It is a close relative of the D2R but its pharmacological properties are peculiar. Although many, albeit not all, common antagonists of the D2R have comparable affinities for the D3 subtypes, many agonists, starting with DA itself, show significantly higher affinities for the D3R than for the D2R (Lévesque et al. 1992; Freedman et al. 1994; Sokoloff et al. 1990, 1992; Perachon et al. 1999). In the presence of GTP, which leads to the dissociation of G protein from the receptor, the D2R is transitioned to the law affinity state characteristic of a free non-G protein-coupled receptor [for a review of the mechanisms, see Park et al. (2008)], with the agonist affinity in the micromolar range. In contrast to the D2R, the D3R is not converted into the low affinity state by GTP, and there is little difference in the affinity of agonists to the D3R in the presence or absence of GTP (Lévesque et al. 1992; Freedman et al. 1994; Sokoloff et al. 1992; Gurevich et al. 1997). This feature has been extensively taken advantage

of to achieve selective labeling of the D3R in radioligand binding experiments (Gurevich et al. 1997, 1999; Gurevich and Joyce 1999; Murray et al. 1994). Originally, when the receptor was first cloned, this phenomenon, i.e., lack of response to GTP, gave rise to the idea that the D3R is not functional. Indeed, it proved difficult to define the signal transduction pathways activated by the D3R. Later studies have attributed multiple critical physiological roles to the D3R distinct from those of the D2R (Gurevich et al. 1997; Bezard et al. 2003; Van Kampen and Eckman 2006; Kiss et al. 2021). However, the exact functions of the D3R expressed in many areas of the brain are yet to be understood.

## 2 The D3 Receptor Expression in the Mature Rodent Brain

It is often said that the D3R has a more restricted distribution than the closely related D2R. Indeed, in the rodent brain, D3 binding sites and mRNA are detectable in a limited number of brain regions: the islands of Calleja, the nucleus accumbens, mostly rostral pole and shell subdivisions, the olfactory tubercle, and the ventral pallidum, bed nucleus of stria terminals, mammillary bodies of the hypothalamus, and substantia nigra (Gurevich et al. 1999; Sokoloff et al. 1990; Bouthenet et al. 1991; Le Moine and Bloch 1996). Of these brain regions, the highest concentration of both D3R mRNA and binding sites is found in the islands of Calleja and shell of the nucleus accumbens, whereas the concentration in the caudate-putamen is quite low. When this special distribution of the D3R was first demonstrated (Sokoloff et al. 1990; Bouthenet et al. 1991), the findings generated significant excitement in the field. The relative abundance of the D3R in the limbic as opposed to the motor striatum suggested that this DA receptor subtype could be selectively targeted for the treatment of schizophrenia (Sokoloff et al. 1990; Gurevich et al. 1997).

These findings also have for the first time attracted attention to the islands of Calleja, the structures, although quite conspicuous in the rodent brain, remained until then largely ignored. The islands of Calleja, together with the olfactory tubercle, receive direct projections from the regions of amygdala connected to the vomeronasal organ, i.e., the system dedicated to sensing pheromones (Novejarque et al. 2011; Lanuza et al. 2008). This suggests that the islands of Calleja are a part of the reward circuit specialized in processing the information related to pheromones. Surprisingly, the pheromone-sensing system does not appear to be under control of the mesolimbic dopaminergic system, which innervates the limbic striatum, although the findings are somewhat ambiguous as to whether DA itself has any modulatory effect (Lanuza et al. 2008). To the best of our knowledge, it has never been investigated whether the D3R in the islands of Calleja plays any role in the pheromone-driven reward mechanisms.

Later research in rodents demonstrated that the D3R is co-expressed with D1R or D2R in neurons that also expressing Substance P or enkephalin, respectively, both in the shell and core of the nucleus accumbens (Le Moine and Bloch 1996). Interestingly, the level of the D3 mRNA and binding in the caudate-putamen, which is so

low as to be below detection level, progressively increases following dopaminergic denervation and subsequent repeated treatment with the DA precursor levodopa in the hemiparkinsonian rat model of Parkinson's disease (Bordet et al. 1997, 2000). This enhancement appears to occur in the D1R-bearing direct pathway MSN and requires D1R stimulation (Bordet et al. 1997, 2000) and could be driven by BDNF released from the cortico-striatal terminals (Guillin et al. 2001). Therefore, the D3R is present in the motor regions of the striatum, albeit at a lower level than in the limbic territories, and its expression there could be modulated by drugs and/or pathological conditions. Based on the studies in hemiparkinsonian rats, it appears that the D3R in the caudate-putamen is restricted to the direct pathway D1R-bearing MSN (Bordet et al. 1997, 2000), whereas in the nucleus accumbens it is present both in the D1R- and D2R-bearing neurons (Le Moine and Bloch 1996). It remains unclear whether these neurons of the nucleus accumbens represent both direct and indirect pathways or belong exclusively to the indirect pathway. In the nucleus accumbens, the direct projections to the substantia nigra (from the core) and ventral tegmental area (from the shell), which are analogous to the direct pathway from the dorsal striatum, originate from substance P/dynorphin and D1R-expressing neurons. The pathway to the ventral pallidum, which then proceeds, directly or through the subthalamic nucleus, to the substantia nigra or ventral tegmental area, starts from both dynorphin-D1R and enkephalin-D2R-expressing neurons (Smith et al. 2013; Humphries and Prescott 2010; Kupchik et al. 2015; Zinsmaier et al. 2021). However, collectively these data suggest that the D3R occupy different functional places in the brain motor and reward circuits.

The data obtained by immunohistochemistry with a D3R-specific antibody demonstrate the presence of D3R on all dopaminergic (tyrosine hydroxylase-positive) neurons in the substantia nigra, ventral tegmental area, and A8 retrorubral field in the rat (Bouthenet et al. 1991; Diaz et al. 2000). However, destruction of the nigral dopaminergic neurons results in only partial reduction in the D3R level in the substantia nigra in hemiparkinsonian rats (Stanwood et al. 2000), supporting the notion that many or all D3R are located on non-DA cells and thus unlikely to serve as autoreceptors.

## 3 The D3 Receptor Expression in the Mature Human Brain

The presence of both the D3R mRNA and binding sites in the primate brain appears more widespread as compared to the rodent. However, this could be misleading resulting simply from a relatively higher level of the D3R expression and easier detection in the brain areas of relatively lower abundance. Thus, the concentration of D3R in the human striatum is approximately 30% of that of the D2R, whereas in the rodent brain it is barely 5% (Gurevich et al. 1999; Murray et al. 1994). Still, as compared to the D2R, the D3R is found in a limited number of the primate brain areas closely connected anatomically and functionally and belonging, with some exceptions, to the brain limbic system.

In primates, including humans, D3R binding sites and mRNA are seen throughout the striatum, including the motor regions, although they are most abundant in the nucleus accumbens and ventral putamen (Gurevich et al. 1999; Murray et al. 1994; Bezard et al. 2003; Suzuki et al. 1998; Meador-Woodruff et al. 1996). These territories are often collectively referred to as the ventral, or limbic, striatum (Haber 2011; Heimer et al. 1999) and are a part of the brain reward circuit. This pattern of expression differs considerably from that of the D2R, which is highly enriched in the human caudate nucleus and putamen (Fig. 1a, d). Nevertheless, the D3R expression overlaps with that of the D2R across most of the striatal territories, and the D3R and D2R mRNAs are often co-expressed in the same neurons in all striatal subdivisions (Gurevich and Joyce 1999). The ventral striatum projects to the substantia nigra pars reticulata, ventral pallidum, and medial part of the globus pallidus internal (Haber 2011; Root et al. 2015), all structures enriched in the D3R mRNA and binding sites (Figs. 1e, f and 2a–d). Interestingly, the D2R sites are quite abundant in the external subdivision of the globus pallidus, which receives dense projections from the motor striatum, whereas the D3 sites are more abundant in the globus pallidus internal (Gurevich and Joyce 1999; Suzuki et al. 1998), particularly its medial part (see Fig. 1b–f), which receives projections from the ventral striatum. The substantia nigra reticulata also expresses a significant amount of the D3R (Figs. 1e and 2c, d). These data suggest that the D3R might coordinate the dopaminergic control over this reward circuit in a functionally relevant manner thus modulating the output of the limbic striatum.

The D3R binding sites are detectable throughout the thalamus, albeit at a low level. The D3R sites are particularly prominent in the anteroventral thalamic nucleus (Fig. 2c, d), a part of the anterior thalamic nuclei group belonging to the brain limbic system. This nuclei group plays an important role in memory and higher cognitive functions [reviewed in Perry et al. (2021); Perry and Mitchell (2019)]. The anteroventral thalamic nucleus receives its main subcortical projections from the mammillary nuclei of the hypothalamus, which themselves are component of the diencephalic memory system (Vann and Aggleton 2004) via the mammillothalamic tract. Damage to the mammillary bodies themselves or to the mammillothalamic tract in humans results in anterograde amnesia, i.e., inability to form new memories, with the damage to the tract being the best predictor to amnesia (Vann and Aggleton 2004; Van Der Werf et al. 2000). This suggests that these two brain structures, the anterior thalamic nuclei and the hypothalamic mammillary nuclei, connected via the mammillothalamic tract are involved in the proper functioning of the short-term memory. Interestingly, the D3R binding sites are detected not only in the anteroventral thalamic nucleus but also in the mammillary nuclei and mammillothalamic tract (Fig. 2c, d). In contrast, the D2R is absent from the mammillothalamic tract. Such anatomical arrangement suggests a role for the D3R in coordinating the dopaminergic influence over the diencephalic memory system via the control over the neuronal activity in the nuclei as well as over the afferents from the mammillary nuclei to the anteroventral nucleus. The human thalamic nuclei, including the anteroventral nucleus, are densely innervated by the dopaminergic axon that originate from various sources not confined to the commonly

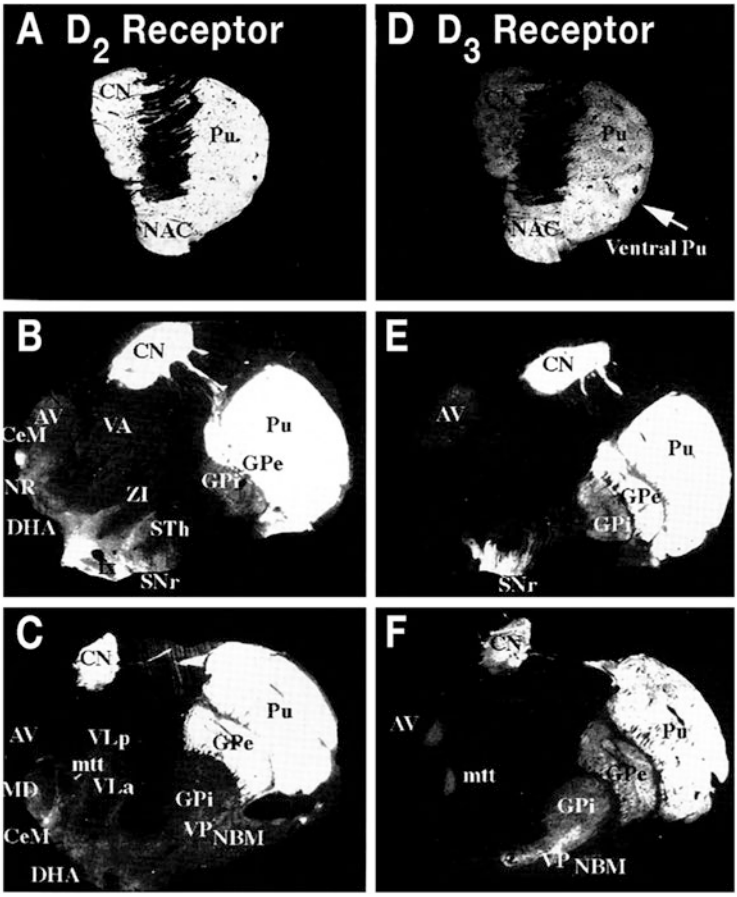


**Fig. 1** Dark-field photomicrographs of [$^{125}$I]epidepride binding to the D2R and D3R in the human brain. The binding in the rostral striatum (**a, d**) and two rostro-caudal levels of the basal ganglia, thalamus, and hypothalamus (**b, e** and **c, f**) are shown. Adjacent sections were labeled with 50 pM [$^{125}$I]epidepride (D2/D3R antagonist) in the presence of 100 μM Gpp[NH]p (non-hydrolysable analog of GTP) and either 100 nM 7-OH-DPAT (to block D3R) to visualize D2R (left panel) or 10 μM domperidone (to block D2R) to visualize D3R (right panel). Photographs represent specific binding. There is an evident gradient of D3R in the rostral striatum (**b**) with the highest binding in the NAC and ventral Pu not evident for D2R (**a**). Arrow in **b** indicates an approximate border of the ventral striatum coincident with the increased D3R binding in the ventral Pu. Autoradiograms in **b**, **c**, **e**, and **f** were deliberately overexposed to allow for visualization of binding to D2R and D3R in extrastriatal areas. Note a relatively high concentrations of D3R in the GPi and SNr as compared to D2R, labeling of the intralaminar thalamic nuclei for D2R but not D3R (**c**), and a much higher concentrations of D2R than D3R sites in the hypothalamus. Also note the presence of D3R and the absence of D2R in mtt. *The basal ganglia*: *CN* caudate nucleus, *Pu* putamen, *NAC* nucleus accumbens, *GPe* globus pallidus external', *GPi* globus pallidus internal, *VP* ventral pallidum; *the thalamus: AV* anteroventral nucleus of the thalamus, *VA* ventral anterior nucleus, *CeM* centrum medianum nucleus, *NR* nucleus reuniens, *VLp* nucleus ventralis lateralis posterior, *VLa* nucleus ventralis lateralis anterior, *MD* mediodorsal nucleus; *other: DHA* dorsal hypothalamic area, *ZI* zona incerta, *STh* subthalamic nucleus, *SNr* substantia nigra pars reticulata, *NBM* nucleus basalis of Meynert. Reproduced with permission, with some modifications, from Gurevich and Joyce (1999)

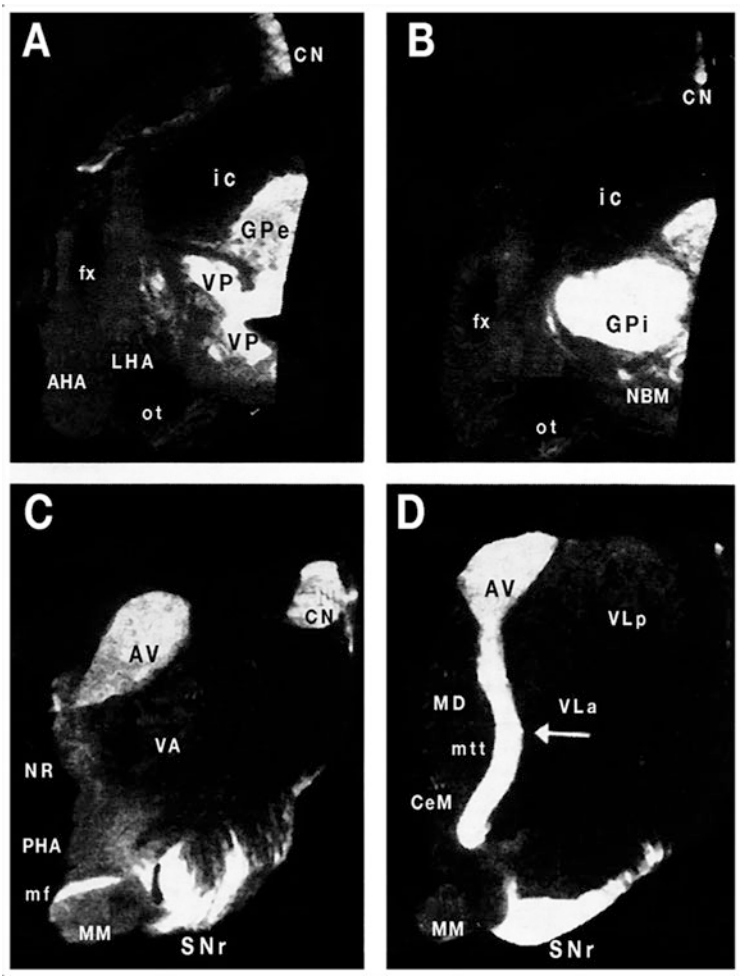


**Fig. 2** Dark-field photomicrographs of [$^{125}$I]7-trans-hydroxy-PIPAT to D3R in the human brain. Adjacent sections were labeled with 0.3 nm. [$^{125}$I]7-trans-hydroxy-PIPAT (selective D3R agonist) in the presence of 100 μM Gpp[NH]p. Coronal sections at four rostro-caudal levels (**a**–**d**) containing the striatum, thalamus, and hypothalamus are shown. Note high concentrations of D3R sites in the VP (**a**), GPi (**b**), and SNr (**c, d**) and the labeling of the AV and mtt in the thalamus (**c, d**). In **d**, arrow indicates the border where elevated concentration of D3R sites coincides with the presence of D3R mRNA positive cells within the mtt. Also note the presence of very low concentration of D3 sites throughout the thalamus and hypothalamus (including the mammillary nuclei, although the D3R sites are seen in mf). *ic* internal capsule, *ot* optic tract; *the hypothalamus*: *fx* fornix, *AHA* anterior hypothalamic area, *LHA* lateral hypothalamic area, *PHA* posterior hypothalamic area, *MM* mammillary nuclei, *mf* mammillary fasciculus. Other abbreviations are the same as in Fig. 1. Reproduced with permission, with some modifications, from Gurevich and Joyce (1999)

recognized nigrostriatal versus mesolimbic/mesocortical systems (Sánchez-González et al. 2005). This is unlike the rodent thalamus, which is reported to have limited dopaminergic input (García-Cabezas et al. 2009). Nevertheless, the D3R binding sites are present in the rat anterior thalamic nuclei and mammillothalamic tract, similar to that seen in the human brain (Gurevich and Joyce 1999) suggesting that the D3R-dependent control of this diencephalic circuitry is evolutionary conserved in mammals.

The D3R binding sites are detectable in the substantia nigra (Fig. 3), and the D3R mRNA is seen in dopaminergic neurons of the substantia nigra often co-localizing with the D2R mRNA (Gurevich and Joyce 1999; Joyce and Gurevich 1999). The D3R is conspicuously absent from the ventral tegmental area structures (Gurevich and Joyce 1999), in contrast to the D2R seen in all midbrain dopaminergic groups (Fig. 3) (Gurevich and Joyce 1999; Joyce and Gurevich 1999; Hurd et al. 2001). This is also different from the situation in the rodent where apparently the D3R is evident throughout the midbrain dopaminergic system (Diaz et al. 2000). Importantly, a large proportion of the D3R-bearing neurons in the primate midbrain are non-dopaminergic, presumably, GABAergic (Gurevich and Joyce 1999; Joyce and Gurevich 1999; Quik et al. 2000).

The recent years have seen the development of radioligands for the in vivo imaging of the D3R in humans. The most promising D3R imaging agent [$^{11}$C]-(+)-PHNO is an agonist with limited selectivity for D3R over D2R (Ginovart et al. 2006; Searle et al. 2010, 2013; Tziortzi et al. 2011; Narendran et al. 2006). The receptor binding studies using an agonist as a radioligand are always more complicated than with an antagonist, for the agonist binding is sensitive to the presence of GTP, which leads to the receptor transition to the low affinity state often undetectable experimentally. This is the case with the D2R, and since in in vivo situations GTP is always present, [$^{11}$C]-(+)-PHNO, when used alone, detects only the high affinity state of the D2R. The ligand also detects total D3R binding, since, as mentioned above (Lévesque et al. 1992; Freedman et al. 1994; Sokoloff et al. 1992; Gurevich et al. 1997), the D3R does not transition into the low affinity state as readily as the D2R does, and thus, both high and low affinity states of the D3R remain detectable. For this reason, the use of an agonist to label the D3R could even be advantageous allowing to increase the binding selectivity to the D3R over the D2R. Thus, when [$^{11}$C]-(+)-PHNO is used with displacing selective D3R antagonists, it allows for the evaluation of the proportion of the signal attributable to the D3R detected by PET measurements analyzed by mathematical modeling with the cerebellum as the reference region (assuming it has no D3R) (Searle et al. 2013).

Using [$^{11}$C]-(+)-PHNO with the selective D3 blocker GSK598809 and manual as well as automated delineation of anatomical regions of interest, it was determined that over half of the signal from the ventral pallidum/substantia innominata, globus pallidus and essentially all the signal from the substantia nigra and hypothalamus were due to the D3R (Tziortzi et al. 2011). A surprising finding was a relatively low fraction due to the D3R in the ventral striatum (19–26%) and lack of the D3R-related signal in the putamen. These discrepancies could be explained by the technical limitations of the in vivo PET approach as compared to in vitro postmortem

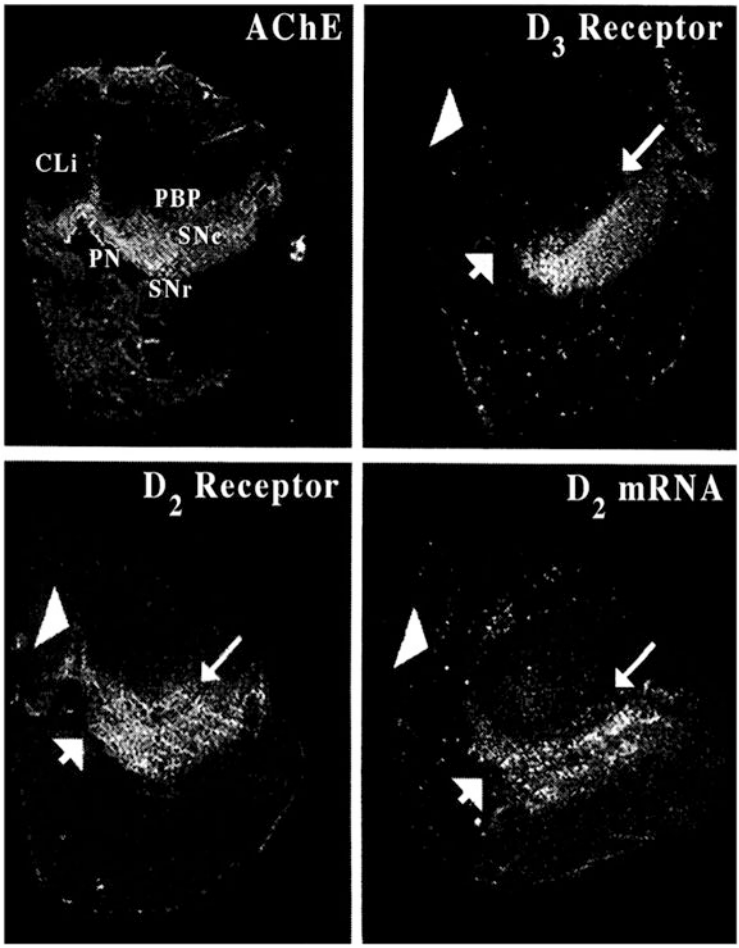


**Fig. 3** Dark-field photomicrographs of the transverse tissue sections of the human midbrain processed for the D2R or D3R binding or in situ hybridization histochemistry. The topography of the midbrain dopaminergic cell groups is shown by the staining for acetylcholine esterase AChE. The D3R sites were labeled with [$^{125}$I] 7-trans-hydroxy-PIPAT, and the D2R was labeled with [$^{125}$I] epidepride in the presence of 7-OH-DPAT (to displace the D3R). The D2R was detected mRNA using $^{33}$P-labeled riboprobe on adjacent sections. Large arrows indicate the position of the PBP; small arrows – the position of the PN; and arrowhead – the position of the CLi. Note the presence of D2R and mRNA both in the SN and VTA structures, including CLi, and the absence of D3R binding sites in the PN and PBP. Also note dense D3 binding in the SNr. *SNc* substantia nigra pars compacta, *SNr* substantia nigra pars reticulata, *PN* paranigral nucleus, *CLi* caudal linear raphe nucleus, *PBP* parabrachial pigmented nucleus. Reproduced with permission, with some modifications, from Gurevich and Joyce (1999)

autoradiographic studies (Searle et al. 2010; Tziortzi et al. 2011). Surprisingly, no D2R high binding was detected in the substantia nigra, although the presence of D2R autoreceptors on nigral neurons is well documented. Furthermore, autoradiographic

and in situ hybridization studies in the human have demonstrated the presence of the D3R binding and mRNA in the dopaminergic neurons of the midbrain (Gurevich and Joyce 1999; Joyce and Gurevich 1999). There is evidence that the majority of the D3R in the primate (and human) substantia nigra are not located on the dopaminergic neurons but rather on the GABAergic nigral cells, particularly in the pars reticulata (Gurevich and Joyce 1999; Joyce and Gurevich 1999; Quik et al. 2000). This notion is supported by the finding that the [$^{11}$C]-(+)-PHNO signal in the substantia nigra attributable to the D3R does not decrease in human PD patients (Boileau et al. 2009), in agreement with in vitro autoradiographic studies in parkinsonian primates, where it also did not decrease (Quik et al. 2000). These data support the notion that D3R does not serve as autoreceptors in dopaminergic neurons in the human brain. This, however, does not explain the lack of the D2R high signal in the substantia nigra on the PET scan since the resolution of the in vivo imaging is insufficient to distinguish the different types of nigral neurons or the subdivisions of the substantia nigra. The most likely explanation would be the technical limitations associated with the use of an agonist for imaging, the need for the radioligand displacement to achieve selectivity as well as an inability to measure in vivo the actual number of the receptor binding sites rather than a complex function related to the ratio of the number of sites to affinity. Despite the technical difficulties, in vivo measurement of the D3R is allowing for the studies of the D3R functional role in a variety of the human normal and pathological conditions (Girgis et al. 2021; Worhunsky et al. 2021; Payer et al. 2014) that were off limits before. Undoubtedly, such studies, with the improvements in the in vivo binding technique, will eventually yield a more profound understanding of the specific roles the regional D3R plays in the neural circuitry.

## 4 The D3 Receptor Expression in the Developing Brain

An interesting feature of the D3 receptor is its expression in the proliferative zones during prenatal and early postnatal development (Gurevich and Joyce 1999; Diaz et al. 1997; Araki et al. 2007). The D3R mRNA appears at prenatal day (E) 14 and by E18 is seen in the proliferative zone throughout the forebrain (Diaz et al. 1997). The D3R mRNA appears in the differentiating neuronal fields of the nucleus accumbens and mammillary bodies at birth. At postnatal day (P) 5 the distribution of the D3R mRNA resembles that of the adult, with its expression in the striatal subventricular proliferative zone, while diminished, continuing throughout the postnatal development and in the adult (Gurevich et al. 1999; Diaz et al. 1997) (Fig. 4a). In then adult rodent brain, the concentration of both D3R mRNA and protein is the highest in the islands of Calleja (Gurevich et al. 1999; Bouthenet et al. 1991). The islands of Calleja are composed of small cells derived postnatally from the subventricular zone (De Marchis et al. 2004). The subventricular zone, as the name implies, is a zone lining the lateral ventricles adjacent to the striatum that houses, among others, proliferating neural stem cells and neuroblasts [reviewed in Nakajima et al.

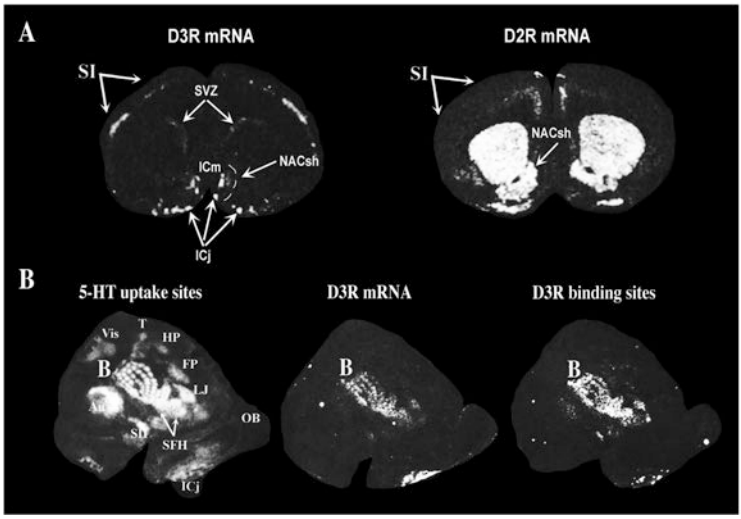


**Fig. 4** Topography of the D3R expression in the developing rodent brain. (**a**) Photomicrographs of the original autoradiograms of in situ hybridization histochemistry with $^{33}$P-labeled riboprobes for D3R and D2R on adjacent coronal from a 7-day-old rat. Note a relatively high level of the D3R mRNA in ICj with a virtual absence in NAC, whereas the D2R mRNA is present throughout the striatum. Also note selective expression of the D3R mRNA in layer IV of SI. In contrast, the D2R mRNAs is somewhat enriched in the deep laminae of the cingulate and secondary motor cortex but is undetectable in layer IV of SI. (**b**) The sections of a 7-day-old rat cut tangentially through layer IV of the flattened cortex were labeled for serotonin (5-HT) uptake sites with [$^{125}$I]RTI-55 to visualize the entire cortical map. The adjacent sections were labeled for D3 mRNA by in situ hybridization histochemistry and D3R binding sites with [$^{125}$I]7-hydroxy-PIPAT. The whiskers on the lateral aspect of the rat snout are represented in the primary somatosensory cortex (SI) as columns of cells spanning layer IV. When viewed in tangential sections prepared from flattened cortices, these whisker barrels appear roughly circular together comprising the barrel field (BF). Note completely overlapping distributions of D3R binding sites and mRNA. D3R binding sites and mRNA correspond precisely to the representations of whiskers, jaws, paws, etc., in the SI. Also, note the presence of both D3 sites and mRNA in the secondary somatosensory and auditory cortex, albeit at a much lower level than in the SI. *Au* auditory cortex, *FP* area of the front paw representation, *HP* area of the hind paw representation, *ICj* islands of Calleja, *ICm* island of Calleja magna, *LJ* area of the representation of the lower jaw, *NACsh* shell subdivision of the nucleus accumbens, *OB* olfactory bulb, *OT* olfactory tubercle, *SFH* area of the representation of short rostral facial hairs, *SI* primary somatosensory cortex, *SII* secondary somatosensory cortex, *T* area of the trunk representation, *Vis* visual cortex. Reproduced with permission, with some modifications, from Gurevich and Joyce (2000)

(2021); Akter et al. (2021)]. The neuroblasts from that zone, in addition to migrating along the rostral migratory stream into the olfactory bulb, migrate extensively into subcortical regions differentiating, among others, into GABAergic granule cells of the islands of Calleja (De Marchis et al. 2004; Inta et al. 2008). In the rat, the D3R mRNA and protein in the islands of Calleja appear postnatally, and at P7 still remain low reaching the near adult level by P14, i.e., significantly earlier than in the neighboring nucleus accumbens, where the D3R expression continues to rise until

adulthood (Gurevich et al. 1999). The level of the D3R expression in the nucleus accumbens at this age remains exceeding low, which is in a sharp contrast with the D2R already expressed at a significant level throughout the striatum (Fig. 4a). The early postnatal appearance of the D3 receptor in the islands of Calleja is well in agreement with the developmental origin of these structures form the subventricular zone, where the expression of the D3R is also evident early in development. The functional role of D3R expressed in the brain proliferative zones remains poorly understood although this expression pattern does suggest a role for the D3 receptor in neurogenesis and brain maturation. Studies have implicated D3R in regulating adult neurogenesis in the brain, the D3R function that might be involved in pathogenesis of schizophrenia or have an impact on the treatment of Parkinson's disease (Van Kampen and Eckman 2006; Inta et al. 2008; Egeland et al. 2012; Kim et al. 2010).

Interestingly, D3R was found to be transiently and selectively expressed in the stellate neurons of the layer IV of the somatosensory cortex of rats and mice (Gurevich and Joyce 2000; Gurevich et al. 2001). The rodent primary somatosensory cortex contains specialized structures in layer IV referred to as "barrels" due to their barrel-like shape on the transverse cortical sections (Woolsey and Van Der Loos 1970; Rice and Van Der Loos 1977; Rice 1985; Schlaggar 1994). Each barrel receives information from one whisker of the rodent's snout, and the arrangement of the barrels mirrors that of the whiskers. The barrel formation is controlled by the thalamo-cortical terminals conveying the somatosensory information to the primary somatosensory cortex, and an early abrogation of these terminals or removal of the whiskers disrupts the barrel formation (Kossut 1992; O'leary et al. 1994). The D3R is expressed by the granule neurons of layer IV and is located on the cell bodies, for the location of the binding sites and mRNA overlap (Gurevich and Joyce 2000) (Fig. 4a, b). The expression becomes evident at P5 (mRNA) or P7 (binding sites), steadily increases until P7 (mRNA) or P14 (binding sites) and then rapidly declines, although some D3R binding remains detectable even in the adult cortex (Gurevich and Joyce 2000). This expression pattern is unique for the D3R; other dopamine receptor subtypes are not expressed in the developing somatosensory cortex. The neonatal lesion of the thalamic ventrobasal complex, which sends projection to the primary somatosensory cortex, results in a total suppression of the elevation of the D3R concentration normally seen between the first and second postnatal weeks (Gurevich et al. 2001). This suggests that the D3R expression is controlled by early developmental activity of the somatosensory system. Unfortunately, the functional significance of the D3R transient expression during the critical period of the somatosensory cortex development so far has not been explored.

## 5 Conclusion

The anatomical distribution of the D3R in the human brain is consistent with its relative segregation, when compared to the D2R, to the limbic striatum, its output structures, its secondary targets as well as its major afferent sources. The highest expression of the D3R is found in the ventral striatum, which projects to the ventral pallidum and substantia nigra reticulata also relatively enriched in the D3R. The anterior thalamic nuclei enriched in D3R sites send projections to the ventral striatum and themselves receive afferents from the substantia nigra reticulata as well as the mammillary nuclei, both structures rich in the D3R sites. The dopaminergic neurons of the ventral tegmental area providing the dopaminergic input to the shell region of ventral striatum but lacking the D3Rs seem to break the pattern. Curiously, however, the rest of the ventral striatum receives dopaminergic input from the dorsal tier of the substantia nigra compacta expressing the D3R. Furthermore, the ventral striatum sends relatively widespread projections to the D3R-expressing substantia nigra compacta (Haber and Fudge 1997; Haber 2003), which in its turn projects to the associative striatal territories, thereby serving to integrate the functional striatal circuits. The D3R enriched in the brain regions associated with the limbic system may prove an important target for a variety of psychotropic drugs. The presence of the D3R in the brain proliferative zones adds yet another layer of intrigue to the functional role of this enigmatic dopamine receptor subtype. The developmental expression of the D3R peaks in the developing brain at the time of intense neurogenesis or during critical periods for the formation of cortical neuronal circuits. Such expression pattern suggests an important role in neural development, and it is regrettable that this role has not yet been better elucidated.

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# Recent Advances in Dopamine D3 Receptor Heterodimers: Focus on Dopamine D3 and D1 Receptor–Receptor Interaction and Striatal Function

**Federica Bono, Veronica Mutti, Zaira Tomasoni, Giulia Sbrini, Cristina Missale, and Chiara Fiorentini**

## Contents

1 Introduction ..... 48
2 G Protein-Coupled Receptors (GPCRs) and Receptor–Receptor Interactions ..... 48
3 GPCRs Heterodimer's Experimental Validation ..... 50
4 Dopamine D3 Receptor (D3R) and Heterodimerization ..... 52
5 D1R-D3R-Heterodimer in the Striatum ..... 56
6 Functional Role of the D1R-D3R Heterodimer in the Striatum ..... 61
7 Conclusions ..... 63
References ..... 64

**Abstract** G protein-coupled receptors (GPCR) heterodimers represent new entities with unique pharmacological, signalling, and trafficking properties, with specific distribution restricted to those cells where the two interacting receptors are co-expressed. Like other GPCR, dopamine D3 receptors (D3R) directly interact with various receptors to form heterodimers: data showing the D3R physical interaction with both GPCR and non-GPCR receptors have been provided including D3R interaction with other dopamine receptors. The aim of this chapter is to summarize current knowledge of the distinct roles of heterodimers involving D3R, focusing on the D3R interaction with the dopamine D1 receptor (D1R): the D1R-D3R heteromer, in fact, has been postulated in both ventral and motor striatum. Interestingly, since both D1R and D3R have been implicated in several pathological conditions, including schizophrenia, motor dysfunctions, and substance use disorders, the D1R-D3R heteromer may represent a potential drug target for the treatment of these diseases.

**Keywords** Dopamine D3 receptor (D3R) · G protein-coupled receptors (GPCRs) · Heterodimerization · L-DOPA-induced dyskinesia (LID) · Striatum

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## 1 Introduction

Starting from the observation of Agnati and Fuxe (Agnati et al. 1980, 1982; Fuxe et al. 1983), the concept of G protein-coupled receptors (GPCRs) heterodimerization, consisting in the ability of GPCRs to physically interact with other receptors to form novel receptor entities, has emerged as a common mechanism for GPCRs function and regulation. Supporting evidence of the existence of GPCRs heterodimers has been greatly increased in the last years, thanks to the development of biophysical techniques and other innovative strategies able to detect the close proximity between proteins. Like other GPCRs, dopamine D3 receptors (D3R) directly interact with various receptors to form heterodimers with other GPCRs or with non-GPCRs. In this chapter, current knowledge about the molecular characteristics and functional roles of complexes involving D3R has been reviewed, focusing on the D3R interaction with the dopamine D1 receptor (D1R) (the D1R-D3R heterodimer) and its relevance in the modulation of striatal function.

## 2 G Protein-Coupled Receptors (GPCRs) and Receptor–Receptor Interactions

G protein-coupled receptors (GPCRs) are the largest and most diverse superfamily of cell surface receptors that comprise more than 800 functional GPCRs encoded by approximately 2% of the total genome (Fredriksson et al. 2003). GPCRs are also called 7 transmembrane (TM) receptors since they all display a seven-transmembrane helical structure, forming the classical receptor unit required for binding an extracellular ligand and for the interaction with intracellular G proteins, that transduce and amplify extracellular signals via the production of second messengers (Fredriksson et al. 2003; Lefkowitz 2013; Flock et al. 2017). Ligands for the GPCRs are extremely variable, including ions, odors, amines, peptides, proteins, lipids, nucleotides, and even photons (Fredriksson et al. 2003). Therefore, GPCRs are fundamental for a plethora of physiological processes, making them the most pursued targets for drug development: more than half of all modern drugs, in fact, are targeted at these receptors (Hauser et al. 2017), and several ligands for GPCRs are found among the worldwide top-100-selling pharmaceutical products (Fredriksson et al. 2003).

Besides the classical GPCRs-G protein model, additional GPCRs modes of action have been described including the possibility to signal through pathways that are independent from G proteins (e.g., beta-arrestin-dependent pathways) (Wootten et al. 2018), to activate signals also when they are located into intracellular sites (Luttrell et al. 1999; Schiaffino et al. 1999), and to interact with other receptors to form novel receptor entities, such as dimer or higher-order oligomers (Agnati et al. 1980). In particular, dimerization between GPCRs with both closely-related and structurally divergent receptors, such as channels, has greatly expanded the

heterogeneity of GPCRs family and their ability to recognize and respond to an enormous variety of ligands; these novel entities are characterized by unique properties (pharmacological, signaling, and trafficking ones) distinct from those of both the interacting receptors that allosterically modulate each other, influencing the various aspects of receptor function (Fuxe and Agnati 1985; Bouvier 2001; Angers et al. 2002; Milligan and Smith 2007).

It is now recognized that dimerization between GPCRs takes place early in the biosynthetic process, occurring within discrete intracellular compartments, such as endoplasmic reticulum (ER) and Golgi, and moving to the plasma membrane as pre-formed mature complexes (Bulenger et al. 2005; Herrick-Davis et al. 2006). Moreover, GPCRs dimerization, that may also require the involvement of co-clustered ancillary proteins (Benkirane et al. 1997; Zhu and Wess 1998; Karpa et al. 2000; Lee et al. 2000; Decaillot et al. 2008), is believed to be part of a general quality control strategy in the GPCRs biosynthetic pathway, thus ensuring that only properly folded proteins are delivered to their sites of action (Bulenger et al. 2005). Interestingly, the possibility for GPCRs and GPCRs complexes to be transferred from cell to cell via exome pathway has been also provided (Agnati et al. 2011; Guescini et al. 2012; Guidolin et al. 2018).

With regard to the molecular mechanisms underlying GPCRs protein–protein interaction, it is generally believed that GPCRs receptor–receptor interactions occur via multiple interfaces. Bioinformatic strategies combined with docking analyses as well as experimental findings have suggested that highly conserved TM helices are required for both homodimeric (between the same GPCR) and heteromeric interactions, with a relevant role for TM4, TM5, and TM6 (Guidolin et al. 2018). Moreover, GPCRs carboxyl tail or intracellular loops (ICL), in particular the ICL3, have been found as required for several GPCRs heterodimers, with a key role of amino-acid residues, such as two or more adjacent arginine on one protomer and two or more adjacent glutamic/aspartic acids, or a phosphorylated residue on the other protomer, that are sufficient to induce electrostatic interactions; these type of interactions, that allow the formation of stable non-covalent (but covalent-like) complexes, likely represent a common mechanism in both heteromerization between GPCRs and between GPCRs and non-GPCRs (Perreault et al. 2014). Moreover, the involvement of extracellular domains in the dimerization mechanisms has been described for those GPCRs characterized by a large N-terminal domain, where disulfide bonds between cysteine residues (AbdAlla et al. 1999; Goldsmith et al. 1999; Kunishima et al. 2000) or posttranslational modifications such as sialylation and glycosylation (Michineau et al. 2006) might play a crucial role.

To date, evidence of heterodimerization has been provided for many families of GPCRs (Farran 2017), thus representing a general mechanism that allows interactions among systems, a crucial determinant of physiological cellular responses. Moreover, GPCRs heterodimers may also strongly impact the pharmacological field when a disorder is associated with dysfunctional GPCRs heterodimers: in these cases, heterodimers should represent targets for drugs with fundamental therapeutic advantages since heterodimers often exhibit discrete distribution and only tissues expressing both the interacting protomers should be targeted by such

molecules with potentially reduced incidence of side effects. In this line, while pre-eclampsia was the first disease associated with pathological GPCRs heterodimer activity (AbdAlla et al. 2001; Hansen et al. 2009), implications of heterodimers in several other disorders, including asthma, acquired immune deficiency syndrome, cardiac failure, schizophrenia, and Parkinson's disease (PD) (Somvanshi and Kumar 2012) have generated a great interest in GPCR heterodimers as new targets for novel drug discovery (Hauser et al. 2017).

## 3 GPCRs Heterodimer's Experimental Validation

In order to define and characterize GPCRs heterodimers, a combination of biochemical, functional, and biophysical approaches is usually performed, most of them carried out in heterologous cell systems expressing appropriately recombinant GPCRs. However, heterodimers need to be validated in physiological contests, since overexpression in hosts cells could generate artifacts; moreover, it is crucial to demonstrate that GPCRs heterodimers exhibit novel biochemical properties that are "biochemical fingerprints" for each specific heterodimer. Therefore, to validate a GPCR's heterodimer, some criteria need to be met as required by the International Union of Basic and Clinical Pharmacology (Fig. 1) (Pin et al. 2007; Gomes et al.

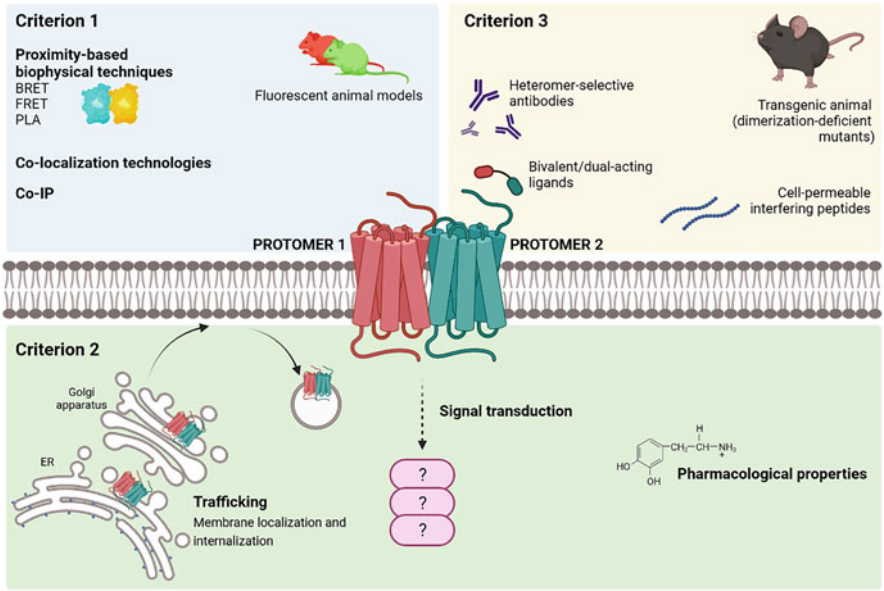


**Fig. 1** Representative cartoon depicting the three criteria used for establishing GPCR-heterodimerization. Created with BioRender.com. *BRET* bioluminescence resonance energy transfer, *ER* endoplasmic reticulum, *FRET* fluorescence resonance energy transfer, *IP* immunoprecipitation, *PLA* proximity ligation assay

2016). First of all, evidence of co-localization and physical interaction between the interacting protomers in native cells or tissue should be provided (Criterion 1). Usually, a combination of different assays, such as classical co-immunoprecipitation and Western Blot experiments, or co-localization techniques (including in situ hybridization, immunoelectron microscopy, and immunohistochemical methods) are used to address this criterion. However, the more recently developed proximity-based biophysical techniques are now the best approaches able to establish close proximity between two interacting protomers: among them, the resonance energy transfer (RET) technique, mainly carried out in transfected cells with properly tagged receptors, and proximity ligation assays (PLA). In particular, fluorescence resonance energy transfer (FRET) and bioluminescence resonance energy transfer (BRET) assay implicate a short-range nonradioactive transfer of energy between donor and acceptor molecules fused with each single protomer, that takes place only if the two species are in close proximity (<10 nm) to each other (Ciruela et al. 2010). In the case of FRET, both the donor and the acceptor are fluorescent molecules, whereas in BRET a bioluminescent molecule acts as the energy donor (Ciruela et al. 2010). FRET could be also adapted to detect heterodimers in native tissue, by incorporating the donor/acceptor pairs into antibodies (antibody-aided FRET) or ligands (ligand-aided FRET) against individual protomer (Cottet et al. 2013). In contrast to RET techniques, PLA has the advantage to monitor the presence of a receptor heterodimer in native tissues; this technique requires primary antibodies able to specifically recognize each of the two interacting receptors and two different secondary antibodies coupled to complementary oligonucleotide sequences, called PLA probes: close proximity between the two receptors (<17 nm), allows the PLA probes ligation, and amplification by the means of fluorescently labeled oligonucleotides thus leading to a signal that can be visualized using fluorescence microscopy (Trifilieff et al. 2011; Bellucci et al. 2014). Besides these widely used techniques, a double fluorescent transgenic mouse engineered to express individual interacting receptors each tagged with a fluorescent protein has been developed as a useful tool to investigate "in vivo" mu-delta opioid heterodimer formation, distribution, and function (Erbs et al. 2015).

That a receptor heterodimer should display distinct properties different from those induced by the single protomers, in terms of pharmacological, signaling, and trafficking ones ("biochemical fingerprints") is a key issue and needs to be deeply investigated and demonstrated (criterion 2). Therefore, a combination of different techniques, from radioligand binding assay to those assays able to measure intracellular signals, and receptor localization and distribution has to be performed taking advantage of cell systems that express both interacting receptors and individual protomers, used as control, and, when available, wild-type animals physiologically expressing both the receptors with the control knock-out counterpart, lacking one of the two protomers (Gomes et al. 2016).

Finally, demonstrating that disruption of a heterodimer specifically alters its properties, including loss of co-localization and function in both physiological and pathological contests, represents criterion 3: a widely used tool is represented by cell-permeable interfering peptides, designed to target and disrupt the predicted

heterodimeric interfaces. Interestingly, transgenic mice expressing mutant receptors unable to dimerize have been developed (González et al. 2012; Moreno et al. 2012; Baba et al. 2013) providing a very useful model to investigate heterodimer function in vivo. Moreover, the possibility to generate ligands selective for a heterodimer (antibodies, bivalent or dual-acting compounds, and small molecules), allowing both the recognition and the modulation of the heterodimers, paves the way for drug design. To date, numerous efforts have been made to pharmacologically target GPCRs heterodimers by designing heterodimer-specific ligands, and, among them, bivalent and dual-acting ligands seem to be the most promising drugs. Bivalent ligands, designed for the simultaneous binding to both protomers of a dimer are usually composed by two pharmacophoric moieties linked by a spacer with a length that is crucial for bridging the receptor dimer (Portoghese 2001); by contrast, dual-acting ligands combine two pharmacophoric units, usually linked by shorter spacers than those of bivalent ligands, with the aim of delivering both ligands simultaneously without the expectation of simultaneous binding (Jörg et al. 2015; Glass et al. 2016). After the development of the first bivalent ligand designed for targeting opioid receptor dimers (Erez et al. 1982), a considerable number of compounds were synthesized, including those targeting serotonin (Halazy et al. 1996; Perez et al. 1998), histamine (Birnkammer et al. 2012), opioid (Daniels et al. 2005), and dopamine (Kühhorn et al. 2011; Huber et al. 2012) receptor heterodimers. Interestingly, bivalent ligands targeting adenosine-dopamine heterodimers, composed of a xanthine and a dopamine analogue, that displayed dual dopamine agonist and adenosine antagonist activity, have been developed as potential treatments for PD (Vendrell et al. 2007).

## 4 Dopamine D3 Receptor (D3R) and Heterodimerization

Dopamine (DA) receptors, widely distributed within the brain, are GPCRs classically subdivided into "D1-like" (D1R and D5R) and "D2-like" (D2R, D3R, and D4R) receptor subtypes (Missale et al. 1998; Beaulieu and Gainetdinov 2011). Like other GPCRs, DA receptors retain the ability to form heterodimers with other DA receptors or other GPCRs as well as with non-GPCRs: among DA receptor-DA receptor heterodimers, evidence showing the formation of the D1R-D2R (Rashid et al. 2007), D2R–D5R (Hasbi et al. 2010), and D2R–D4R (Borroto-Escuela et al. 2011) heterodimers have been provided. Moreover, DA receptors have been shown to interact with the A1 and A2 receptors for adenosine (A1R-D1R heterodimer: Gines et al. 2000; A2R-D2R heterodimer: Hillion et al. 2002, and with the H3 receptor for histamine (D1R-H3R heterodimer, Ferrada et al. 2009; D2R-H3R heterodimer: Ferrada et al. 2008) as well as with non-GPCRs, such as the NMDA receptor for glutamate (D1R-NMDAR heteromer; (Fiorentini et al. 2003, 2008b); Lee et al. 2002; D2R-NMDAR heteromer:, Liu et al. 2006).

With regard to the D3R, a series of D3R-containing heterodimers has been demonstrated thus pointing to this receptor as a highly promiscuous entity in forming

**Table 1** Heterodimeric complexes containing the D3R

| Receptor complex | Criterion 1 | | Criterion 2 | Criterion 3 | Reference |
|---|---|---|---|---|---|
| | Techniques | Cell/tissue | | | |
| *DA receptors* | | | | | |
| D1R–D3R | FRET/BRET<br>CO-IP<br>PLA | Heterologous expression systems<br>Rat striatum<br>Rat and monkey striatum<br>Mouse primary striatal neurons | D1R-D3R heterodimer internalization and recycling upon coincident D1R and D3R stimulation<br>D1R interaction with D3R synergistically enhances second messenger signaling<br>D3R stimulation enhanced D1R affinity for DA and DA agonist | The cell-permeable interfering peptide TM5 counteracts both the positive cross-talk of D1R and D3R receptor agonists at the Erk1/2 levels and the quaternary structure of the heterodimer | Fiorentini et al. (2008a, b)<br>Marcellino et al. (2008)<br>Farré et al. (2015)<br>Present work |
| D2R–D3R | CO-IP | Heterologous expression systems | In the presence of excess D3R, the properties of partial D2R agonists transformed to antagonists | – | Scarselli et al. (2001)<br>Novi et al. (2007)<br>Maggio and Millan (2010) |
| *Other GPCRs* | | | | | |
| D3R–A2AR | FRET | Heterologous expression systems | A2AR reduced both the D3R affinity for agonist and the D3R ability in inhibiting AC activity | – | Torvinen et al. (2005) |
| D3R–MT1R/MT2R | BRET<br>PLA | Heterologous expression systems<br>Human non-pigmented ciliary body epithelial cells | Heterodimerization may result in the blockade of D3R-Gi coupling and in a reduction of the signaling to the Erk1/2 pathway | – | Reyes-Resina et al. (2020) |
| D3R-NTSR1 | BRET | Heterologous expression systems | NTSR1-controlled internalization of D3R into endosomes via recruitment of β-arrestin | Bivalent ligands stabilize and induce the interaction between the two receptors | Budzinski et al. (2021) |
| D3R-GPR143 | FRET | Heterologous expression systems | GPR143 may reduce D3R Affinity for DA | – | Bueschbell et al. (2021) |

(continued)

**Table 1** (continued)

| Receptor complex | Criterion 1 | | Criterion 2 | Criterion 3 | Reference |
|---|---|---|---|---|---|
| | Techniques | Cell/tissue | | | |
| *Ion channels* | | | | | |
| D3R–nAChR | BRET<br>PLA | Heterologous expression systems<br>Mouse primary mesencephalic DA neurons and midbrain sections<br>hiPSC-derived DA neurons | D3R-nAChR heteromer stimulation induces a strong and sustained activation of the PI3K-Erk1/2-Akt pathways, leading to the expression of the immediate-early gene c-Fos and to sustained phosphorylation of p70S6K | HyNDA-1, a dual-acting compound significantly modulates structural plasticity on both mice and hiPSC-derived DA neurons, possibly by acting on D3R–nAChR heteromer<br>Cell-permeable interfering peptides that avoid the interaction between D3R and nAChR, abolish structural plasticity mediated by the heteromer | Bontempi et al. (2017)<br>Bono et al. (2019)<br>Matera et al. (2019)<br>Bono et al. (2021)<br>Mutti et al. (2022) |

*Akt* thymoma viral proto-oncogene, *A2AR* adenosine A2A receptor, *BRET* bioluminescence resonance energy transfer, *CO-IP* co-immunoprecipitation, *DA* dopamine, *D1R* dopamine D1 receptor, *D2R* dopamine D2 receptor, *D3R* dopamine D3 receptor, *Erk1/2* extracellular regulated kinase 1/2, *FRET* fluorescence resonance energy transfer, *GPCRs* G-protein coupled receptors, *GPR143* G protein-coupled receptor 143, *hiPSC* human induced pluripotent stem cell, *MT1R/MT2R* melatonin receptor 1 and 2, *nAChR* nicotinic acetylcholine receptor, *NTSR1* neurotensin receptor 1, *PI3K* phosphatidylinositol 3-kinase, *PLA* proximity ligation assay, *p70S6K*, p70 ribosomal S6 kinase

receptor heteromers (Table 1) (Perreault et al. 2014; Bono et al. 2020). D3R are found both post- and pre-synaptically in the nervous system, being expressed in DA neurons of the substantia nigra (SN) and ventral tegmental area (VTA), and in medium spiny neurons (MSN) of the ventral (Le Moine and Bloch 1996) and dorsal striatum (Sokoloff et al. 1990). Classically, the activity of the D3R is mediated by the recruitment of Gi/o proteins which inhibit adenylate cyclase (AC), and by the regulation of calcium and potassium channels activity (Missale et al. 1998). In addition, it was reported that D3R may also signal through other specific intracellular pathways, that include the extracellular signal regulated kinase 1/2 (Erk1/2) and the Akt cascades in a G protein-dependent or independent manner (Beaulieu and Gainetdinov 2011).

Among the D3R-containing heterodimers D2R-D3R heterodimer was first detected in transfected cell models using chimeras generated from receptor fragments of the D2R and D3R in combination with co-immunoprecipitation (Scarselli et al. 2001). More recently, the existence of the D2R-D3R heterodimer has been demonstrated in transfected cell lines by using SNAP and CLIP tagging technologies, combined with time-resolved FRET (TR-FRET) (Pou et al. 2012); this technology, by labeling surface proteins with TR-FRET compatible fluorophores, allows a quantitatively analysis of protein–protein interactions at the surface of a living cell (Maurel et al. 2008). A putative role for the D2R-D3R heterodimer in the pathogenesis of schizophrenia and PD has been suggested, thus indicating that this heterodimer could be a target for novel drugs (Novi et al. 2007; Maggio and Millan 2010).

D3R heterodimerization with the adenosine A2A receptor (A2AR) has been demonstrated by both co-localization and FRET experiments in transiently co-transfected A2AR/D3R cell lines (Torvinen et al. 2005); by using these in vitro models, the observation that activating A2AR reduced both the D3R affinity for agonist and the D3R ability in inhibiting AC activity has suggested that the activation of the A2AR within the heterodimer could represent a novel strategy to antagonistically modulate D3R in antipsychotic treatments (Torvinen et al. 2005).

Moreover, a possible interaction between D3R and the GPCRs for neurotensin (NTS), a 13 amino-acid neuropeptide involved in the modulation of numerous processes, including locomotion, memory, and cognition (Sarret and Cavelier 2017) has been described (Koschatzky and Gmeiner 2012; Budzinski et al. 2021). In particular, while for the NTSR2 the interaction with the D3R still remains to be demonstrated (Koschatzky and Gmeiner 2012), a specific interaction between D3R and NTSR1 has been recently reported (Budzinski et al. 2021). An overlapping expression of D3R and NTSR1 in the brain, especially in the islands of Calleja was detected by autoradiography using D3R- or NTS1R-selective radioligands (Budzinski et al. 2021). Moreover, by using BRET analysis in transfected cell lines, specific interaction of the D3R with the NTSR1 was detected (Budzinski et al. 2021). Interestingly, bivalent ligands targeting the D3R-NTSR1 heterodimer have been designed and characterized for their ability to stabilize and increase the interaction between the two receptors as well as to induce heterodimer internalization via recruitment of β-arrestin, with a potential value in the pharmacological strategies for counteracting drug addiction (Budzinski et al. 2021).

D3R heterodimerization with the melatonin GPCRs MT1R and MT2R has been also recently reported in both transfected cells, by using BRET analysis, and in human eye post-mortem tissues, as demonstrated by PLA experiments (Reyes-Resina et al. 2020). Functional experiments, performed in both transfected cells and in human non-pigmented ciliary body epithelial cells, showed that for both the heterodimers, co-stimulation led to a decrease in the overall intracellular signaling (Reyes-Resina et al. 2020). Interestingly, both these heterodimers may be involved in the control of intraocular pressure induced by melatonin and DA.

Very recently, the orphan receptor G protein-coupled receptor 143 (GPR143), an atypical GPCR expressed in several brain regions with multiple roles in pigment

cells, has been shown as a new interacting partner for D3R (Bueschbell et al. 2021); this interaction has been demonstrated by using FRET in co-transfected cells (Bueschbell et al. 2021).

The ability of D3R in interacting with non-GPCRs has been demonstrated by the existence of a heteromer composed of the D3R and the α4β2 nicotinic acetylcholine receptor (nAChR) subtype, first identified in transfected cells by BRET analysis and then validated in primary cultures of mouse mesencephalic neurons, in mouse mesencephalic brain sections and in human DA neurons derived from induced pluripotent stem cells (iPSCs) by using PLA (Bontempi et al. 2017; Bono et al. 2019). Further characterization of this heteromer has shown its involvement in the neurotrophic and neuroprotective effects induced by nicotine (Bontempi et al. 2017; Bono et al. 2018, 2019), indicating that the D3R-nAChR heteromer is a functional unit supporting DA neuron plasticity and survival (Bontempi et al. 2017; Bono et al. 2018, 2019). A unique intracellular signaling has been recently associated with the D3R-nAChR heteromer activation: a strong and persistent activation of the PI3K-Erk1/2-Akt pathway, leading to the expression of c-Fos and to a sustained phosphorylation of cytosolic p70 ribosomal S6 kinase (p70S6K,) has been described as key molecular effectors critical for DA neuron dendritic remodeling and protection (Mutti et al. 2022).

Intriguingly, the analyses of iPSCs-derived DA neurons obtained from two Parkinson's disease (PD) patients carrying the G2019S LRRK2 mutation have shown a reduced expression of the D3R-nAChR heteromer at the plasma membrane level, a molecular alteration that may crucially contribute to the specific vulnerability of DA neurons in this pathology (Bono et al. 2021, 2022). Moreover, a dual-acting compound, named HyNDA-1, composed of the selective α4β2 nAChR agonist, A-84543, and the D3R preferential agonist, ropinirole, linked together by means of a spacer, was designed to selectively target and activate the D3R-nAChR heteromer with the aim to protect and support DA neuron against injury (Matera et al. 2019).

## 5 D1R-D3R-Heterodimer in the Striatum

DA transmission occurs broadly throughout the brain with a special impact on the activity of the striatum, the input structure of the basal ganglia, that is greatly influenced by DA released by SNpc VTA midbrain nuclei: sending a massive output to the striatum and receiving input from the striatum, these DA neurons are to be considered as an integral part of the corticobasal circuitry that is crucially involved in the control of the various aspects underlying goal-directed behaviors, such as emotions, motivations, and cognition that drive them (Haber 2014; Chen et al. 2021). Classically, DA neurons of the SNpc control voluntary movements primarily projecting to the dorsal striatum (nigrostriatal pathway), mostly composed of GABAergic medium spiny neurons (MSNs), divided into two major efferent pathways based on molecular and anatomical evidence: about half of MSNs give rise to

the striatonigral D1R-expressing neurons of the excitatory "direct pathway," which directly connect the striatum with the output structures, while the other half, the striatopallidal D2R neurons of the inhibitory "indirect" pathways that project to the external segment of the globus pallidus, indirectly connect the striatum to the output nuclei (Gerfen and Surmeier 2011). As the D1R and D2R act in an opposing way in terms of intracellular messengers, with D1R increasing and D2R decreasing intracellular cyclic AMP (cAMP) (Missale et al. 1998), DA increases the excitability of the "direct pathway" and decreases that of the "indirect pathway," thus allowing motor activity (Gerfen and Surmeier 2011). VTA DA neurons project to the ventral striatum, including the ventral and medial parts of the dorsal striatum, the olfactory tubercle system and the nucleus accumbens (NAc) (Schultz 2007; Haber 2014) via the "mesolimbic pathway" and regulate different aspects of motivated behavior, including adaptive responses to both positive and negative reinforces (Bromberg-Martin et al. 2010; Haber 2014; Morales and Margolis 2017; Chen et al. 2021). As in dorsal striatum, both the core and shell of the NAc are also comprised of D1R- and D2R-expressing MSNs that predominantly project to the midbrain and ventral pallidum, respectively (Gerfen and Surmeier 2011). Classically, D1R-expressing MSNs in NAc have been associated with positive reinforcement, while D2R-expressing MSNs with negative reinforcement (Durieux et al. 2009; Hikida et al. 2010; Lobo et al. 2010). However, to date, a clear distinction between D1R-expressing and D2R-expressing MSNs is hardly to delineate, prompting the suggestion of abolishing the use of "direct" and "indirect" pathway terminology in the NAc (Cazorla et al. 2014; Kupchik et al. 2015; Soares-Cunha et al. 2016). Similarly, optogenetic studies have shown that inter-pathway communications also occur in the dorsal striatum of mice leading to the notion that both D1R-expressing- and D2R-expressing MSNs pathways likely contribute to the various aspects of motor action (Jin and Costa 2010; Tecuapetla et al. 2010; Cui et al. 2013).

While the D1R- and D2R-expressing pathways are primary constituents of both the dorsal and ventral striatum, other DA receptors are behaviorally and clinically relevant, including the D3R, with a characteristic pattern of distribution mainly confined to the ventral part of the striatum, particularly in the neurons of the shell of the NAc (Sokoloff et al. 1990) but at low levels of expression in the MSN of the dorsal striatum (Ariano and Sibley 1994; Surmeier et al. 1996; Schwartz et al. 1998; Nicola et al. 2000). Functionally, D3R has been involved in the control of locomotion (Marcellino et al. 2008), motivated behaviors (Heidbreder 2008), and various aspects of cognitive functions (Nakajima et al. 2013). Interestingly, in both ventral and dorsal striatum, D3R have been shown to be co-localized with D1R (Le Moine and Bloch 1996; Ridray et al. 1998; Schwartz et al. 1998; Nicola et al. 2000) indicative of the possibility that these two receptor systems may regulate the MSNs function in an integrated way; therefore, the possibility that co-expressed D1R and D3R could result in the formation of a heterodimer composed by the physical interaction of D1R with D3R at the membrane level has been investigated as an additional level of interaction between the two receptor subtypes, useful to decode the D1R/D3R functional interplay.

According to Criterion 1 described in Gomes et al. (2016), co-localization was first demonstrated by using the co-immunoprecipitation, carried out in both Hek 293 cells co-expressing D1R and D3R and rat striatal membranes (Fiorentini et al. 2008a). D1R co-localization with D3R was also suggested by immunofluorescence experiments and confocal analyses in transfected Hek 293 cells (Fiorentini et al. 2008a; Marcellino et al. 2008). Strong methodological support for the study of protein–protein interaction inside living cells comes from biophysical techniques such as FRET and BRET techniques, both used to provide evidence of the existence of direct interaction between D1R and D3R in living cells (Fiorentini et al. 2008a; Marcellino et al. 2008). Moreover, BRET experiments carried out in Hek293 cells expressing the fusion proteins D1R-RLuc and D3R-GFP have shown that the levels of expression of the D1R-D3R heterodimer were unchanged upon agonist stimulation, suggesting that this heterodimer is a constitutive entity, likely transported to the plasma membrane as a pre-formed structure (Fiorentini et al. 2008a). Interestingly, the D1R-D3R heterodimer was visualized in primary cultures of striatal neurons obtained from wild-type mice, but not in neurons obtained from D3R knock-out mice (D3R-KO), by using PLA (Fig. 2), a powerful tool for detecting with high specificity and sensitivity close proximity between proteins in their native environments without the need for fusion proteins (Fredriksson et al. 2003; Bellucci et al. 2014).

A heterodimer is defined as an entity characterized by pharmacological, signaling, and trafficking properties that are different from those of each protomer forming the complex (Criterion 2; Gomes et al. 2016). Pharmacological studies carried out in Hek293 cells co-expressing D1R- and D3R-interacting receptors have shown that upon D1R and D3R stimulation, while D1R affinity for agonists was increased, the binding characteristics of the agonist for the D3R were unchanged, indicating the existence of allosteric/not reciprocal interactions between the two DA receptors (Marcellino et al. 2008); a D3R-mediated modulation of D1R agonist binding was also measured in membrane preparations of striatal tissue by using binding radioligand competition experiments (Marcellino et al. 2008). Interestingly, behavioral evidence of this pharmacological observation has been highlighted in the reserpinized mice model, classically used to dissect the activity of postsynaptic striatal DA receptors; in this model, stimulating the D3R led to a potentiation of the D1R agonist-induced locomotor activity (Marcellino et al. 2008), thus suggesting that in addition to a classical view of motor activation as the result of modulation of the "direct/indirect pathways" by DA or DAR agonist, a behavioral synergy of the D1R- and D2R-expressing pathways could also occur at the plasma membrane level of D1R-expressing MSNs co-expressing D3R by the means of the D1R-D3R heterodimer. In line with this observation, a potentiation of D1R-mediated stimulation of cAMP formation has been measured in Hek 293 cells expressing both D1R and D3R (Fiorentini et al. 2008a), an effect likely associated with D1R signaling pathway, since when individually expressed, D1R and D3R exert opposite effects on AC activity, with the D1R interacting with the Gs/Golf protein to activate AC and D3R inhibiting this pathway (Missale et al. 1998). In contrast with these results, the canonical negative interaction between D1R

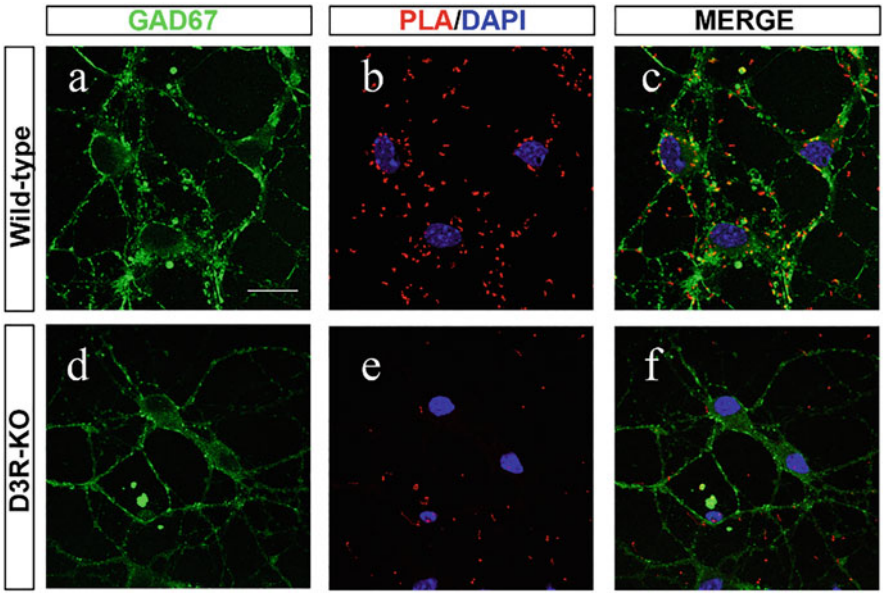


**Fig. 2** Detection of the D1R-D3R-heterodimer by in situ PLA in mouse primary striatal neurons. PLA was performed in primary striatal cultures derived from both wild-type mice and mice knock out for the D3R (D3R-KO), by using the Duolink In Situ reagents kit (O-LINK Bioscience, Upsalla, Sweden), following the manufacturer directions. Commercial primary antibodies were used to detect the D1R (1:100; Sigma Aldrich) and D3R (1:50; Santa Cruz Biotechnology). The GAD67 marker (green, 1:500; Millipore) was used to detect striatal neurons. Nuclei were stained with Dapi. Scale bar = 20 μm. Images were captured using a Zeiss LSM 510 Meta confocal microscope equipped with Plan-Apochromat 63x/1.4 numerical aperture oil objective and LSM 510 Meta Software, version 3.5 (Carl Zeiss AG, Oberkochen, Germany). Panel a–c: PLA signals in wild-type neurons, visible as red spots, observed in approximately 24% of GAD-67-positive neurons, each having 17,8 red spot/cell; Panel d–f: PLA signals (undetectable) in striatal neurons derived from D3R-KO mice

and D3R on cAMP accumulation was described by the group of Guitart et al. (2014), when the AC subtype 5, poorly represented in HEK cells but required for D3R activity (Robinson and Caron 1997) was overexpressed. In this line, a combination of Complemented Donor Acceptor (CODA)-RET and molecular fluorescence complementation/BRET experiments in transfected Hek 293 cells has shown that upon stimulation, D1R interacts with Gs protein and D3R with Gi protein when forming heterodimer and that the D1R-D3R heterodimer arranges as a pre-coupled macromolecular complex consisting of two D1R and D3R homodimers, each able to signal with their preferred G protein; since two protomers in a GPCR oligomer are likely insufficient to simultaneously accommodate two trimeric G protein (Maurice et al. 2011), the heterotetrameric structure provides the structure for canonical antagonistic interaction at the AC level (Guitart et al. 2014). Whether a synergy in activating the G protein-related cAMP accumulation by the D1R-D3R heterodimer needs to be more investigated, a synergistic activation at the level of MAPK-Erk1/2 signal has

been clearly demonstrated (Guitart et al. 2014, 2019) interestingly, while stimulation of D1R resulted in a G protein-dependent activation of Erk1/2, D1R and D3R co-stimulation switched toward a G protein-independent/beta arrestin-dependent Erk1/2 activation (Guitart et al. 2019); accordingly, BRET experiments in cells expressing D3R fused to YFP, beta-arrestin 1-RLuc, and D1R revealed that only upon co-stimulation strong beta-arrestin recruitment was detected (Guitart et al. 2019). Moreover, a cross-antagonism at the Erk1/2 level is another property of the D1R-D3R heterodimer, as a result of allosteric interactions by which an antagonist of one protomer, within the heterodimer, blocks the agonist-induced activation of the partner receptor. Interestingly, a heterodimer-induced synergistic activation of Akt, another G protein-independent/beta arrestin-dependent signaling pathway associated with D2-like receptors (Beaulieu et al. 2007) has also been observed in both transfected cells and reserpinized mice following D1R and D3R co-stimulation (Guitart et al. 2019). Of note, in the ventral striatum of reserpinized mice, a significant and selective increase in Akt phosphorylation, but not of Erk1/2, was correlated with the synergistic locomotor activity induced by D1R and D3R co-stimulation, (Guitart et al. 2019).

Finally, the trafficking properties of the D1R/D3R heterodimer in response to agonist stimulation, determinant for the heterodimer availability at the plasma membrane, were also well defined (Fiorentini et al. 2008a). It is well known that D1R stimulation results in a rapid receptor internalization, while the D3R is only marginally modified by agonist stimulation (Kim et al. 2005); a series of experiments carried out in transfected Hek 293 cells have shown that, within the D1R-D3R heterodimer, only the coincident D1R and D3R stimulation, and not the singular one's, allowed the heterodimer internalization (Fiorentini et al. 2008a), representing an additional mechanism of synergy between the two receptors that are both locked at the plasma membrane. These experiments also showed that beta-arrestin was required for co-agonist-induced internalization of D1R-D3R heterodimer, that afterward recycled back to the plasma membranes, thus mirroring the D1R trafficking properties (Fiorentini et al. 2008a).

While selective compounds for the D1R/D3R heterodimer have not been designed yet, membrane-permeable peptides targeting the predicted heterodimeric interface have been designed (Guitart et al. 2014, 2019) and used as tools to further explore and support heterodimerization studies (Criterion 3; Gomes et al. 2016). In particular, peptides with the amino-acid sequence of the transmembrane domain (TMs) 5, 6, and 7 of the D1R were fused to the cell-penetrating HIV trans-activator of transcription (TAT) peptide to allow intracellular delivery; interestingly, TM5, but not TM6 TM7 peptides, used as heterodimer-destabilizing agents in "in vitro" and "in vivo" experiments, had the ability to counteract both the positive cross-talk of D1R and D3R receptor agonists at the Erk1/2 levels and the quaternary structure of the heterodimer, thus pointing to the D1R TM5 as a crucial domain involved in the heterodimer formation (Guitart et al. 2014, 2019). In addition, the VK4-116 compound, a recently discovered selective D3R antagonist (Kumar et al. 2016), displayed a biased activity against D1R-D3R heterodimer since its ability to block, with higher potency, G-protein-dependent (cAMP accumulation) then G protein-

independent signals (MAPK/Erk1/2, beta-arrestin recruitment, and locomotor activity) (Guitart et al. 2019); thus, this compound may represent an additional tool for examining the heterodimer activity, especially in "in vivo" models.

## 6 Functional Role of the D1R-D3R Heterodimer in the Striatum

Even if the existence of this heterodimer remains to be clearly determined, results from in vitro and in vivo models likely indicate that D1R interaction with D3R stabilizes D1R in the membrane, increases D1R affinity for DA and DA agonists, and synergistically enhances second messenger signaling (Fig. 3) (Fiorentini et al. 2008a; Marcellino et al. 2008; Guitart et al. 2014, 2019). While in the ventral striatum, where D3R are abundantly expressed, the D1R-D3R heterodimer may

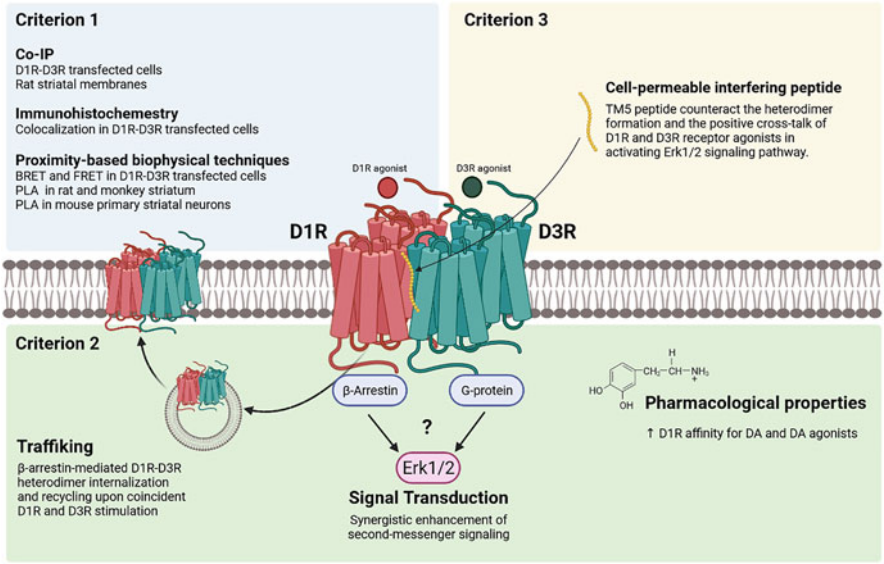


**Fig. 3** D1R-D3R heterodimer fulfill the three criteria for heterodimerization. The presence of the D1R-D3R heterodimer was demonstrated by several approaches, using both co-localization and proximity-based technologies, as well as with co-immunoprecipitation assay in transfected cell lines and in native tissue (Criterion 1). From a functional point of view, the heterodimer displays new pharmacological, trafficking, and signaling properties since the D1R interaction with D3R stabilizes D1R in the membrane, increases D1R affinity for DA and DA agonists, and synergistically enhances second messenger signaling (Criterion 2). Finally, a support for the D1R-D3R heterodimerization and its functional relevance was provided by using the TM5, a cell-permeable interfering peptide (Criterion 3). Created with BioRender.com. *BRET*, bioluminescence resonance energy transfer, *DA* dopamine, *D1R* dopamine D1 receptor, *D3R* dopamine D3 receptor, *Erk 1/2* extracellular regulated kinase 1/2, *FRET* fluorescence resonance energy transfer, *IP* immunoprecipitation, *PLA* proximity ligation assay

physiologically operate (Guitart et al. 2019), in the dorsal striatum an association with heterodimer abnormal expression and the development of L-dopa induced dyskinesia (LID), a common, severe, and irreversible side effect of long-term L-DOPA in Parkinson's disease patients, has been widely postulated. A growing body of evidence has shown that LID is attributed to the hyperactivation of the D1R-mediated transmission, resulting in increased levels of cAMP/PKA activity and ERK1/2 signaling (Bastide et al. 2015). The observation that abnormal activation of the tyrosine phosphatase Shp-2, a D1R-interacting protein crucially involved in the D1R-mediated activation of Erk1/2 in striatal neurons (Fiorentini et al. 2011), also characterized the MSNs in an animal model of LID (Fiorentini et al. 2013), and that knocking-down Shp-2 reduced LID (Fiorentini et al. 2016), also confirmed the pivotal role of D1R signaling in the mechanisms underlying LID.

Similarly, the role of D3R in LID pathogenesis has been almost clearly defined: starting from the observation that chronic-DOPA induced an ectopic expression of D3R mainly occurring in the D1R-expressing MSNs of the dorsal striatum of animal models of PD (Bordet et al. 1997, 2000), several other evidence further remarked this issue; among them, in the 6-OHDA rodent model, blocking D3R upregulation via intrastriatal infusion of oligonucleotide antisense to D3R mRNA attenuated the development of LID (Van Kampen and Jon Stoessl 2003). Moreover, in various PD models, D3R-preferring antagonists/partial agonist attenuated the development and the expression of LID (Bézard et al. 2003; Kumar et al. 2009; Visanji et al. 2009; Mela et al. 2010; Solís et al. 2015). LID was also decreased in D3R knock-out mice, a behavioral effect accompanied by decreases in well-accepted LID molecular markers, including active Erk1/2, in D1R-expressing MSNs only (Solís et al. 2015); more recently, by using the miRNA strategy in a rat model of PD, silencing of D3R in D1R-expressing MSNs resulted in attenuation of LID development without compromising L-DOPA's therapeutic benefits (Lanza et al. 2021); interestingly, overexpressing D3R in the dorsal striatum of rats in combination with L-DOPA was sufficient for developing dyskinetic behavior, also in the absence of DA depletion (Cote et al. 2014). Remarkably, by using the D3R-preferring PET radiotracer [(11)C]-(+)-PHNO, D3R upregulation in globus pallidus has been also shown in PD patients receiving chronic L-DOPA and developing LID but not in PD patients without LID or healthy subjects (Payer et al. 2016).

Therefore, it is reasonable to assume that an association between LID development and abnormal expression of striatal D1R-D3R heterodimer could exist, leading to increased efficiency of D1R coupling to AC, exaggerated cAMP/PKA activation, and abnormal Erk1/2 phosphorylation. In this line, radioligand binding studies and PLA have shown that in the striatum of animal models of PD, L-DOPA and LID development were associated with increased levels of D3R and D1R–D3R heterodimer, also correlated with higher D1R binding affinity in the presence of a D3R agonist (Farré et al. 2015). Moreover, using quantitative autoradiograph assay and in situ mRNA hybridization in human brain samples derived from patients suffering from different neurodegenerative conditions, a correlation between striatal D1R plus D3R densities, but not D1R and D3R alone, and PD survival, treatment, and progression has been described (Yang et al. 2021). The role of D1R- and

D3R-interacting receptors in LID has been also supported by experiments carried out on LID rats: the systemic co-administration of D1R and D3R agonists resulted, in fact, in synergistic increases in both dyskinesia and striatal expression of active Erk1/2 (Lanza et al. 2018). Intriguingly, in the same rat model of PD and LID, anti-dyskinetic strategies were able to prevent both LID and key molecular events associated with LID, including increased levels of striatal D1R–D3R heterodimers (Fanni et al. 2019). It is also relevant to underline that while the mechanism by which D3R is upregulated in LID is not fully understood, evidence of a link between higher striatal brain-derived neurotrophic factor (BDNF), increased level of D3R and behavioral sensitization has been provided (Guillin et al. 2001; Scheggi et al. 2020): in rat PD models, in fact, BDNF overexpression not only exacerbated D1R-agonists-induced LID but also increased both striatal D3R and D1R-D3R heterodimer levels, together with increased levels of active Erk1/2 (Scheggi et al. 2020).

Besides the pivotal role of abnormal D1R-mediated increases in Erk1/2 signals as molecular events underlying LID, changes in the Akt/GSK3beta pathway in both rat and monkey PD/LID models have been demonstrated (Bychkov et al. 2007; Morissette et al. 2010). In this line, dysregulation of Akt/GSK3β signaling has been associated with the pathogenesis of various neurological and neuropsychiatric disorders associated with DA dysfunction (Beaulieu et al. 2009). Interestingly, inhibition of GSK3β ameliorates L-DOPA-induced dyskinesia in 6-OHDA-lesioned rats (Xie et al. 2016). Therefore, the involvement of D1R-D3R heterodimer in LID could also be associated with its ability to over-activate the Akt pathway (Guitart et al. 2019).

## 7 Conclusions

The GPCRs superfamily of receptors are ubiquitously expressed among all cell types and are implicated in very numerous functions. Therefore, the discovery that many, if not all, of the GPCRs can physically associate with other receptors to form new entities may have important functional consequences and provide novel targets for more selective drugs in the future. To date, D3R have been found to interact with several other GPCRs and non-GPCRs, including the D1R-D3R heterodimer, very abundant in the ventral part of the striatum and physiologically acting as an entity that synergistically enhances the intracellular signaling induced by heterodimer activation. By contrast, in the dorsal part of the striatum, an abnormal expression of the D1R-D3R heterodimer has been associated with the development of pathological dyskinetic behaviors induced by long-term L-DOPA in PD patients. It is well known that while D1R hyperactivity in the MSNs of the DA-denervated dorsal striatum is a crucial target for counteracting the development of LID, blocking D1R signaling is a pharmacological strategy that affects the symptomatic benefits of L-DOPA treatment. In seeking new strategies for counteracting LID development in PD patients, those acting on D1R-D3R heterodimer, able to module its activity

thus affecting abnormal Erk1/2 and Akt signaling, may thus represent novel and more selective drugs; moreover, since both D1R and D3R have been implicated in several other pathological conditions, including schizophrenia, and substance use disorders, the D1R–D3R heterodimer may also be considered as a potential drug target for the treatment of these diseases.

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# Involvement of DA D3 Receptors in Structural Neuroplasticity of Selected Limbic Brain Circuits: Possible Role in Treatment-Resistant Depression

**Emilio Merlo Pich, Laura Cavalleri, Caterina Toma, and Ginetta Collo**

## Contents

1 Introduction: Cellular and Molecular Mechanisms of Neuronal Structural Plasticity ..... 74
2 Defective Neuronal Structural Plasticity in Brain of Patients with Mood Disorders ..... 75
3 Dopamine D3 Receptors (D3R): Biological and Pharmacological Profile ..... 76
3.1 D3R Role in Post-Synaptic Non-dopaminergic Neurons and Glia ..... 76
3.2 D3R Role in Presynaptic DAergic Neurons ..... 77
4 D3R Mediates Structural Plasticity in DAergic Neurons Engaging Neurotrophic Pathways ..... 78
4.1 Studies in Mesencephalic DA Neurons of Rodents ..... 78
5 Studies in Human DA Neurons Differentiated from Inducible Pluripotent Stem Cells (iPSC) ..... 79
6 Effects on DA Neuron Structural Plasticity Produced by the Activation of D3R Could Be Beneficial in Patients with Treatment-Resistant Depression ..... 81
7 D3R Signaling Is Involved in Structural Plasticity Produced by the Antidepressant Ketamine on Human and Mice Mesencephalic DAergic Neurons ..... 82
8 Conclusions ..... 82
References ..... 83

**Abstract** Structural neuroplasticity in the adult brain is a process involving quantitative changes of the number and size of neurons and of their dendritic arborization, axon branching, spines, and synapses. These changes can occur in specific neural circuits as adaptive response to environmental challenges, exposure to stressors, tissue damage or degeneration. Converging studies point to evidence of structural plasticity in circuits operated by glutamate, GABA, dopamine, and serotonin neurotransmitters, in concert with neurotrophic factors such as Brain Derived Neurotrophic Factor (BDNF) or Insulin Growth Factor 1 (IGF1) and a series of modulators that include circulating hormones. Intriguingly, most of these endogenous agents trigger the activation of the PI3K/Akt/mTOR and ERK1/2

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intracellular pathways that, in turn, lead to the production of growth-related structural changes, enhancing protein synthesis, metabolic enzyme functions, mitogenesis for energy, and new lipid-bilayer membrane apposition. The dopamine (DA) D3 receptor has been shown to play a specific role by inducing structural plasticity of the DAergic neurons of the nigrostriatal and mesocorticolimbic circuit, where they are expressed in rodents and humans, via activation of the mTORC1 and ERK1/2 pathways. These effects are BDNF-dependent and require the recruitment of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors to allow the structural changes. Since in mood disorders, depression and anhedonia have been proposed to be associated with impaired neuroplasticity and reduced DAergic tone in brain circuits connecting prefrontal cortex, ventral striatum, amygdala, and ventral mesencephalon, activation of D3 receptors could provide a therapeutic benefit. Sustained improvements of mood and anhedonia were observed in subjects with an unsatisfactory response to serotonin uptake inhibitors (SSRI) when treated with D3-preferential D2/D3 agonists such as pramipexole and ropinirole. The recent evidence that downstream mTOR pathway activation in human mesencephalic DA neurons is also produced by ketamine, probably the most effective antidepressant currently used in subjects with treatment-resistant depression, further supports the rationale for a D3 receptor activation in mood disorders.

**Keywords** Antidepressants · Dopaminergic neurons · iPSC · Ketamine · Mood disorders · mTOR

## 1 Introduction: Cellular and Molecular Mechanisms of Neuronal Structural Plasticity

Neuronal structural plasticity is a critical feature of adult mammalian brain and not only a prerogative of the developing brain. It consists of changes of density of macro-processes such as neuronal soma, axons, and dendritic arborizations, as well as of micro-processes such as synapses and dendritic spines within a given circuit or brain nuclei in response to stressors, damage, or long-term functional adaptation (Koleske 2013; Duman et al. 2016).

During brain development, the neuronal dendrites branch and form spines, the latter being the target of synapses coming from other neurons, the cornerstone of neuronal communication, turning over dynamically to fulfill an ontogenetic program. By contrast, in the adult brain, most dendrite branches and spines are tendentially stable over long spans of time, turning over mostly functionally in response to activation or hyperactivation coming from specific circuits, or due to local factors e.g., mediators of inflammation, circulating stress hormones, interaction with glial cells and failure of intracellular organelles such as mitochondria. Convergent findings indicate that stability of dendritic arborization and synaptic spines has a key role in the functioning of the adult brain, loss of stability, and structural deficits

being associated with psychiatric or neurodegenerative disorders (Koleske 2013; Duman et al. 2016), while reactive structural neuroplasticity aimed to adaptively normalize those circuits was proposed to be triggered by the re-engagement of neurodevelopmental programs (Castrén and Rantamäki 2010). Recent findings have provided better understanding of the molecular mechanisms that underlie the adaptive structural plasticity implicated in the stabilization of dendritic arborization, pointing to major players such as the intracellular pathways activated by Brain Derived Neurotrophic Factor (BDNF) and Insulin Growth Factor 1 (IGF1), by the level of activity of NMDA-mediated glutamate neurotransmission, of circulating glucocorticoids, the local expression of adhesion molecules, new protein synthesis and the integrity of energy-producing machinery associated with the mitochondria (Mattson et al. 2008; Liston and Gan 2011; Koleske 2013, Duman et al. 2016; Castrén and Monteggia 2021).

Here we will review the available evidence supporting the specific role for Dopamine D3 receptors (D3R) in producing and maintaining structural plasticity of the nigrostriatal and mesolimbic DAergic circuits in the adult brain, of potential relevance for mood disorders and, in particular, for treatment-resistant depression (Collo and Merlo Pich 2018).

## 2 Defective Neuronal Structural Plasticity in Brain of Patients with Mood Disorders

Defective structural plasticity in circuits of frontal cortex, hippocampus, amygdala, and ventral mesencephalon has been consistently described in patients with mood disorders (Drevets et al. 2008) and in rodents after chronic stress (Christoffel et al. 2011). This reduced neuroplasticity is paralleled by reduced activity of the mTOR pathway, whose phosphorylation cascade controls cell survival and growth (Jernigan et al. 2011). Reversal of the reduced structural plasticity observed in dendrites produced by stress and depression was described in cortico-limbic circuits of rodents and humans exposed to clinically-effective electroconvulsive therapy (Chen et al. 2009; Dukart et al. 2014) and pharmacological treatments with ketamine or, to lesser extent, serotonin uptake inhibitors (SSRI) (Duman et al. 2016; Bessa et al. 2009). The antidepressant actions of these treatments involve the increase of BDNF levels and the activation of the BDNF-TrkB signaling that activate the main neurotrophic pathways in neurons, leading to enhanced structural plasticity at synaptic and dendritic levels, indicating that defective BDNF/TrkB could be a critical mechanism in determining the impaired structural plasticity in major depressive disorder (Duman et al. 2016; Castrén and Monteggia 2021). D3R appears to be a player in this process, being controlled by BDNF-TrkB signaling as part of the DA sensitization mechanisms (Guillin et al. 2001) and, in turn, acting as a trigger for the release of BDNF from the DAergic neurons (Yang et al. 2020). This reciprocal interaction was further explored in Chap. 4 “D3R Mediates Structural Plasticity in DAergic Neurons Engaging Neurotrophic Pathways”.

## 3 Dopamine D3 Receptors (D3R): Biological and Pharmacological Profile

The two most common dopaminergic receptors in mesolimbic and nigrostriatal circuits are the D3R and the D2R, the latter consisting of the "short" and the "long" subtypes. Even if they share a large sequence homology and several signaling pathways, they show differences in their action and regulation. The expression patterns of D2R and D3R partially differ in the mammalian brain, with differences between rodents and primates (Gurevich and Joyce 1999, Gurevich, chapter in the present book). At cellular level, D2R subtype and D3R are expressed either presynaptically as autoreceptors in DAergic neurons in the ventral mesencephalon, e.g., in substantia nigra (Diaz et al. 2000), or postsynaptically in neurons with various phenotypes present in the terminal regions of the DAergic projections, e.g., GABAergic neurons in striatum and putamen, and in substantia nigra (Gurevich and Joyce 1999, Gurevich, chapter in the present book) or glutamatergic neurons of prefrontal cortex layer 5 (Clarkson et al. 2017). Interestingly, when present in the same brain regions, as in prefrontal cortex of rodents, functional segregation of D1R, D2R, and D3R can be observed, affecting different circuits (Clarkson et al. 2017). In addition, pharmacological antagonism of either D2R or D3R in frontal cortex disrupts and promotes cognitive function, respectively (Watson et al. 2012). Further functional differences between D2R and D3R were observed in mice whose genes were knockout (KO). The endophenotype of D2R KO mice displayed behavioral hypoactivity, insensitivity to the D2R/D3R agonist quinpirole, and low extracellular DA levels in the striatum (Baik et al. 1995). Conversely, D3R KO mice showed the opposite phenotype, displaying behavioral hyperactivity, responded to quinpirole response and spontaneous high extracellular DA level in the striatum (Accili et al. 1996; Koeltzow et al. 1998). Finally, D3R were observed in glial cells of striatum, cortex, and substantia nigra (Miyazaki et al. 2004; Zhang et al. 2014), where they contribute to local inflammation (Montoya et al. 2019).

### *3.1 D3R Role in Post-Synaptic Non-dopaminergic Neurons and Glia*

D2R and D3R being members of the 7-transmembrane domain receptors display different coupling with the subunits of the G protein-coupled receptor (GPCR): DA D3R engages the βγ subunits, while D2R uses the Gαo subunits (Beom et al. 2004). Moreover, desensitization of D2R is associated with phosphorylation mediated by the G protein-coupled receptor kinase (GRK) and by interaction with β-arrestin for internalization, while D3R undergoes protein kinase C (PKC)-mediated phosphorylation, internalization and degradation, or translocation into membrane hydrophobic domains (Cussac et al. 1999; Kim et al. 2001; Beom et al. 2004). Indeed, in GABAergic medium spiny neurons of striatum, the activation of both D2R and D3R

increases phosphorylation of the MAPK/ERK pathways, while D3R appears to be able to drive the selective activation of the Akt-mTOR signaling pathway produced by D2R/D3R agonists, since the latter was completely blocked by pretreatment with S-33084, a highly selective D3R antagonist (Salles et al. 2013). During development D3R begins its expression at the early embryological stages in neuronal precursors and immature oligodendrocytes (Bongarzone et al. 1998). In astrocytes of the adult mouse midbrain/striatum Montoya et al. (2019), showed that exposures to DA or to the D3-preferential DA agonist PD128907 were able to increase inducible Nitric Oxide Synthase (iNOS) to a similar extent to a systemic LPS administration, generating a pro-inflammatory-like response and increasing the expression of Glial Fibrillary Astrocytic Protein (GFAP). These effects were not observed in glial cells of D3R-KO mice, suggesting a possible permissive role of D3R neurotransmission in neuroinflammation. Intriguingly, LPS toxin exposures were shown to reduce the expression of D3R, suggesting a negative feedback possibly aimed to attenuate the local contribution of DA-dependent signals of inflammation.

## *3.2 D3R Role in Presynaptic DAergic Neurons*

In DAergic neurons of substantia nigra (SN) and ventral tegmental area (VTA), both D2R-short and D3R are expressed in neuronal soma and functionally defined as "autoreceptors" (Diaz et al. 2000; Ford 2014). The role of "autoreceptors" has been seen as associated to negative feedback control of synaptic activities, in this case control of neuron firing rate and control of neurotransmitter release, mostly via modulatory effect on Ca++ efflux and/or via direct interaction with the Dopamine Transporter (DAT). Dopamine or D3R-preferential D2R/D3R agonists such as quinpirole, ropinirole, or pramipexole by binding to presynaptic D3R reduce DA uptake by interacting with DAT functions (Joyce et al. 2004). Interestingly, D3R requires functional D2R autoreceptors to exert its control on DA release (Zapata and Shippenberg 2005). Similar effects were observed when indirect DA agonists, such as amphetamine or cocaine, were tested on mesencephalic DAergic neurons. Cocaine increased extracellular DA by blocking DAT, an effect potentiated by the blockade of D3R using the selective antagonist SB-277011-A, but not by the selective D2R antagonist L-741,626 (McGinnis et al. 2016). Recent in vivo microdialysis studies support the evidence that D3R activation increases DA release from rat substantia nigra/VTA (Rodríguez-Sánchez et al. 2019). Previously, Schwarz et al. (2004) reported that the selective D3R antagonist SB-277011-A was able to potentiate pharmaco-Magnetic Resonance Imaging (MRI) response to amphetamine challenges in the ventral mesencephalon of rats, affecting the brain functional connectivity and suggesting a presynaptic effect. Another series of studies has recently profiled the activation of other D3R-mediated intracellular pathways: they are reviewed in the following paragraph.

# 4 D3R Mediates Structural Plasticity in DAergic Neurons Engaging Neurotrophic Pathways

## *4.1 Studies in Mesencephalic DA Neurons of Rodents*

Based on the evidence collected by Diaz et al. (2000) on the localization of D3R in mesencephalic DAergic neurons, Du et al. (2005) studied the effects of D3R activation by incubating rodent primary mesencephalic culture containing astrocytes with D3R-preferential DA agonists, such as pramipexole and ropinirole, showing neurotrophic-like effects and increase in number of DAergic neurons. Similar results were obtained in mouse primary mesencephalic cultures that were maintained under conditions to prevent astrocytes growth using D3R-preferential D2R/D3R agonist such as 7-OH-DPAT and quinpirole at low doses, whose effects were blocked by selective D3R antagonist SB-277112-A (Collo et al. 2008). These data supported the tenet a neuronal-mediated D3R-dependent effect. Similar increases of dendritic arborization and soma size were also observed with the indirect DA agonist amphetamine, producing effects that involved the activation of MAPK/ERK pathways via presynaptic D3R, probably due to the increased extracellular DA produced by amphetamine (Collo et al. 2008). These effects were in keeping with the dendritic outgrowth observed postmortem in VTA of rats repeatedly exposed to amphetamine (Mueller et al. 2006). Few years later, using both in vitro and in vivo studies on mice, Collo et al. (2012) show that exposure to cocaine, another indirect DA agonist, produced D3R-dependent increases of structural plasticity in mesencephalic DAergic neurons. These effects were seen in vitro on primary cultures of DAergic neurons from mouse embryo, where cocaine-induced increase of dendritic arborization and soma size were antagonized by the non-selective D2R/D3R antagonist sulpiride and by the selective D3R antagonists SB-277011-A and S-33084. These effects of cocaine were mediated by the activation of the ERK1/2 and Akt-mTOR pathways, since preincubation with selective phosphorylation blockers completely inhibited structural plasticity induced by cocaine. Moreover, when primary cultures of mesencephalic DA neurons from D3R KO mice were challenged with cocaine, no change in dendritic arborization was observed and no activation of ERK1/2 and Akt pathway phosphorylation was observed. These observations were corroborated in vivo by morphometric assessment of mesencephalic dopaminergic neurons of P1 newborns exposed to cocaine from E12.5 to E16.5. The experiments were performed in wild-type and D3R KO mice. Cocaine increased the soma area of wild-type but not of D3R KO mice, supporting the translational value of primary culture (Collo et al. 2012). Other in vivo studies support structural plasticity effects of D3R signaling: van Kampen and Eckman (2006) evaluated rats exposed to 6-OHDA acute neurotoxic damage of nigrostriatal DAergic pathways after a chronic treatment with D3-preferential DA agonist 7-OH-DPAT. They observed a significant induction of cell proliferation in the substantia nigra pars compacta with a time-dependent adoption of DAergic phenotype. Retrograde tracing revealed a restoration

of striatal innervation from mesencephalic DAergic neurons and persistent recovery of locomotor function, a demonstration of induction of structural plasticity in vivo.

## 5 Studies in Human DA Neurons Differentiated from Inducible Pluripotent Stem Cells (iPSC)

The relatively recent observation that inducible Pluripotent Stem Cells (iPSC) from human donors can be differentiated in DAergic neurons (Kriks et al. 2011; Fedele et al. 2017) (Fig. 1a) has open the possibility to study their pharmacological phenotype in vitro (Fig. 1b). The changes of dendritic arborization and soma size induced by D3R-preferential agonists were studied in human iPSC-derived DAergic neurons (Collo et al. 2018) following a procedure schematically showed in Fig. 1b. An example of the application of this procedure is shown in Fig. 1c where the structural plasticity effects of pramipexole were quantified, resulting in a dose-dependent increases of maximal dendritic length, number of primary dendrites and soma size. Similar effects were also observed with ropinirole and antagonized by the selective D3R antagonists SB-277011-A and S-33084 (Collo et al. 2018). Visualization of phosphorylated p70S6 kinase indicated the recruitment of the mTOR pathway, a critical mediator of cell growth and structural plasticity. Phosphorylation of p70S6 kinase and structural plasticity induced by ropinirole and pramipexole were blocked by the kinase inhibitors LY294002 and by rapamycin, an mTORC1 inhibitor, confirming the involvement of the mTOR pathway. Since Ras-ERK and PI3K-mTOR pathways are also constitutive elements of the BDNF-TrkB signaling, different modalities of BDNF-TrkB pathway disruption previously used in rat telencephalic neurons (i.e., immunoneutralization of BDNF, inhibition of TrkB receptor and blockade of MEK-ERK signaling) (Jourdi et al. 2009) were applied to human DA neurons exposed to ropinirole, all procedures blocked D3R-dependent structural plasticity. These effects are consistent with the regulation of dendritic morphogenesis by Ras-PI3K-Akt-mTOR and Ras-MAPK signaling pathways previously described in the rat telencephalic neurons (Kumar et al. 2005). These data also indicate that active BDNF-TrkB signaling is necessary for D3R-dependent structural plasticity in human DA neurons. Interestingly, the behavioral relevance of reciprocal crosstalk between these two crucial pathways in DA neurons was demonstrated in rats with a unilateral nigrostriatal lesion of DA projections, showing structural recovery of axonal innervation and novel dendritic spine formation (Razgado-Hernandez et al. 2015).

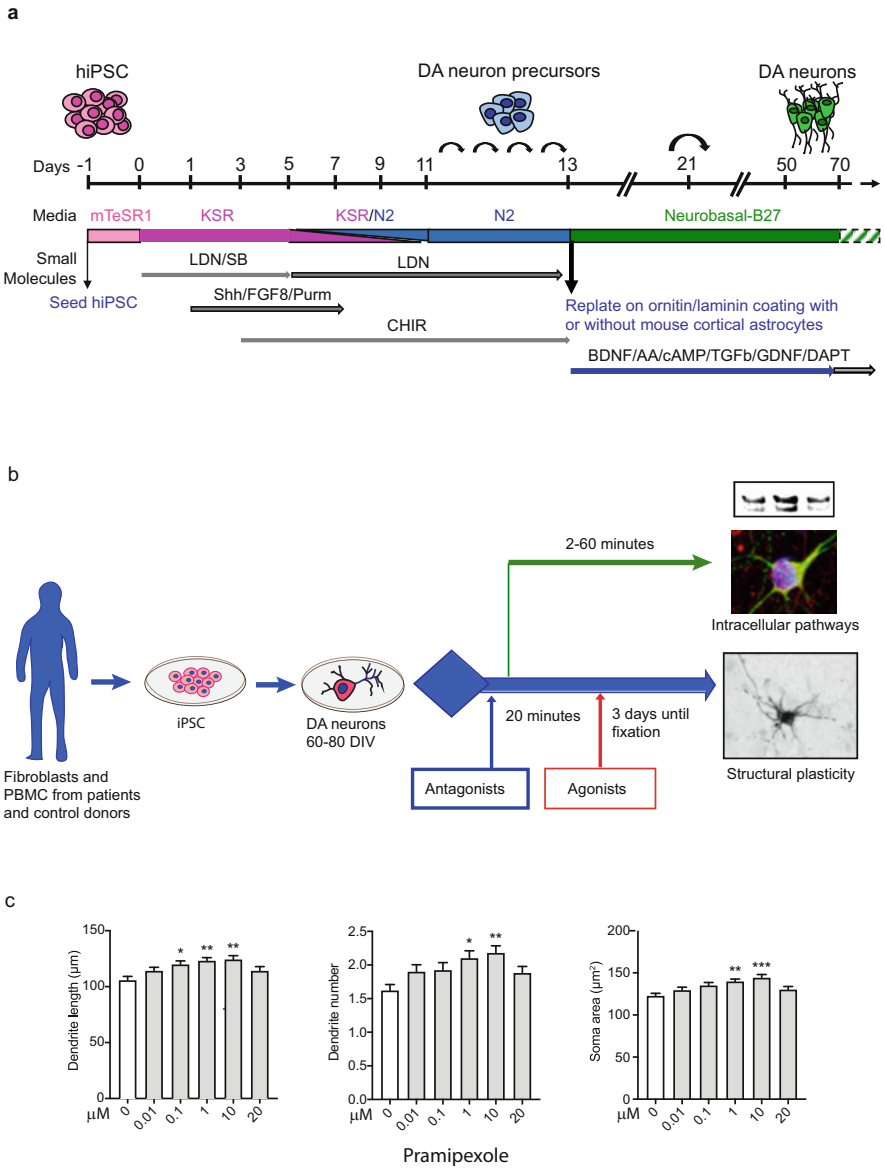


**Fig. 1** Human iPSC-derived dopaminergic neurons methodological approach to study neuroactive compounds. (**a**) Schematic representation of the differentiation procedure used to obtain in vitro human DAergic neurons. Neuronal precursors derived from iPSCs are exposed to successive mix of growth factors and small molecules aimed to develop the A9-like DAergic phenotype, according to Kriks et al. (2011) and Fedele et al. (2017). KSR, knockout serum replacement; LDN, LDN193189; SB, SB431542; Shh, Shh C25II; FGF8, fibroblast growth factor 8; Purm, purmorphamine; CHIR, CHIR99021; BDNF, brain-derived neurotrophic factor; AA, ascorbic acid; cAMP, dibutyryl cAMP; TGFβ, transforming growth factor type β3; GDNF, glial cell line-derived neurotrophic factor. (**b**) Cartoon of the procedure used to test iPSC-derived human DA neurons with various pharmacological agents, performing activation and inhibition studies with a variety of concentrations of agonists and antagonists as described in Collo et al. (2018). The outcomes were imaging or

## 6 Effects on DA Neuron Structural Plasticity Produced by the Activation of D3R Could Be Beneficial in Patients with Treatment-Resistant Depression

Dysfunction of dopaminergic neurotransmission within the mesolimbic and nigrostriatal systems, where D3R are expressed, may contribute to anhedonia, loss of motivation, and psychomotor retardation in severe depressive disorders that partially respond to treatment, and targeting D3R has been considered a possible therapeutic approach (Leggio et al. 2013). Preclinical studies showed association between low levels of BDNF in ventral mesencephalon with anhedonia, a core symptom of major depressive disorder (Der-Avakian et al. 2014). Low levels of TrkB expression were observed in postmortem striatum of patients with mood disorders (Reinhart et al. 2015). Indeed, increased BDNF signaling was recognized as a necessary step for the antidepressant effects of ketamine (Autry et al. 2011) and, partially, of SSRI (Bessa et al. 2009), leading to the concept of normalizing defective structural plasticity and dendritic arborization stability through a BDNF-TrkB orchestrated intracellular growth pathways activation (Castrén and Rantamäki 2010; Castrén and Monteggia 2021). Hence, the engagement of BDNF-TrkB signaling in mediating structural plasticity in DA neurons driven by D3R-preferential D2R/D3R agonists, such as 7-OH-DPAT, ropinirole or pramipexole, may be seen as a common feature involving D3R-mediated neurotransmission to address the problem of treatment-resistant depression. Accordingly, cariprazine, a D2R/D3R partial agonist with a 10-fold preferential affinity to D3R, improved symptoms in subjects with major depressive disorder that were poorly responsive to standard-of-care (Durgam et al. 2016). The intrinsic activity of cariprazine at D3R (Emax 70%) is comparable to that of aripiprazole, another D3R-preferential D2R/D3R partial agonist, that was approved for adjunctive treatment of major depressive disorder (Berman et al. 2007). Antidepressant effects of pramipexole were described in preclinical studies (Breuer et al. 2009), as well in clinical studies in Parkinson's patients with diagnosis of depression (Barone et al. 2010) and as add-on to SSRI in individuals with treatment-resistant depression (Fawcett et al. 2016; Tundo et al. 2022).

**Fig. 1** (continued) biochemical parameters, aimed to characterize structural plasticity on dendrites and soma profiles and the molecular pathways involved in these responses. (**c**) Histograms representing the quantification of the morphological changes of dendrites and soma produced by pramipexole dose-dependent D3R activation as described in Collo et al. (2018)

## 7 D3R Signaling Is Involved in Structural Plasticity Produced by the Antidepressant Ketamine on Human and Mice Mesencephalic DAergic Neurons

Ketamine is probably the most effective rapid-acting antidepressant for patients suffering from treatment-resistant depression (Schwartz et al. 2016; Zanos and Gould 2018; Collo and Merlo Pich 2018). Structural remodeling of prefrontal, hippocampal neurons involving dendritic arbors and spines has been proposed as a key neurobiological mechanism underlying antidepressant properties of ketamine (Duman et al. 2016; Zanos and Gould 2018). Ketamine-induced increase of dendritic arborization and soma size was also observed in mouse mesencephalic primary cultures and human iPSC-derived DAergic neurons (Cavalleri et al. 2018). These authors showed that the critical molecular mechanisms involved downstream activation of AMPA receptors which in turn trigger mTOR pathway-dependent structural plasticity via BDNF-TrkB activation. Both structural plasticity and neurotrophic pathway activation were blocked by MEK inhibitor PD98059, by PI3K inhibitor LY294002, and by rapamycin, an mTOR signaling inhibitor. The effects of ketamine were abolished by AMPA receptor antagonists and were mimicked by the AMPA positive allosteric modulator CX614, as shown also in telencephalic neurons (Li et al. 2010; Duman et al. 2016). Inhibition of BDNF-TrkB signaling achieved with various modalities prevented the induction of structural plasticity produced by ketamine. Intriguingly, ketamine effects on mesencephalic DAergic neurons required functional D3R, since its effects were abolished by pretreatment with selective D3R antagonists and were absent in D3R KO mice DAergic neurons (Cavalleri et al. 2018). These data are in line with the results of behavioral experiments in rodents using Forced Swim Test to assess depressive-like behavior, showing that the combined administration of sub-effective doses of ketamine and pramipexole exerted antidepressant-like effects compared with each drug alone (Li et al. 2015). The cartoon in Fig. 2 describes a working hypothesis about the molecular and cellular mechanisms involved in the interaction between ketamine and D3R-preferential DA agonist in producing beneficial effects in patients with treatment-resistant depression.

## 8 Conclusions

The present review summarized the findings supporting a role for D3R activation through pharmacological agents such as pramipexole or, indirectly, ketamine to increase structural plasticity in human DA neurons via recruitment of BDNF-TrkB and the activation of the MAPK/ERK and mTOR signaling pathways. Given the evidence of disrupted stability and reduced plasticity of dendritic arborization in several brain circuits in mood disorders, the structural effects produced by pharmacological activation of D3R can be seen as a reasonable treatment approach for a combination treatment, not implemented yet in the clinics. D3R activation could,

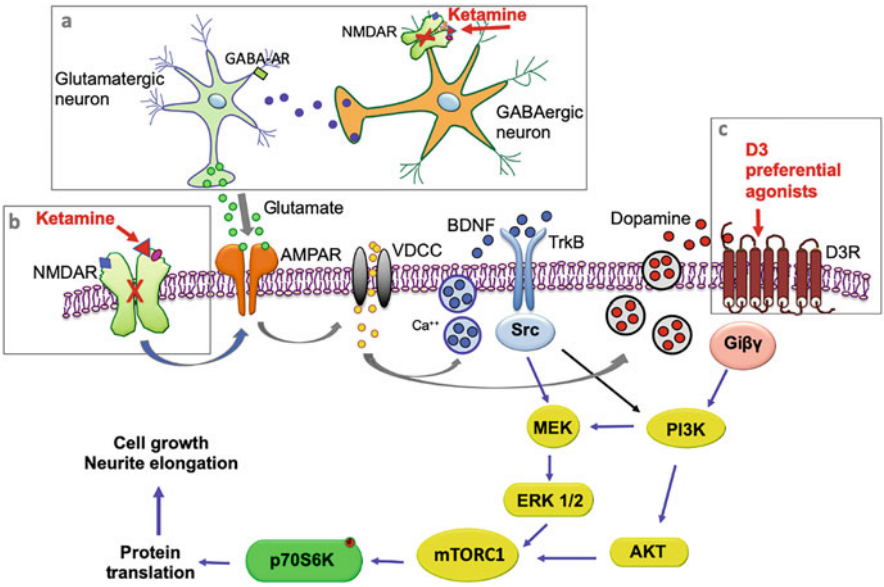


**Fig. 2** Schematic representation of the working hypothesis of molecular mechanisms involved in mediating the interaction between ketamine and D3R-preferential DA antagonists in producing structural plasticity-mediated changes in DAergic neurons suggested to improve depression in treatment-resistant depression. (**a**) This inset represents the hypothetical key steps mediating ketamine action on DAergic neurons via NMDA receptors expressed on GABAergic interneurons, as occurring in telencephalic neurons and summarized in Duman et al. (2016). (**b**) This inset represents an alternative hypothesis of ketamine action by a direct interaction on NMDA receptors expressed on DAergic neurons, as originally proposed for cortical neurons by Miller et al. (2016). Note that both proposed mechanisms upregulate synaptic AMPA receptors. (**c**) In this inset the exposure with D3R-preferential DA agonists, such as pramipexole or ropinirole (Collo et al. 2018), is highlighted as a way to drive a possible interaction with the ketamine-triggered intracellular pathways of relevance for structural changes, as proposed by Collo and Merlo Pich (2018)

therefore, contribute to the enhancement of structural plasticity necessary to improve depression, providing a reasonable interpretation of the clinical effects observed with pramipexole or ropinirole as add-on treatment in patients with treatment-resistant depression.

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# Dopamine D3 Receptor in Parkinson Disease: A Prognosis Biomarker and an Intervention Target

**Jinbin Xu**

## Contents

1 Introduction ..... 90
2 Dopamine and Dopamine Receptors ..... 90
2.1 Dopamine ..... 90
2.2 Dopamine Receptors ..... 91
2.3 Differential Regulations of D2R and D3R ..... 92
2.4 Development of [$^3$H]WC-10 as a Novel D3R Radioligand ..... 92
2.5 Quantitative Autoradiography Measure of Binding Affinity of [$^3$H]WC-10 to D3R in Human Striatal Tissues ..... 93
2.6 Quantitative Autoradiography Assay for D2R and D3R Densities ..... 93
2.7 Striatal and Extrastriatal Distribution of D2R and D3R in Postmortem Human Brain ..... 95
3 D3R in PD Intervention, Prognosis, and Progression ..... 96
3.1 D3R Agonists Alleviate PD Symptoms ..... 96
3.2 Possible Interventional Mechanisms of D3R Activation ..... 97
3.3 Neuroimaging Biomarkers in PD Pathogenesis ..... 97
3.4 D3R Changes for Predicting Prognosis of PD Treatment ..... 98
3.5 D1R, D2R, and D3R Expression in Control, AD and LBD Brains ..... 98
3.6 D1R + D3R Density Correlates with PD-Associated Symptomatic and Therapeutic Features ..... 99
3.7 Impact of Striatal D3R Density on Overall Survival ..... 100
4 Conclusions and Perspectives ..... 101
References ..... 102

**Abstract** Parkinson disease (PD) dementia, pathologically featured as nigrostriatal dopamine (DA) neuronal loss with motor and non-motor manifestations, leads to substantial disability and economic burden. DA therapy targets the DA D3 receptor (D3R) with high affinity and selectivity. The pathological involvement of D3R is evidenced as an effective biomarker for disease progression and DA agnostic interventions, with compensations of increased DA, decreased aggregates of α-synuclein (α-Syn), enhanced secretion of brain-derived neurotrophic factors

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(BDNF), attenuation of neuroinflammation and oxidative damage, and promoting neurogenesis in the brain. D3R also interacts with D1R to reduce PD-associated motor symptoms and alleviate the side effects of levodopa (L-DOPA) treatment. We recently found that DA D2 receptor (D2R) density decreases in the late-stage PDs, while high D3R or DA D1 receptor (D1R) + D3R densities in the postmortem PD brains correlate with survival advantages. These new essential findings warrant renewed investigations into the understanding of D3R neuron populations and their cross-sectional and longitudinal regulations in PD progression.

**Keywords** BDNF · Dopamine D3 receptor · Neuroinflammation · Parkinson disease · Progression · Survival · α-Synuclein

## 1 Introduction

Lewy body diseases (LBDs): Parkinson disease (PD), dementia with LB (DLB), and PD dementia (PDD), classified as Alzheimer disease and related disorders (ADRD), are neurodegenerative disorders that relentlessly and progressively lead to substantial disability. Pathologically, abnormal α-Syn deposition occurs in cytoplasmic inclusions called Lewy bodies located in pigmented brainstem nuclei such as substantia nigra pars compacta and are also deposited in dystrophic neurons in the striatal and cortical regions (Lewy neurites) (Braak et al. 2004; Lach et al. 1992). PD, the second leading progressive neurodegenerative disorder following AD, clinically manifests resting tremor, bradykinesia, rigidity, and postural instability (Kalia and Lang 2015). PD affects ∼1% population over 60 years old (Macdonald et al. 2018). PD is diagnosed by motor symptoms and pre-symptom non-motor features, including depression, sleep problems, and loss of smell (Srivanitchapoom et al. 2018). Current treatments improve motor symptoms but without halting disease progression. Ideally, earlier interventions are needed to modify the disease. Dopamine therapies have been concentrated on the activation of D1 receptor (D1R) and dopamine D2 receptor (D2R) (Lewis et al. 2006), but the critical role of D3R in PD pathogenesis and target engagement is less discussed. This chapter discusses D3R characteristics and regulations in PD pathogenesis, progression, and targeted DAergic therapies.

## 2 Dopamine and Dopamine Receptors

### *2.1 Dopamine*

Dopamine (DA), chemically defined as 3-hydroxytyramine, was first synthesized and tested in 1910 and was named 40 years later (Marsden 2006). DA transmission is

critically involved in the central nervous system (CNS) functions of movement, cognition, emotion, memory, reward, drug addiction, and a broad panel of neurodegenerative disorders. The motor manifestations of PD have been well characterized as loss of nigral DAergic cell bodies in the substantia nigra pars compacta (SNpc) and the subsequent striatal DA dysfunction in DA neuron terminals (Girault and Greengard 2004). DA does not penetrate the blood-brain barrier, while precursor levodopa (L-DOPA) can, and L-DOPA therapy has clinical benefits for PD patients but also the motor complications like "on-off" fluctuations, which have narrowed its clinical application (Wijeyekoon and Barker 2009).

## *2.2 Dopamine Receptors*

DA binds to the two major classes of G protein-coupled receptors (GPCRs) named dopamine receptors (DRs): the D1-like receptors (D1R, D5R) and the D2-like receptors (D2R, D3R, D4R). It is commonly believed that D1Rs couple to $G\alpha_{s/olf,}$ and agonist stimulation activates adenylate cyclase. Agonist stimulation of D2Rs (a) inhibits adenylate cyclase activity and (b) increases the release of arachidonic acid and phosphatidylinositol hydrolysis (Luedtke and Mach 2003; Neve et al. 2004). As summarized and reviewed previously, these DRs differ in their distribution, expression, affinity, and functional properties (Yang et al. 2020). It is worth restating that DA binds to D3R with the highest affinity ($K_i$ of ~2 nM) and selectivity among all DR subtypes.

D2Rs exist in two interconvertible affinity states for their natural agonist DA: a high- and a low-affinity state (Sibley et al. 1982). Under physiological conditions, DA binds predominantly to the high-affinity state and mediates the activation of the second-messenger cascade. Although autoradiography studies using the D2R/D3R agonists, [$^3$H]7-OH-DPAT and [$^3$H]quinpirole, under conditions minimizing binding to the D2R, suggest that D3R localizes in the ventral striatum and the islands of Calleja (Gehlert et al. 1992; Kaichi et al. 2000; Levesque et al. 1992), other data indicates that the density of D3R measured with agonists [$^3$H]7-OH-DPAT and [$^3$H] PD128907 is higher in the adult rat caudate-putamen than in the islands of Calleja (Hillefors and von Euler 2001; Hillefors et al. 1999). The high-affinity state is believed to be functionally important for agonist actions (George et al. 1985; Leff 1995). Nevertheless, the low-affinity state of D2R, and its conversion to a high-affinity form, need to be further investigated to better understand its regulatory functions in both diseased and healthy individuals (Briand et al. 2008; Graff-Guerrero et al. 2009; King et al. 2009; Skinbjerg et al. 2009). Cell culture studies of D2R and D3R demonstrate that both are subject to internalization upon agonist stimulation (Guo et al. 2010; Min et al. 2013), which challenges the interpretation of potential receptor measures using D2R/D3R imaging tracers.

## 2.3 Differential Regulations of D2R and D3R

Substantial evidence has shown that D2R and D3R differentially concert with DAergic tones. For example, Ryoo et al. (1998) reported a 45% *reduction* in D3R in the ventral striatum and a 15% *increase* in D2R in the postmortem PD caudate/putamen, which is consistent with animal PD models' 6-OHDA and MPTP lesioning studies (Levesque et al. 1995; Morissette et al. 1998). In addition, several positron emission tomography (PET) studies in human subjects with a chronic history of cocaine abuse have revealed a reduction in D2-like receptors relative to age-matched controls (Volkow et al. 1990, 1993). Others have shown that D2R is reduced in autoradiography (Moore et al. 1998) and PET imaging (Morgan et al. 2002; Nader et al. 2006) studies of rhesus monkeys with self-administered cocaine. However, autoradiography studies conducted by Staley and Mash (1996) (Staley and Mash 1996) reported an upregulation of D3R in human cocaine overdose victims in comparison to age-matched controls. These data suggest that D2R and D3R become "dysregulated" under conditions of increased (cocaine) and decreased (PD) DAergic tones, resulting in a change of D2R:D3R ratio in some DAergic system related disorders. This difference in the D2R:D3R balance may play a vital role in the behavioral consequences of the CNS syndromes characterized by altered D2-like receptor function. Therefore, it is necessary to measure the density of D2R and D3R independently to thoroughly understand the role of altered DR function in various CNS disorders.

## 2.4 Development of [$^3$H]WC-10 as a Novel D3R Radioligand

The lack of potent and selective D3R ligands has hampered the D3R research since its discovery in 1990 (Sokoloff et al. 1990). Obtaining ligands specifically selective for D2R or D3R has been difficult due to the high degree of amino acid homology in the helical transmembrane spanning regions of the receptors. Selective D3R agonists (7-OH-DPAT and PD128947) and D2R agonist (PHNO) are available, but selective D2R or D3R antagonists are not well documented (Ginovart et al. 2006; Vasdev et al. 2007).

4-(dimethylamino)-*N*-(4-(4-(2-methoxyphenyl)piperazin-1-yl)butyl)benzamide (WC-10), a *N*-phenyl piperazine analog, a higher affinity and binding selectivity D3R antagonist (Chu et al. 2005) was radiolabeled with tritium and the binding properties of [$^3$H]WC-10 to genetically cloned human and rat D2R and D3R were evaluated in vitro (Xu et al. 2009). The chemical structure of [$^3$H]WC-10 and its dissociation constant ($K_d$) values to human D2R (76 nM) and D3R (1.16 nM) are shown in Fig. 1. Although the preliminary data have indicated that [$^{11}$C]WC-10 is not an ideal PET tracer; human neuroimaging studies require a more potent D3R imaging probe (Laforest et al. 2016; Mach et al. 2011; Nabulsi et al. 2008; Peng et al. 2015). [$^3$H]WC-10 proved to be a valuable probe for in vitro autoradiography for

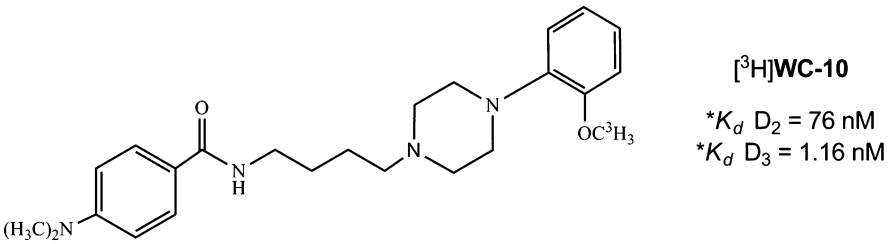


**Fig. 1** Chemical structure of [$^3$H]WC-10 and the binding affinities to D3R and D2R. $K_d$ values were obtained through the saturation binding of [$^3$H]WC-10 to cloned human D3R and D2R receptors expressed in HEK cells. *Taken from (Xu et al. 2010)

understanding the regulation of the D3R in PD progression and evaluating potential novel D3R PET tracers. WC-10 is an antagonist and measures both high and low-affinity D3R states.

## 2.5 *Quantitative Autoradiography Measure of Binding Affinity of [$^3$H]WC-10 to D3R in Human Striatal Tissues*

To measure the binding affinity of [$^3$H]WC-10 to D3R in monkey and human brain tissue, direct autoradiography saturation binding studies have been conducted, $K_d$ values in monkey caudate (1.3 nM) and putamen (1.1 nM) are found to be consistent with that in engineering cloned human D3R. Furthermore, autoradiography saturation binding of [$^3$H]WC-10 was performed in the striatum of a cognitively healthy control male brain. Autoradiograms showed that the total and nonspecific bindings of [$^3$H]WC-10 (range from 0.4 to 13 nM) to D3R (Fig. 2) in the caudate and putamen, nonlinear one-site binding curve fit determined the $K_{d,}$ values of the receptor-radioligand binding of [$^3$H]WC-10 to human striatum are shown in Fig. 2. [$^3$H]WC-10 binds with $K_d$ of 1.23 nM and the binding density ($B_{max}$) of 79.5 fmol/mg tissue to D3R in human caudate and 1.27 nM and 67.6 fmol/mg tissue in the putamen. It is interesting to note that the binding affinity of [$^3$H]WC-10 to D3R measured from monkey and human brains is in consistent agreement with that estimated using cloned human D3R.

## 2.6 *Quantitative Autoradiography Assay for D2R and D3R Densities*

Subsequently, we developed a mathematical model for calculating the absolute densities of D2R and D3R based on in vitro binding data obtained from [$^3$H]WC-10 and D2R preferring antagonist [$^3$H]raclopride (Xu et al. 2010).

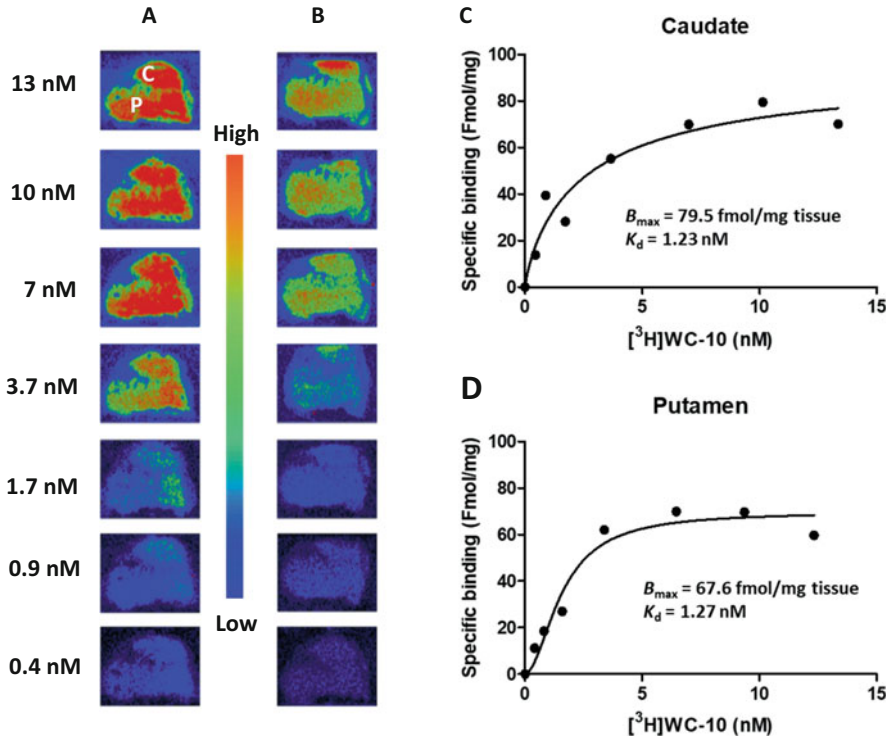


**Fig. 2** Quantitative autoradiography saturation binding of [$^3$H]WC-10 to D3R in the caudate and putamen of a male cognitively healthy case. Autoradiograms show the binding of [$^3$H]WC-10 to D3R in caudate (C) and putamen (P) under different radiotracer concentrations where 10 nM WAY-100635 is present to block the 5-HT$_{1A}$ receptor (panel **a**). Nonspecific binding was determined from the adjacent tissue section, which contained 1 uM S(−)-eticlopride to mask the D3R (panel **b**). Quantitative autoradiography analysis of the saturation binding in caudate (panel **c**) and putamen (panel **d**) and nonlinear binding isotherm curve-fittings were used to derive the binding densities ($B_{max}$ values) and the dissociation constants ($K_d$ values)

We developed a quantitative autoradiography-based mathematical model for calculating the absolute densities of D2R and D3R using in vitro binding data obtained from D3R preferring antagonist [$^3$H]WC-10 (Xu et al. 2009) and [$^3$H] raclopride (Xu et al. 2010). [$^3$H]WC-10 and [$^3$H]raclopride bind to D2R and D3R with different labeling proportions. The specific bound amount of receptors of a single concentration of [$^3$H]WC-10 or [$^3$H]raclopride binding can be expressed by the formulas:

$$\left[^3\mathrm{H}\right]\mathrm{WC\text{-}10} : a_1D_2 + b_1D_3 = B_1$$

$$\left[^3\mathrm{H}\right]\mathrm{raclopride} : a_2D_2 + b_2D_3 = B_2,$$

where $a_1$ and $b_1$ are the fractional occupancies of [$^3$H]WC-10 to D2R and D3R; $B_1$ is the apparent receptor binding density ($D_2 + D_3$) directly measured from autoradiography studies of [$^3$H]WC-10; $a_2$, $b_2$, and $B_2$ are the same parameters for [$^3$H] raclopride; $D_2$ and $D_3$ are the absolute densities of D2R and D3R, respectively. The absolute densities of D2R and D3R were calculated by solving the simultaneous equations:

$$D_2 = \frac{b_2 B_1 - b_1 B_2}{a_1 b_2 - a_2 b_1}$$

$$D_3 = \frac{a_1 B_2 - a_2 B_1}{a_1 b_2 - a_2 b_1}$$

This novel assay has been effectively used for evaluating D2R and D3R alterations in sleep deprivation and attention deficiency (Brown et al. 2011; Lim et al. 2011) and genetic PD models of DJ-1 and Pink-1 knockout rats (Sun et al. 2013b).

## 2.7 *Striatal and Extrastriatal Distribution of D2R and D3R in Postmortem Human Brain*

We completed the first comprehensive biomarker analysis of presynaptic and postsynaptic DRs in the striatal and extrastriatal regions in aged cognitively healthy human brains (Sun et al. 2012). The differential distribution and reported density of D1R, D2R, and D3R are shown in Fig. 3**.** (Sun et al. 2012). We found that the D3R density is higher than the D2R density in the thalamus, red nucleus, substantia nigra, and globus pallidus internal part in these brains (Sun et al. 2012). These data are important and assist in explaining the clinical imaging using nonselective D2R/D3R tracers, where the significant changes of tracer binding potential occurred in the extrastriatal regions: thalamus, substantial nigra, and globus pallidus—where the densities of D3R are greater than D2R in schizophrenia (Kessler et al. 2009), methamphetamine polydrug users (Boileau et al. 2012), and drug-naïve PD patients (Boileau et al. 2009). The measured dominant alterations are to D3R but not to D2R. The changes in D3R binding potential in the striatal regions might be overwhelmed by the D2R binding signal in these studies. Subsequently, we compared healthy controls with a small sample size of PD dementia cases ($n = 5$, including 1 PDD and 4 DLB); we found that D3R was upregulated in the striatum, whereas D1R and D2R were not, and downregulated in the substantia nigra of PDD/DLBs. The observed striatal D3R upregulation may reflect a compensatory change upon DAergic denervation. In contrast, the nigral D3R density reduction reflects DAergic neuronal loss in the nigrostriatal system of PDD/DLBs (Sun et al. 2013a).

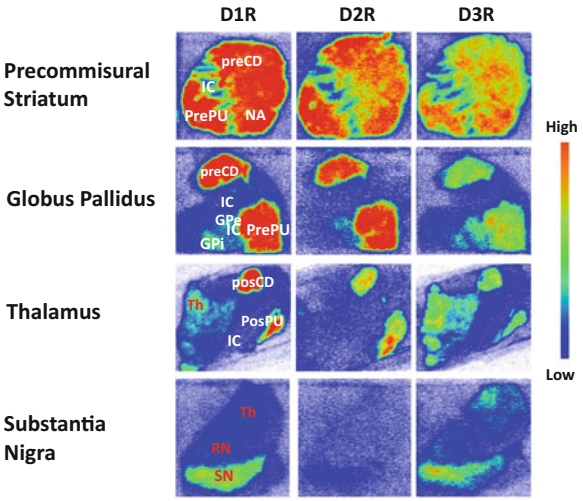


**Fig. 3** Autoradiograms show the differential distribution of D1R, D2R, and D3R in the striatal and extrastriatal regions of aged human brain tissue sections. The following CNS anatomical regions have been denoted: Precommissural putamen (PrePu); Precommissural caudate (PreCd); Nucleus accumbens (NAc); Internal capsule (IC); Globus pallidus external part (GPe); Globus pallidus internal part (GPi); Postcommissural putamen (PostPu); Postcommissural caudate (PosCd); Thalamus (Th); Substantia nigra (SN); Red nucleus (RN). D3R density is higher than D2R in Th, RN, SN, and GPi. Adapted from (Yang et al. 2021)

## 3 D3R in PD Intervention, Prognosis, and Progression

### *3.1 D3R Agonists Alleviate PD Symptoms*

D3R agonists have been routinely used to treat PD in both in vivo and in vitro models. In animal studies, D3R agonists alleviated striatal DA depletion in the striatum, attenuated substantial nigral DAergic neuron cell, ameliorated microglial activation, and improved behavioral performance in Parkinsonism mice; these effects were not observed in D3R KO mice (Lao et al. 2013; Li et al. 2010). In clinical research trials, D3R preferential agonist rotigotine's efficacy has been demonstrated in a series of phase III clinical studies with good tolerability and safety. Studies included patients with early and advanced stages of PD and established a dose-response relationship between escalating doses of rotigotine and improvements in PD symptoms (Benitez et al. 2014; Elmer et al. 2012; LeWitt et al. 2007; Pham and Nogid 2008).

In comparison to D1R and D2R, D3R may be an appropriate alternative target, supported by the facts: 1.) although the striatal D3R density is two–three times lower than D1R and D2R in the aged human brain (Sun et al. 2012), DA binds to D3R with greater than 100-fold higher affinity than it binds to D2R or D1R; 2) the endogenous

concentrations of DA in extracellular (5–10 nM) and synaptic (50 nM) spaces (Prieto 2017) are much lower than the $K_i$ of DA binding at D1R or D2R, but higher than its $K_i$ at D3R.

Although DR subtype-selectivity (D2R or D3R) has not been clearly defined, D2R/D3R agonists have shown significant beneficial effects for improving motor deficits in PD (Magnard et al. 2016). The most commonly used DA agonists are pramipexole, apomorphine, ropinirole, and rotigotine. Pramipexole was first reported to bind to D1R/D2R (Kaneko et al. 1990) and was later proved to be a potent D3R agonist (Chen et al. 2014) and preferentially activates D3R with high D2R/D3R selectivity of 24–800 (Cortes et al. 2016) with much lower affinity (>1,000 nM) to D1R (Wood et al. 2015). Pramipexole not only shows benefits in early-stage PD but also slows the occurrence of dyskinesia in poor L-DOPA therapy responders (Antonini et al. 2010). Pramipexole also works as a secondary intervention in advanced PD (Antonini et al. 2010; Schapira et al. 2011) and should be established as a treatment option for all stages of PD patients (Frampton 2014). Apomorphine, ropinirole, and rotigotine, first reported as D2R agonists (Eden et al. 1990; Umegaki et al. 1997; Van der Weide et al. 1988), were subsequently discovered as high-affinity D3R preferring agonists with D3R/D2R selectivity of 2–4, 19–94, and 19–20, respectively (Cortes et al. 2016). Thus D3R activation may represent the major pathway for these traditional DR agonists.

## *3.2 Possible Interventional Mechanisms of D3R Activation*

D3R agonists show promises and efficiency in the treatment of PD, although the principal for activation via D3R agonists in PD treatment is still obscure. The potential D3R activation mechanisms have been reviewed and discussed in detail (Yang et al. 2020), extensively covering their effects in increasing DA content through inhibition of DA reuptake and breakdown, decreasing α-Syn aggregates, promoting α-Syn clearance through autophagy, enhancing BDNF secretion, ameliorating oxidative stress and neuroinflammation, promoting neurogenesis, and interacting with other DRs. Besides, targeting D3R also helps in relieving some PD-associated non-motor symptoms such as depression, anxiety, psychosis, impulse control disorders (ICDs), and cognitive defects, as well as slowing down the side effects of L-DOPA, manifested as L-DOPA-induced dyskinesia (LID) or L-DOPA-induced abnormal involuntary movements (AIMs).

## *3.3 Neuroimaging Biomarkers in PD Pathogenesis*

Two PD pathological features, α-Syn aggregation nigrostriatal DA neuron projection loss, are well characterized in the postmortem brain (Dauer and Przedborski 2003; Zhang et al. 2018). Currently, no in vivo imaging biomarkers of α-Syn are available

for PD diagnosis or therapy evaluation. The development of therapies to slow LBD progression requires an objective biomarker of disease severity. Thus one of the current imaging strategies focuses on the nigrostriatal pathway. Several presynaptic biomarker PET radiotracers have been used to evaluate presynaptic DAergic nigrostriatal neurons in human subjects (Brooks et al. 2003). [$^{18}$F]FluoroDOPA (FD) PET primarily reflects the neuronal activity of decarboxylase that converts FD into [$^{18}$F]DA (Martin and Perlmutter 1994). [$^{11}$C]DTBZ, a marker for vesicular monoamine transporter type 2 (VMAT2), and [$^{11}$C]CFT, a marker for DA active transporter (DAT), correlate well with striatal DA and striatal DA fiber density but only correlate with nigral DA neurons when nigral cell loss does not exceed 50% (Karimi et al. 2013; Pate et al. 1993; Tabbal et al. 2012; Tian et al. 2012). These findings are consistent with a flooring effect of presynaptic DAergic measure observed in postmortem PD patients with moderate disease (Kordower et al. 2013) and a longitudinal PET study in PD patients (Kuramoto et al. 2013). Hence, these are suitable neuroimaging biomarkers of presynaptic neurons for early PD. The changes of postsynaptic DRs could serve as better biomarkers for the PD disease progression in the late stage.

## *3.4 D3R Changes for Predicting Prognosis of PD Treatment*

Studies using D2R/D3R ligands raclopride and fallypride (Le Foll et al. 2014; Mukherjee et al. 2015) showed increased striatal uptake in the early-stage PDs (Fisher et al. 2013; Rinne et al. 1995) and MPTP-intoxicated monkeys (Ballanger et al. 2016), which suggest that not only D2R but also D3R potentially contribute to the striatal receptor changes. Importantly, D3R is suggested as a PD prognosis maker. D3R elevation maintains the positive response to DAergic drugs, and D3R loss is correlated with the poor response to DAergic drugs (Joyce et al. 2002). In an animal study, DR agonists showed better improvement in alleviating PD symptoms in animals with striatal overexpression of both D2R and D3R when compared to those with overexpression of only D2R (Matsukawa et al. 2007).

## *3.5 D1R, D2R, and D3R Expression in Control, AD and LBD Brains*

Using the mathematical model for measuring D2R/D3R densities via radiotracers [$^{3}$H]Raclopride and [$^{3}$H]WC-10 described above, together with the D1R radiotracer [$^{3}$H]SCH23390, we comprehensively studied these DRs in a large cohort of 21 cognitively healthy controls, 34 AD, 11 PD, 16 DLB, and 10 PDD cases whose antemortem clinical features and postmortem pathological manifestations were well characterized, the PD progression duration of 37 LBD (PD/DLB/PDD) cases

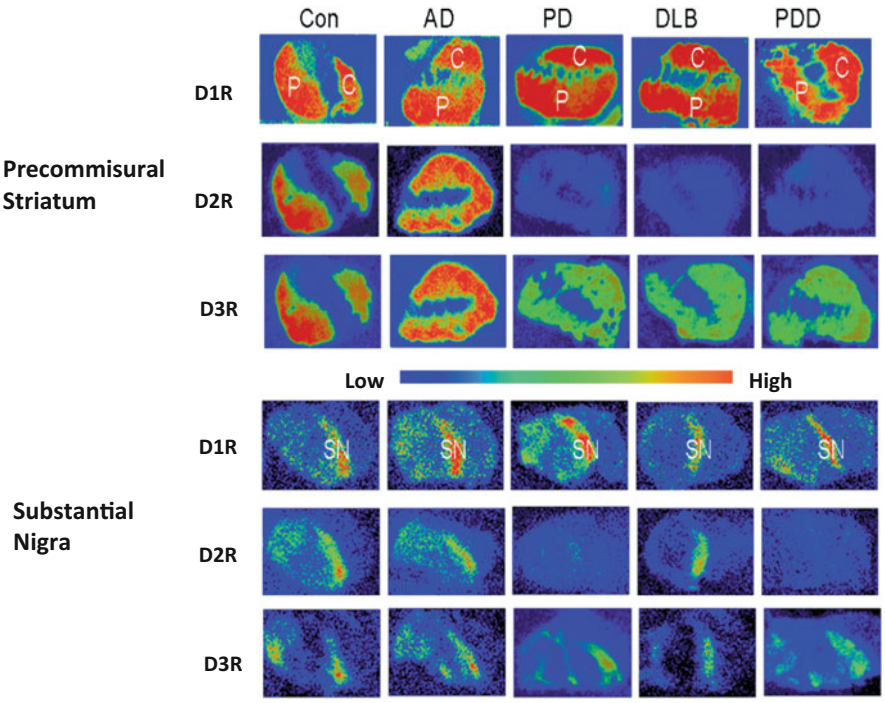


**Fig. 4** Autoradiograms show the differential distribution of D1R, D2R, and D3R in the striatal and substantia nigra regions of human brain sections. A: Autoradiograms D1R, D2R, and D3R in the precommissural caudate (C) and putamen (P) (upper panel) and substantia nigra (SN) (lower panel) of control (Con), AD, PD, DLB, and PDD groups. Adapted from (Yang et al. 2021)

ranges from 5 to 35 years (Yang et al. 2021). We statistically compared the densities of D1R, D2R, and D3R in different regions: striatal caudate/putamen and substantia nigra across all groups (Fig. 4). All the three DR subtype densities are lower in the substantia nigra than in the striatum in all five groups. Their expression in the striatum and substantia nigra was similar in PD, DLB, and PDD groups. D2R, but not the D1R or D3R, was found to be significantly reduced in LBD (PD/DLB/PDD) groups compared to the control and AD groups.

## *3.6 D1R + D3R Density Correlates with PD-Associated Symptomatic and Therapeutic Features*

D1R, D2R, and D3R densities are similar between caudate and putamen. We took the average of caudate/putamen measures as striatal density and conducted systematic correlation analyses between striatal and substantia nigra DR densities with PD-associated clinical manifestations: age of onset, hallucination, dyskinesia, and

PD stage, and with the PD-associated therapeutic features: DA responsiveness and survival time. We found that most features significantly correlate with striatal DR densities. Striatal D1R density significantly correlates with DA responsiveness, whereas striatal but not the substantia nigra D3R density correlates with survival time. The combination of striatal but not the substantia nigra D1R and D3R (D1R + D3R) is closely correlated with age of onset, PD stage, DA responsiveness, and survival time.

## *3.7 Impact of Striatal D3R Density on Overall Survival*

We randomly grouped the 37 LBD patients by receptor densities (low, medium, and high grade) and performed a Kaplan–Meier survival analysis. We discovered for the first time that the striatal but not the substantial nigra D3R grade and D1R + D3R grade significantly correlated with the survival rate. D1R + D3R density is more helpful in predicting clinical manifestations of LBD patients than D1R or D3R alone (Fig. 5). Although a significant reduction of D2R was observed in the striatum of later-stage LBD patients by comparison with age-matched controls and AD patients, D3R or D1R + D3R densities showed great values reflecting the PD features. Therefore, to precisely determine the patients' stage and design the individualized interventions, we need to systematically measure the densities of D1R, D2R, and D3R in the striatum. These findings are encouraging; nevertheless, the postmortem brains used in our studies endured a prolonged disease course, received differing medications, and died for distinct reasons. Therefore, more advanced studies are needed to validate our postmortem findings in animal models and the clinic setting.

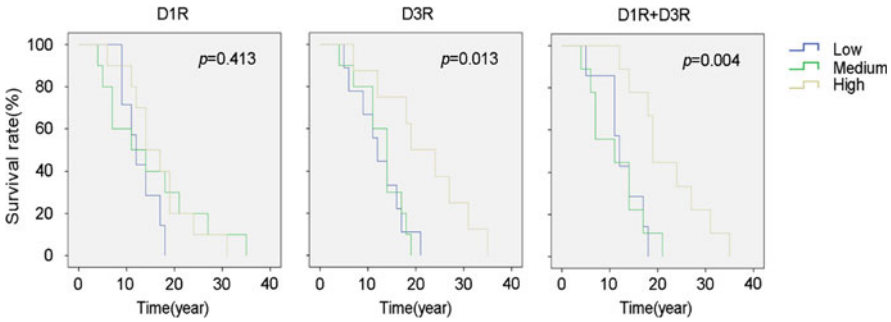


**Fig. 5** Kaplan–Meier survival curves. Relationship between PD progression and the striatal D1R, D3R, and D1R + D3R densities in low-, medium-, and high-density groups. Adapted from (Yang et al. 2021)

## 4 Conclusions and Perspectives

D2R declines in late-stage LBDs and could serve as a pathologic biomarker for PD. However, we found that the D3R or D1R + D3R receptor densities in the striatum correlate with LBD progression and responsiveness to DA treatment in aged LBD patients. These new essential findings warrant further investigations of the role of D3R neurons in PD progression.

Taking collectively, D3R roles as a biomarker for predicting early-stage PD occurrence and later-stage progression, D3R is the critical target for PD interventions. Furthermore, D3R imaging, together with D1R and D2R, will provide helpful information on PD diagnosis, progression, and DA therapy efficacy. To this end, the development of a potent D3R imaging agent for clinical application is in urgent need.

Tremendous progress has been made in understanding the role of DA neurotransmission and D3R functions in the CNS in health and diseases. However, it is still largely unknown on the precise mechanisms and locations along the axons and dendrites where DA activates D3R, the structure, organization, and neuron subpopulations of D3R, the role of glial cells in DA and D3R remodeling, the longitudinal and cross-sectional patterns of DA release and D3R regulations at a single synapse and across large brain areas, and the time scale of DA and D3R modulation on intrinsic neuronal excitability and synaptic plasticity.

The potent antioxidant synoxizyme (previously called carboxyfullerene or $C_3$) was found to salvage nigrostriatal function in nonhuman primates (NHPs) after an intra-carotid infusion of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), a DA neuronal toxin, which models the nigrostriatal injury that occurs in PD. The favorable response to $C_3$ suggests that ROS and oxidative damage contribute to ongoing injury in this NHP model (Dugan et al. 2014). Although fraught with controversy, studies have reported that DA receptor agonists bromocriptine and pramipexole may afford neuroprotection by scavenging ROS (Danzeisen et al. 2006; Muralikrishnan and Mohanakumar 1998). A postmortem study reported that PD patients with amyloid-beta (Aβ) pathology, in addition to cortical synucleinopathy, progress faster (Kotzbauer et al. 2012). This raises questions about whether those with Aβ have different pathologic changes (such as more inflammation) underlying their speedier progression. Our recent immunohistochemistry (IHC) and translocator protein (TSPO) autoradiography studies indicate microglia dystrophy/senescence exists in late-stage PD patients, and astrocytosis in white matter may contribute to demyelination in AD, PD, and normal aging brains (Han et al. 2019; Li et al. 2020; Xu et al. 2019) and that the oxidative insults correlate with DA in the striatum during disease progression (Li et al. 2020). Furthermore, we recently found that TSPO in the putamen correlates with faster disease progression in PD dementia (PDD) patients, that the AD patients with lower striatal 8-oxo-2-′-deoxyguanosine (8-oxo-dG, a DNA damage marker) and myeloperoxidase (MPO, an inflammation marker) levels had a survival advantage, and that 8-oxo-dG in the caudate positively correlates with onset age of DLB patients

(Li et al. 2022; Li and Xu 2020). These important findings suggest that D3R and its function in PD-related neuroinflammation merit in-depth investigation for developing novel PD interventions.

Emerging novel molecular imaging, genetics, bioinformatics, and proteomics techniques, such as super-resolution microscopy, imaging mass spectrometry (IMS) and cytometry (IMC), CRISPR gene editing, cryogenic electron microscopy (cryo-EM), single-cell RNA sequencing, and multi-omics, have already permitted advances and are expected to improve our understanding of DA and its receptor system, DR neuron populations and their roles in disease and health, and facilitate the development of novel D3R pharmaceutical interventions for a variety CNS disorders.

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**Conflict of Interest** The author has no conflicts of interest.

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# Dopamine D3 Receptors: A Potential Target to Treat Motivational Deficits in Parkinson's Disease

**Mathieu Favier, Carole Carcenac, Marc Savasta, and Sebastien Carnicella**

## Contents

1 Beyond the Motor Symptoms of Parkinson's Disease ..... 110
2 Apathy as a Pivotal Symptom in PD ..... 111
3 How to Define Apathy in PD? ..... 111
4 Apathy and its Relation with the Dopaminergic State of PD Patient ..... 113
5 Neurobiological Mechanisms Underlying Apathy: A Pivotal Role of the Dopaminergic System ..... 114
6 How to Disentangle Potential Implication the Mesocorticolimbic and Nigrostriatal Dopaminergic Systems? ..... 115
7 Bilateral and Partial Dopaminergic Lesion of the Nigrostriatal Projection Specifically Affects Motivated and Affective-Related Behaviors ..... 115
8 Pharmacological Reversion of the Behavioral Deficits of SNc-Lesioned Rats Highlights a Critical Involvement of Dopamine $D_3$ Receptor ..... 119
9 Autoradiographic Analysis Reveals Down-Regulation of $D_3R$ in the Dorsal Striatum of SNc-Lesioned Rats ..... 120
10 Intracerebral Infusions of a $D_3R$ Antagonist Confirm the Causal Implication of Dorsostriatal $D_3R$ in the Motivational Deficits of SNc-Lesioned Rats ..... 124
11 Distinct Functional Roles of $D_3R$ in the Dorsal Striatum and Nucleus Accumbens ..... 126
12 Conclusion ..... 128
References ..... 128

**Abstract** Parkinson's disease (PD), which is traditionally viewed as a motor disorder involving the degeneration of dopaminergic (DA) neurons, has recently been identified as a quintessential neuropsychiatric condition. Indeed, a plethora of non-motor symptoms may occur in PD, including apathy. Apathy can be defined as a lack

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of motivation or a deficit of goal-directed behaviors and results in a pathological decrease of self-initiated voluntary behavior. Apathy in PD appears to fluctuate with the DA state of the patients, suggesting a critical role of DA neurotransmission in the pathophysiology of this neuropsychiatric syndrome. Using a lesion-based approach, we developed a rodent model which exhibits specific alteration in the preparatory component of motivational processes, reminiscent to apathy in PD. We found a selective decrease of DA $D_3$ receptors ($D_3R$) expression in the dorsal striatum of lesioned rats. Next, we showed that inhibition of $D_3R$ neurotransmission in non-lesioned animals was sufficient to reproduce the motivational deficit observed in our model. Interestingly, we also found that pharmacologically targeting $D_3R$ efficiently reversed the motivational deficit induced by the lesion. Our findings, among other recent data, suggest a critical role of $D_3R$ in parkinsonian apathy and highlight this receptor as a promising target for treating motivational deficits.

**Keywords** Apathy · Dopamine D3 receptors · Dorsal striatum · Motivation · Non-motor symptoms · Parkinson's disease

## 1 Beyond the Motor Symptoms of Parkinson's Disease

Parkinson's disease (PD) is classically described as a motor disorder (hypokinesia, rigidity, and resting tremor) consecutive to the degeneration of dopaminergic (DA) neurons from the substantia nigra pars compacta (SNc) (Samii et al. 2004). Beyond classical motor symptoms, motivational and affective deficits are frequently observed in PD, dramatically impairing the quality of life of patients (Aarsland et al. 2009a). A wide variety of non-motor symptoms have been described, including sleep disturbance, cognitive impairments, psychosis, anxiety, depression, apathy, or impulsive/compulsive disorders (Aarsland et al. 2009a; Chaudhuri et al. 2006; Voon and Dalley 2011). Fatigue is also common among parkinsonian patients which induces a lack of energy and turns into difficulty initiating and supporting voluntary activity (Chaudhuri and Behan 2004). PD is usually not diagnosed until motor symptoms develop, although non-motor manifestations including depression, sleep problems and loss of smell, typically begin years earlier (Srivanitchapoom et al. 2018). It is now recognized that this cluster of non-motor disorders negatively affects PD patients and their caregivers to the same extent as motor symptoms (Chaudhuri et al. 2006; Chaudhuri and Schapira 2009), making their understanding a central element of therapeutic management.

## 2 Apathy as a Pivotal Symptom in PD

Among neuropsychiatric symptoms, apathy (generally defined as a motivational deficit) appears to be a typical psychiatric feature of PD (Aarsland et al. 2009a; Chaudhuri and Schapira 2009). Apathy is the most common psychiatric disorder in the early stages of untreated PD and can therefore be considered as a hypodopaminergic syndrome, which also includes anxiety and depression (Aarsland et al. 2009b; Shiba et al. 2000). Apathy incidence in PD varies from 16.5 to 70%, depending on the assessment scale used and the population studied (Aarsland et al. 2009a; Dujardin et al. 2008; Starkstein et al. 1992; Brown and Pluck 2000). Most patients affected by PD can exhibit apathy in parallel with the progression of the disease (Aarsland et al. 2009a; Starkstein and Brockman 2011). However, it is difficult to accurately establish the prevalence of apathy in PD, as signs of apathy may overlap with signs of other cognitive dysfunctions (Dujardin et al. 2014). Finally, incidence can vary significantly depending on the non-motor fluctuations: during the off period, up to 75% of patients exhibited apathetic behavior (Fox and Lang 2008; Nissenbaum et al. 1987).

## 3 How to Define Apathy in PD?

A clear definition of apathy is difficult to find in the literature. Apathy is described as a frequent associated feature of PD, like anxiety and depression, two affective impairments that are prevalently associated with apathy in this neurodegenerative disorder (Aarsland et al. 2009a; Chaudhuri et al. 2006; Thobois et al. 2010). Apathy may therefore be regarded as a non-specific symptom, emerging from a general degradation of cognitive functions, with negligible implications for assessment or treatment. However, several pieces of evidence suggest that apathy is a true clinical construct (Marin 1990; Drijgers et al. 2012). Robert Marin in the 1990s defined apathy in neurological disorders as a symptom or a distinct psychiatric syndrome (Marin 1990, 1991). Marin first proposed diagnostic criteria for apathy syndrome based on the construct of deficits in goal-directed behaviors or a primary lack of motivation (Marin et al. 1991). Marin structured the clinical expression of apathy around behavioral, cognitive, and emotional domains that were operationalized as follows:

1. Diminished goal-directed behavior, with a lack of effort, energy, initiative, and productivity.
2. Diminished goal-directed cognition, with decreased interests, lack of plans and goals, and lack of concern about one's personal problems.
3. Diminished emotional concomitants of goal-directed behavior, with a flattened effect and lack of emotional response to positive or negative events.

It was also stated that the symptoms cause clinically significant distress or impairment in social, occupational, or other important areas of functioning.

Since Marin's work, several psychometric scales of apathy were developed, especially for PD (Starkstein et al. 2009; Lhommee et al. 2012; Sockeel et al. 2006). All these scales rely on the behavioral and cognitive dimensions of apathy, but opinions differ on the inclusion of an emotional dimension. For Marin's scale, the term "goal-directed behavior" should be viewed as a "behavior directed toward a goal" and not as a "behavior directed by a goal", as the second has a strong theoretical connotation referring to a specific psychobiological process and putative functional sub-compartmentalization of the dorsal striatum (Yin and Knowlton 2006; Belin et al. 2009).

Levy and Dubois then proposed that the term motivation should not be used to defined apathy, pointing out that such definition would be a psychological interpretation of a behavioral change (Levy and Dubois 2006). Instead, they defined apathy as a quantifiable behavioral syndrome, consisting of a quantitative reduction of self-generated voluntary and purposeful behaviors. This aspect of lack of self-generated action is pivotal, as apathetic patients are able to function properly and to perform daily activities if they receive repeated external stimulation (Levy and Dubois 2006; Isella et al. 2002), indicating a clear deficit in their capacity to initiate and maintain behaviors toward a specific goal. Levy and Dubois also suggested that apathy should not be considered as a unique syndrome, but rather proposed multiple forms of apathetic states subscribing different dysfunctions of the cortico-striatal circuits involved in the chain of processes, from intention to action (Levy and Dubois 2006). Thus apathy could be related to a) disruption of emotional and affective processing (the reduction of goal-directed behaviors is due to an inability to associate these signals with ongoing and forthcoming behaviors), b) alteration of cognitive processing (impairment of the cognitive functions needed to plan and carry out goal-directed behaviors), or c) an auto-activation deficit (difficulties in activating thoughts or initiating the motor programs necessary to perform the behavior) (Levy and Dubois 2006). Accordingly, in an elegant study using an implicit incentive task, Schmidt and colleagues reported that apathetic patients (in a PD and non-PD population) show a strong alteration in the motivational processes responsible for translating an expected reward into effort and action, but no change in the perception of reward value (Schmidt et al. 2008). Specifically, apathetic patients were unable to modulate hand-grip force in order to obtain a monetary incentive, in function of its size, while their sensitivity to the relative value of the incentive was preserved (Schmidt et al. 2008). Hence, these data and recent clinical observations reveal that apathetic state in PD may be specifically linked to the anticipatory component of anhedonia, which is a lack of association between pleasure and a specific action, without any change in consummatory responses reflecting the capacity of patients to experience pleasure when engaged in an enjoyable activity (Loas et al. 2012; Der-Avakian and Markou 2012).

Overall, these studies indicate that at least some forms of apathy in PD are related to dysfunctions of preparatory, but not consummatory, subcomponent of motivated behaviors.

## 4 Apathy and its Relation with the Dopaminergic State of PD Patient

Apathetic symptoms as well as mood disorders such as depression or anxiety are often reported even before the onset of motor symptoms, or early in the disease, in untreated PD patients (Aarsland et al. 2009a, b; Pedersen et al. 2009; Poewe 2008). Apathy incidence and severity are also typically rising in patients undergoing surgical treatment of PD (deep brain stimulation of the subthalamic nucleus, STN-DBS) (Starkstein and Brockman 2011; Houeto et al. 2002), particularly in cases of strong reduction of DA medication (Thobois et al. 2010). At the opposite, apathetic symptoms can be treated at different stages of the disease using the DA precursor levodopa or DA $D_2/D_3$ receptor ($D_2/D_3R$) agonists, such as ropinirole or pramipexole (Chaudhuri and Schapira 2009; Thobois et al. 2010; Czernecki et al. 2002, 2008; Ishizaki and Mimura 2011; Leentjens et al. 2009; Volkmann et al. 2010). These clinical observations therefore indicate that, at least some forms of apathy in PD depend on the DA state of the patients, suggesting an important role of DA neurotransmission in the pathophysiology of this non-motor symptom (Thobois et al. 2010; Volkmann et al. 2010; Krack et al. 2010). In line, functional imaging studies in humans have reported an association between PD-related apathy, as well as anxiety and depression, and the extent of DA denervation in several brain regions, including the ventral and dorsal striatum and the prefrontal cortex (Thobois et al. 2010; Weintraub et al. 2005; Remy et al. 2005).

Thereby, the resurgence of apathy observed during STN-DBS might be the consequence of a DA withdrawal-like syndrome, secondary to the reduction of pharmacotherapies (Thobois et al. 2010). However, as previously described, several forms of apathy resulting from different neurobiological dysfunctions exist, and at least two types of apathy can be observed in PD: (1) a fluctuating apathetic state directly related to a hypodopaminergic state resulting from the DA neuronal loss, and that can be revealed by the decrease in DA medication associated with STN-DBS, and (2) a more protracted apathy, resistant to DA medication and observed in the chronic stage after surgery (Starkstein and Brockman 2011; Drapier et al. 2006; Voon et al. 2006). Some researchers have proposed that postoperative apathy may be directly linked to STN-DBS (Starkstein and Brockman 2011; Volkmann et al. 2010; Drapier et al. 2006). In two longitudinal studies, Verin and coworkers found no association between the increase of apathy following STN-DBS surgery and the reduction in the DA medication dose, arguing against the aforementioned hypothesis (Drapier et al. 2006; Le Jeune et al. 2009; Kirsch-Darrow et al. 2011). In Drapier's study, the stimulation electrodes were placed more ventrally in the STN of apathetic than non-apathetic DBS patients (Drapier et al. 2006), suggesting that apathy could be due to the diffusion of electric current to the limbic sub-compartment of the STN (Le Jeune et al. 2009). In addition, other neurotransmitters than DA may be involved in the pathogenesis of apathetic symptoms, including the well-described degeneration of noradrenergic and serotoninergic systems in PD patients (Castrioto et al. 2016).

The pathogenesis of apathy in PD remains difficult to decipher. For instance, there are no clear mechanisms of action of DBS on the basal ganglia loops that could account for a direct effect on mood and motivation (Krack et al. 2010; Temel et al. 2009). Moreover, it is almost impossible to clearly distinguish the role of the DA denervation and the effect of DBS since it has been reported that STN-DBS also directly impacts DA function (Deniau et al. 2010; Carcenac et al. 2015). Nevertheless, exploring this question with preclinical approaches makes it possible to separately examine potentially interacting factors. In the laboratory, we recently showed that chronic STN-DBS in rats diminishes reward seeking and basal activity in both control animals and in a model of PD-neuropsychiatric symptoms, without any DA medication (Vachez et al. 2020). Thus, our study provides evidence that STN-DBS by itself could promote loss of motivation reminiscent to apathy in PD. Of note, we also demonstrated that pramipexole was effective to reverse the STN-DBS motivational deficit, arguing for a mechanism involving $D_2/D_3$ receptors, in line with our previous observation of a decreased expression of these receptors under acute STN-DBS (Carcenac et al. 2015).

## 5 Neurobiological Mechanisms Underlying Apathy: A Pivotal Role of the Dopaminergic System

Most studies have linked apathy, and its related affective disorders, to the DA denervation of the mesocorticolimbic system occurring in PD patients (Krack et al. 2010; Agid et al. 1984). Clinically, estimation of DA neuronal loss within the VTA in post-mortem studies varied from 30 to 60% (Tong et al. 2000), leading to a ~ 50% DA denervation of the ventral head of the caudate nucleus of PD patients in the late stages of the disease (Kish et al. 1988). Interestingly, Torack and Morris (Torack and Morris 1988) found a partial DA loss in the mesolimbic system exclusively in depressed, but not in non-depressed PD patients. Functional imaging studies also suggest that apathy, anxiety, and depression in PD are associated with a DA hypofunction within the ventral striatum (Thobois et al. 2010; Remy et al. 2005). Using complete mesolimbic lesions in animal models, studies have shown a reduction of motivated behaviors (Le Moal and Simon 1991; Nieoullon and Coquerel 2003), consistently with the pivotal role of DA mesolimbic system in motivational processes (Wise 2004; Salamone et al. 2016).

On the other hand, several data suggest that the partial loss of DA neurons in the VTA is not severe enough, especially in the early stages of the disease, to induce strong neuropsychiatric symptoms such as apathy (Levy and Dubois 2006). Instead, parkinsonian apathy could arise directly from the loss of DA neurons in the SNc, the main nucleus initially affected in the disease. In fact, beyond its role in motor functions, the DA nigrostriatal system is actually strongly implicated in the control of motivated behaviors (Yin and Knowlton 2006; Belin et al. 2009; Belin and Everitt 2008; Bromberg-Martin et al. 2010; Palmiter 2008). Strikingly, depression and

anxiety symptoms have been also found specifically associated with a greater DA denervation affecting the putamen of PD patients (Weintraub et al. 2005).

## 6 How to Disentangle Potential Implication the Mesocorticolimbic and Nigrostriatal Dopaminergic Systems?

In the laboratory, we developed a lesion-based model using stereotaxic bilateral injections of the neurotoxin 6-hydroxydopamine (6-OHDA) into discrete areas of the rat SNc or VTA, to selectively induce degeneration of the DA nigrostriatal and/or mesocorticolimbic systems, respectively. SNc and VTA 6-OHDA lesions resulted in distinct, non-overlapping, complementary patterns of DA denervation (Fig. 1) and DA loss throughout striatal territories (Drui et al. 2014; Favier et al. 2014). Importantly, infusion of the neurotoxin can lead to a 40–60% tyrosine hydroxylase immunoreactivity (TH-IR) loss in the nucleus accumbens (NAc), reproducing the partial denervation of the ventral striatum observed in PD (Kish et al. 1988). Our study was aimed at determining whether such limited DA loss would impact motivational processes.

The SNc lesions also led to a partial DA denervation in the dorsal striatum (Fig. 1). The loss of TH-IR was predominant in the lateral striatal portion, but remained below 80% (Drui et al. 2014), leading to a 70% decrease in basal extracellular DA levels in the dorsal striatum, with no changes in the NAc (Favier et al. 2014). This partial denervation was crucial for preventing the severe alterations of the motor functions that usually occur for denervation around or above 80% (Kirik et al. 1998; Brizard et al. 2006). This approach allowed us to study specifically the role of the nigrostriatal DA system in motivational and affective processes, in the absence of a potential bias related to major locomotor impairments. Using an exhaustive battery of tests, we consistently found that our partial DA SNc lesion did not induce significant motor alterations (Drui et al. 2014; Favier et al. 2014).

## 7 Bilateral and Partial Dopaminergic Lesion of the Nigrostriatal Projection Specifically Affects Motivated and Affective-Related Behaviors

Because it is suggested that apathy in PD is related to preparatory, but not consummatory, behavioral deficits, we used various non-operant and operant tasks to distinguish between the potential effects of the lesions on these two sub-components of motivated behaviors.

We showed that bilateral and partial DA lesions of the SNc, but not of the VTA, dramatically impaired operant responding for obtaining a sucrose solution (Fig. 2).

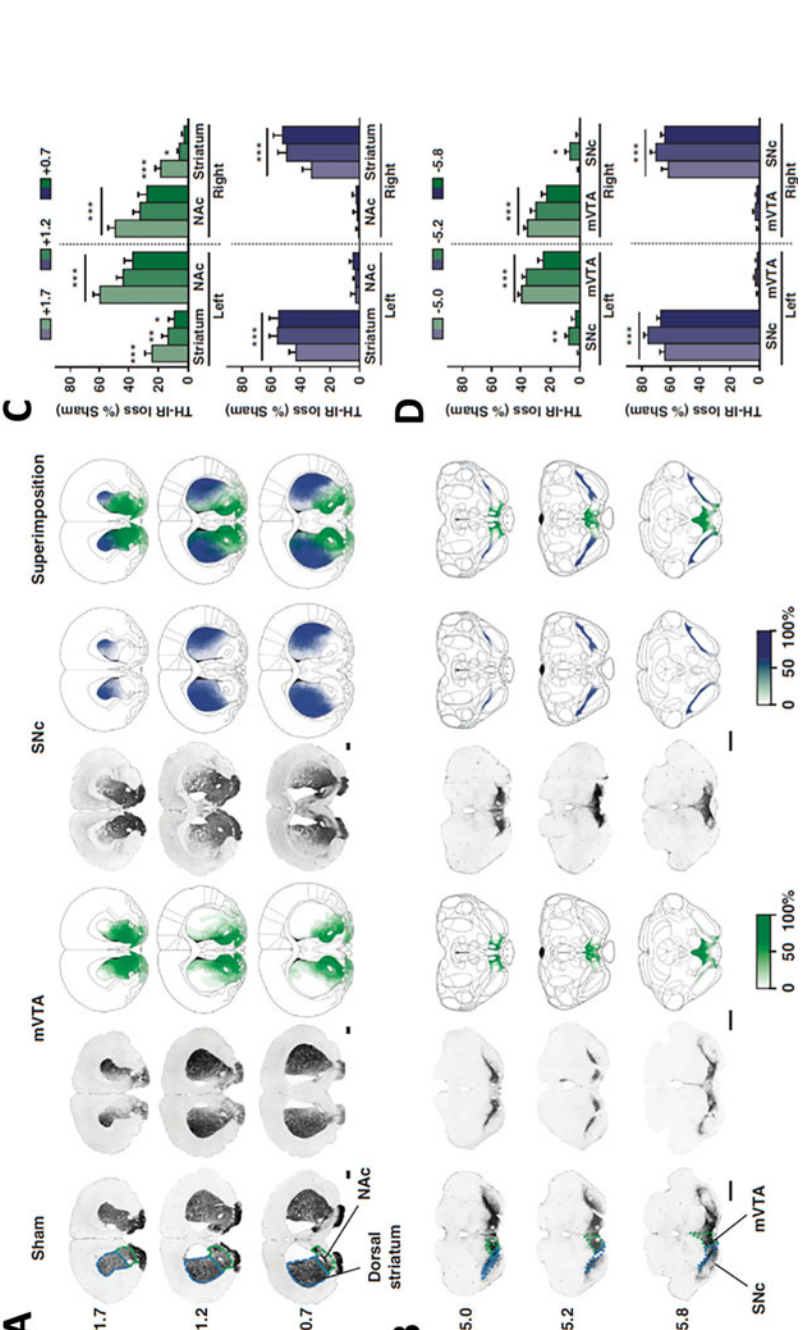


**Fig. 1** Bilateral partial lesions of the mVTA or SNc result in distinct, non-overlapping, complementary patterns of dopaminergic (DA) depletion throughout striatal territories. (**a**, **b**) Representative photomicrographs of coronal sections stained for tyrosine hydroxylase (TH) in striatal (1.7 to 0.7 mm anterior to bregma) (**a**) and mesencephalic (−5 to −5.8 mm anterior to bregma) (**b**) regions according to the stereotaxic atlas of Paxinos and Watson. Bar: 1 mm. The intensity of the gradient of color (white to green or white to blue) in schematic sections corresponds to the measured DA-lesioned area in the different brain structures studied for

each lesion performed. mVTA lesion is shown in green and SNc lesion in blue. The highest intensity of green or blue color (100%) indicates that all animals had lesions in the corresponding area, whereas the lowest color intensity (white, 0%) corresponds to a non-lesioned or denervated area. (**c**, **d**) Quantification of the loss of TH staining in the different mesencephalic (**c**) and striatal (**d**) structures, expressed as percentage of the mean value obtained for sham-operated animals. Two-way ANOVAs revealed significant interactions between the lesion and the brain region considered (Fs $> 9.49$, Ps $< 0.001$). $n = 22$–28, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. *IR* immunoreactivity, *mVTA* medial ventral tegmental area, *NAc* nucleus accumbens, *SNc* substantia nigra pars compacta. From Drui et al. (2014)

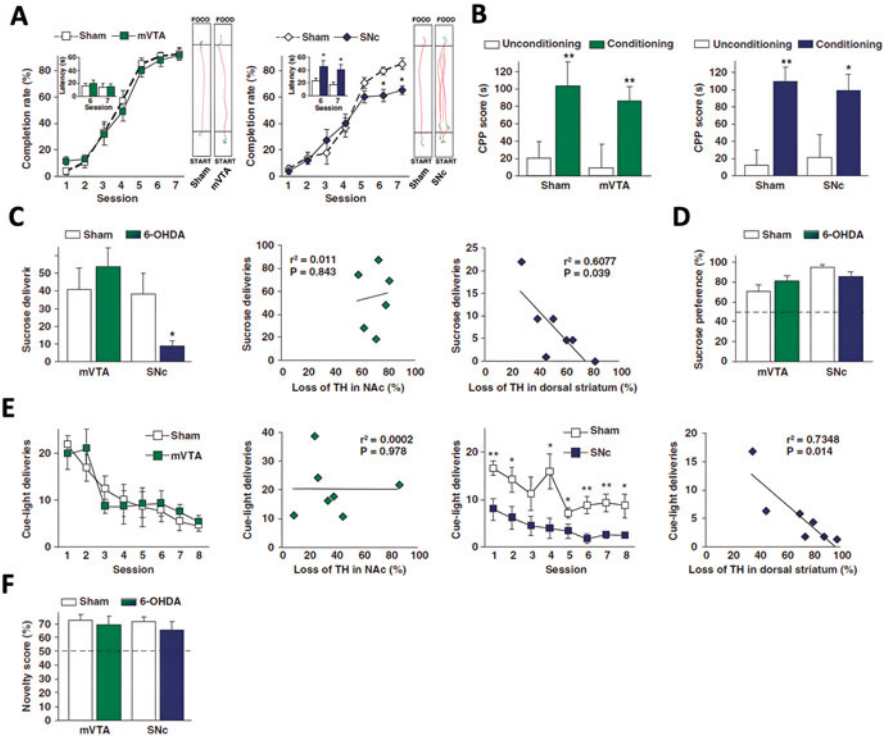


**Fig. 2** Bilateral partial 6-hydroxydopamine (6-OHDA) lesions of the substantia nigra pars compacta (SNc), but not of the medial ventral tegmental area (mVTA), impair motivated behaviors. (**a**, **b**) SNc (lesion × session interaction: $F_{6,108} = 2.65$, $P < 0.02$, $n = 19$–$22$) but not mVTA (lesion x session interaction: $F_{6,66} = 0.84$, $P = 0.55$, $n = 12$–$15$) lesions increased the latency to reach a palatable food in a runway paradigm at the asymptotic level (**a**), with no incidence on conditioning place preference (CPP) for the same reward (**b**, effect of conditioning: $F_s > 11.61$, $P_s < 0.001$, no effect of lesion: $F_s < 0.50$, $P_s > 40.48$ and no interaction: $F_s < 0.16$, $P_s > 0.69$, $n = 10$–$17$). (**c**, **d**) SNc (*$P < 0.05$) but not mVTA ($P = 0.41$) lesions decreased operant sucrose self-administration (**c**, representation of the mean of the three last fixed ratio 1 sessions (first graph) and linear regressions between sucrose deliveries and the loss of tyrosine hydroxylase (TH) in the nucleus accumbens (NAc) (second graph) and the dorsal striatum (third graph) for mVTA and SNc lesions, respectively, $n = 6$–$9$), while having no significant effect on sucrose preference in a two-bottle choice procedure (**d**, $P_s > 0.08$, $n = 12$–$19$). (**e**) SNc but not mVTA lesions (effect of lesion: $F_{1,98} = 14.1$, $P < 0.01$ and $F_{1,91}.0.01$, $P = 0.94$, respectively, $n = 7$–$8$) reduced self-activation of a cue-light during an operant procedure. Linear regressions between cue-light deliveries and the loss of TH in the NAc and the dorsal striatum for mVTA and SNc lesions, respectively. (**f**) No effect of the 6-OHDA lesions was found on the preference for a novel environment ($P_s > 0.73$), $n = 6$–$8$. *$P < 0.05$, **$P < 0.01$, sham-operated vs lesioned. From Drui et al. (2014)

The absence of effect of the partial DA mesocorticolimbic lesion on motivation was confirmed under a progressive-ratio schedule of reinforcement (Drui et al. 2014). This result is in line with other data showing that complete lesions of the mesocorticolimbic system are necessary to decrease motivated behaviors (Le Moal

and Simon 1991; Nieoullon and Coquerel 2003). In addition, we found a robust negative correlation between operant performances and the loss of TH-IR within the dorsal striatum (Fig. 2), strongly supporting the implication of the DA nigrostriatal system in motivational processes.

The reduced behavioral response of SNc-lesioned animals could not be attributed to an impairment in instrumental learning since their capacities to discriminate between a reinforced and non-reinforced lever were preserved (Drui et al. 2014). Moreover, this behavioral deficit was observed after the full acquisition of the instrumental task, confirming that a learning impairment did not account for this effect (Favier et al. 2014). Furthermore, the reduced behavioral response of SNc-lesioned rats could not be attributed to a decrease in the sensitivity to the rewarding properties of sucrose since rodents demonstrated a clear preference for the sucrose solution in a two-bottle choice procedure (Fig. 2). This indicates that partial DA SNc lesions do not affect hedonic, consummatory processes, but rather selectively alter preparatory behaviors. This selective effect of the SNc DA lesion on preparatory behaviors was also confirmed in two other operant tasks, namely the runway task and cue-light self-administration. In a runway task (evaluating the progressive reduction in the latency to reach a palatable food at the end of a straight alley), acquisition was similar for all groups but SNc DA lesions exhibited decreased asymptotic performance compared to controls (Fig. 2). This indicates that motivation to reach the goal but not learning was affected by the DA SNc lesion. Finally, cue-light acted as a robust reinforcer in both VTA-lesioned and controls animals, but not in SNc-lesioned rats (Fig. 2).

In contrast, neither VTA nor SNc lesions impaired preference for a novel environment in a non-instrumental novelty place preference procedure (Fig. 2). The marked motivational deficit observed in animals with partial DA SNc lesions was only present when an instrumental preparatory action was required.

As aforementioned, apathy in PD is frequently associated with anxiety and depression. We then also investigated affective-related behaviors. We found that SNc-, but not VTA-, lesioned rats displayed reduced social interaction, anxiety-related behaviors in an elevated plus-maze, and a dark/light avoidance test, as well as a depression-related behavior, as reflected by an increase in the time spent immobile in a forced-swim test (Drui et al. 2014).

## 8 Pharmacological Reversion of the Behavioral Deficits of SNc-Lesioned Rats Highlights a Critical Involvement of Dopamine $D_3$ Receptor

The various functions of DA are mediated by different DA receptor subtypes and depend on their neuronal and brain localizations (Beaulieu et al. 2015). Moreover, expression and function of these DA receptors can be strongly affected by DA denervation, with potential pathophysiological implications in PD (Rangel-Barajas

et al. 2015; Hurley and Jenner 2006). To further examine the role of DA in our rodent model of parkinsonian apathy, we tested whether sub-chronic systemic administration of different pharmacological DA agents classically used in PD could correct the behavioral impairments induced by partial DA SNc lesions.

Anxiety- and depressive-like symptoms displayed by SNc-lesioned rats were fully reversed by L-Dopa and the $D_2/D_3R$ agonist ropinirole, as indicated by a reversal of the reduction in time spent in the open arms of the elevated plus-maze and immobility in the forced-swim test (Fig. 3) (Drui et al. 2014). In addition, ropinirole was the only pharmacological agent that significantly improved instrumental performances of SNc-lesioned rats under a fixed- or a progressive-ratio of reinforcement, in an operant sucrose self-administration procedure (Fig. 3 and (Drui et al. 2014)). The efficacy of $D_2/D_3R$ agonists in reversing the motivational deficits induced by the SNc lesions was confirmed with the use of pramipexole in the same operant procedure (Favier et al. 2014). Notably, discontinuation of pramipexole treatment induces the resurgence of motivational deficits (Favier et al. 2014), thereby mimicking the re-emergence, or worsening, of apathetic symptoms when DA medication is reduced or withdrawn in PD patients (Thobois et al. 2010).

The beneficial effects of $D_2/D_3R$ agonists on the motivational deficits induced by DA SNc lesions are likely to be mediated specifically by the $D_3R$ subtype. Indeed, while sub-chronic administration of a $D_1R$ (SKF-38393), $D_2R$ (Sumanirole), or $D_3R$ agonist (PD-128907) fully reversed the anxiety- and depression-related behaviors induced by the SNc lesions (Fig. 3 and Carnicella et al. 2014), only the $D_3R$ preferential agonist reversed the deleterious impact of the SNc lesion on operant sucrose self-administration (Fig. 3) The absence of effect of SKF-38393 and Sumanirole was particularly striking, as both agonists dose-dependently improved the instrumental performances of sham animals (Fig. 3), indicating that the lack of effect was most likely not due to insufficient dosage. Moreover, the effect of PD-128907 in SNc-lesioned animals was selectively blocked by the $D_3R$ antagonist SB-277011A, but not the $D_2R$ antagonist L-741,626 (Carnicella et al. 2014), confirming the $D_3R$-mediated action of the agonist.

Taken together, these data strongly suggest a pivotal role of $D_3R$ in motivational processes. This is consistent with earlier evidence that this receptor contributes to the control of affective and motivated behaviors and mediates the therapeutic effect of DA medication on the neuropsychiatric symptoms in PD (Sokoloff et al. 2006).

## 9 Autoradiographic Analysis Reveals Down-Regulation of $D_3R$ in the Dorsal Striatum of SNc-Lesioned Rats

Using our rodent model reminiscent of parkinsonian apathy, we performed a semi-quantitative autoradiographic analysis to evaluate the changes of $D_1$, $D_2$, and $D_3$ receptors ($D_1R$, $D_2R$, and $D_3R$) expression induced by SNc lesion (Fig. 4) (Favier et al. 2014). We found a selective decrease in $D_3R$ levels within the dorsal striatum

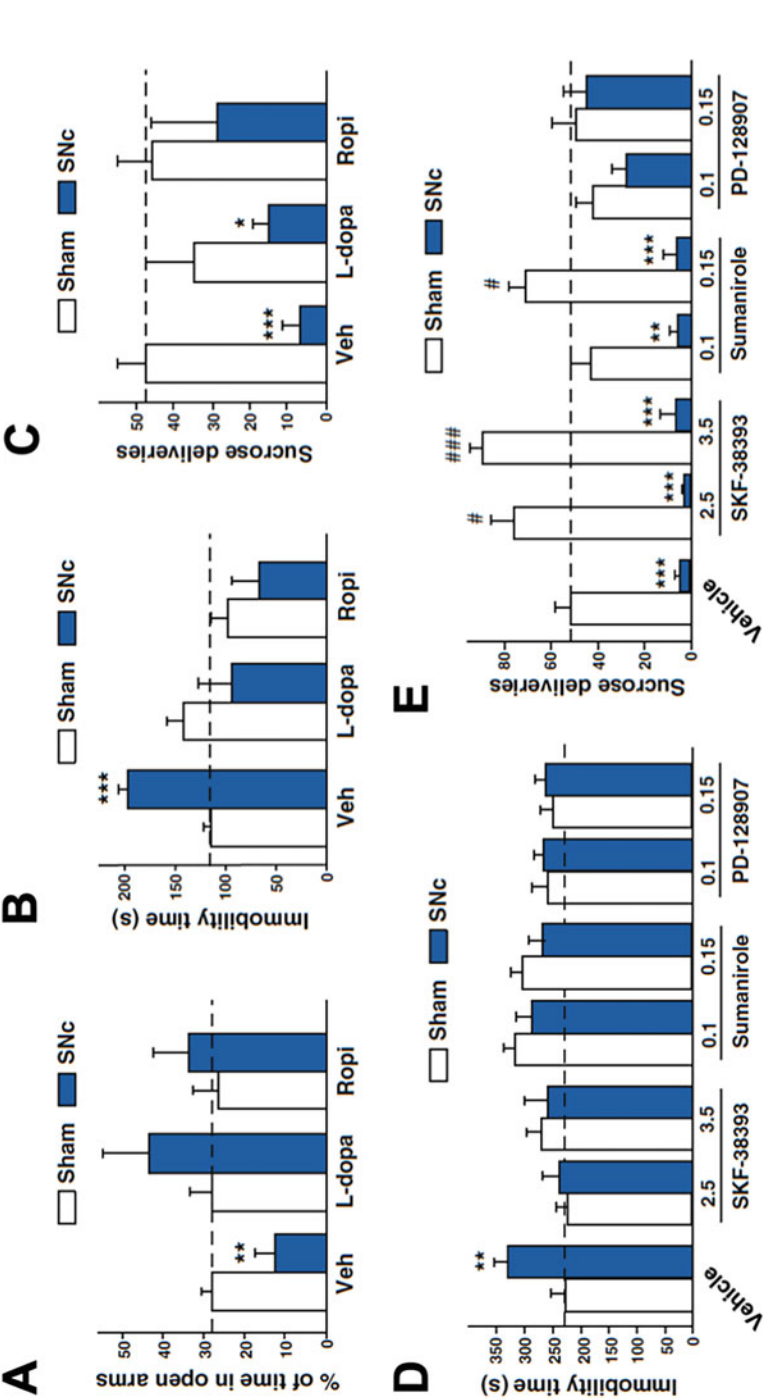


**Fig. 3** Pharmacological reversion suggests a pivotal role of $D_3R$ in motivational deficits of SNc-lesioned rats. (**a–e**) Effects of i.p. sub-chronic administration of L-Dopa (12.5 mg/kg), Ropinirole (Ropi, 1 mg/kg) or vehicle (veh) were evaluated in an elevated plus-maze (**a**), a forced-swim test (**b**) and in an operant sucrose self-administration procedure (**c**), $n = 6–11$. (**d–e**) Effects of i.p. sub-chronic administration of SKF-38393 (2.5 and 3.5 mg/kg), Sumanirole (0.1 and 0.15 mg/

of SNc-lesioned rats. Neither $D_1$ nor $D_2$ receptors expression was modified by the nigrostriatal lesion. In physiological conditions, $D_3R$ expression is high in the NAc shell, intermediate in the NAc core and lower, but detectable, in the dorsal striatum (Bouthenet et al. 1991; Jeanblanc et al. 2006). Due to this low expression, the study of the possible modification of $D_3R$ expression in the dorsal striatum following PD-related DA lesions remains challenging and has given conflicting results. For instance, previous studies reported a decrease or no change in dorsostriatal $D_3R$ expression in DA-depleted rats, depending on the method of detection and analysis (Bezard et al. 2003; Guillin et al. 2001; Morissette et al. 1998). In this study, using an adapted protocol to detect changes of expression in brain areas where $D_3R$ expression is low (Kung et al. 1994; Stanwood et al. 2000), we demonstrated that $D_3R$ is specifically downregulated in the DLS after bilateral SNc 6 OHDA-lesion (Fig. 4).

In the striatum, $D_3R$ can mediate the action of DA as a post-synaptic receptor or exert an inhibitory effect on DA release as a putative pre-synaptic receptor (Beaulieu et al. 2015). However, $D_3R$ autoradiographic binding and mRNA levels are highly colocalized in the striatum, thereby suggesting that these localizations primarily correspond to dendrites or soma of striatal neurons rather than to long axon terminals from distant neurons (Levesque et al. 1992) and the decreased $D_3R$ expression in the present study is associated with a phenotype related to a loss of DA function (observed in SNc-lesioned rats), reproduced by the pharmacological blockade of dorsostriatal $D_3R$. Thus, our data suggest that the loss of $D_3R$ was due, at least in part, to changes of expression in post-synaptic neurons. The implication of the $D_3R$ subtype in PD-related non-motor deficits has rarely been investigated in animals (Moraga-Amaro et al. 2014). Because modulation of the $D_3R$ function influences motivated behaviors (Le Foll et al. 2005; Beninger and Banasikowski 2008), we hypothesized that downregulation of this receptor within the dorsal striatum may participate in the motivational deficits induced by the DA SNc lesion.

**Fig. 3** (continued) kg), PD-128907 (0.1and 0.15 mg/kg), or vehicle were evaluated in a forced-swim test (**d**) and in an operant sucrose self-administration procedure (**e**), $n = 8$–21. Data are expressed as mean Å} SEM. Dotted lines represent the mean of the behavioral performances of vehicle-treated sham animals. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$, sham-operated versus lesioned within the same treatment and #$p < 0.05$, ###$p < 0.001$ between the treatments for sham-operated and lesioned conditions, respectively. *SNc* substantia nigra pars compacta, *TH* tyrosine hydroxylase, *i.p.* intraperitoneal. Adapted from Drui et al. (2014) and Carnicella et al. (2014)

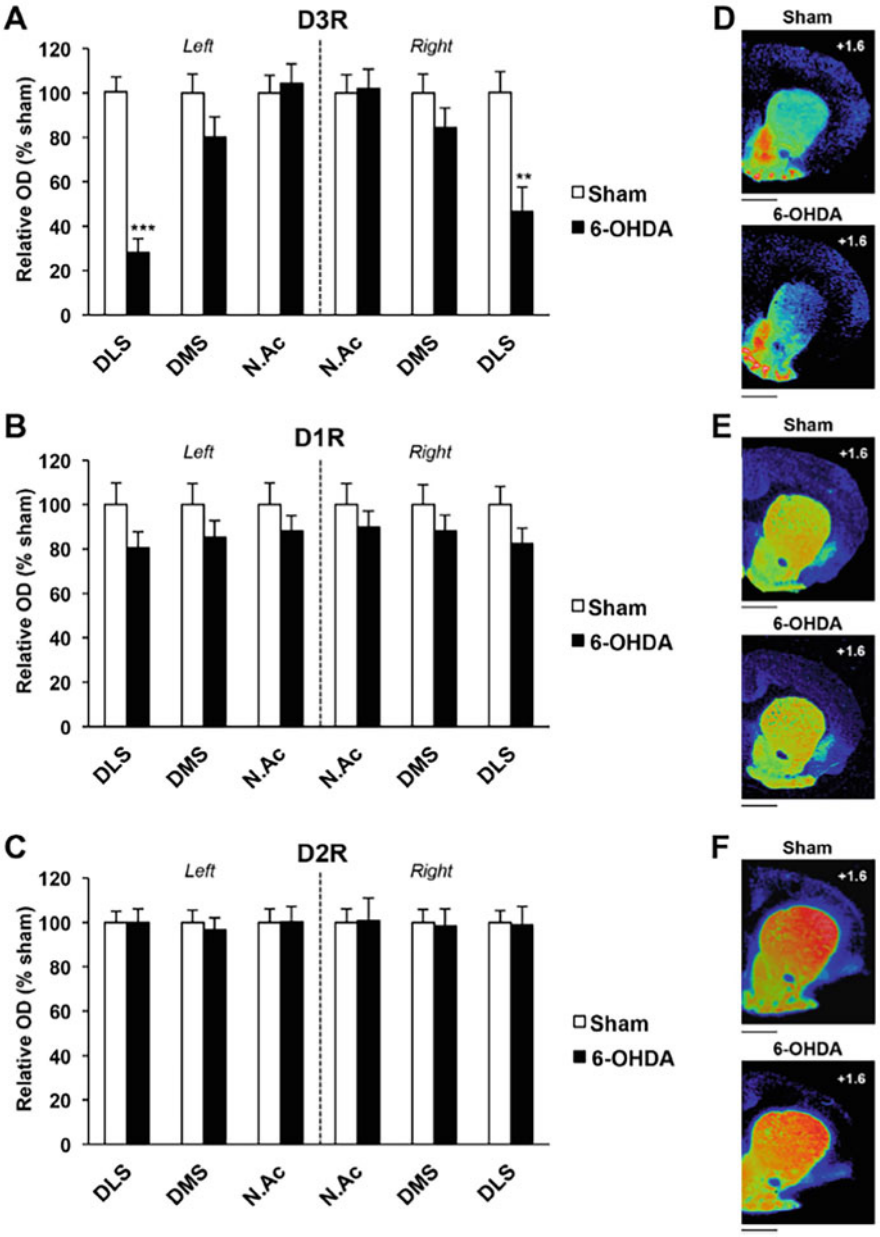


**Fig. 4** Bilateral 6-OHDA SNc lesion induces a selective decrease of $D_3R$ expression in dorsolateral striatum. (**a–c**), Mean ± SEM optical density (expressed as arbitrary units) of $D_3R$, $D_1R$ and $D_2R$ receptor binding density at striatal level, as measured by semi-quantitative autoradiography in sham and 6-OHDA lesioned rats. SNc lesions induced a marked decrease of $D_3R$ binding, specifically within the dorsolateral striatum. Two-way ANOVAs and post-hoc analyses with the method of contrasts were used. $^{**}p < 0.01$, $^{***}p < 0.001$, sham ($n = 8$) vs 6-OHDA ($n = 7$). *DLS* dorsolateral

## 10 Intracerebral Infusions of a $D_3R$ Antagonist Confirm the Causal Implication of Dorsostriatal $D_3R$ in the Motivational Deficits of SNc-Lesioned Rats

In order to test the hypothesis, we decided to functionally reproduce the decrease in expression of $D_3R$ in control rats. We found that pharmacological blocking of the $D_3R$ within the dorsal striatum, but not in the NAc, in non-lesioned rats mimicked the behavioral deficits induced by partial and bilateral SNc DA lesions, suggesting that the motivational deficits observed in SNc-lesioned rats are causally related to the functional downregulation of dorsostriatal $D_3R$. Indeed, our data show that selective blockade of $D_3R$, with the antagonist SB-277011A, within the dorsal striatum in non-lesioned rats impaired operant sucrose self-administration, and specifically the maintenance of the operant response, without affecting the rewarding properties of the reinforcer, as observed in lesioned rats (Fig. 5). In addition, we confirmed that the behavioral effects of SB-277011A were not mediated by $D_2R$, as infusion of a selective $D_2R$ receptor antagonist, L-741,626 (Kulagowski et al. 1996), in the dorsal striatum, had no effect on the operant performances of the animals (Favier et al. 2014).

Although both blockade of $D_3R$ in the dorsal striatum and SNc DA lesions impaired the maintenance of operant behaviors, the effect on operant performance of the latter (Drui et al. 2014; Favier et al. 2014) was stronger than of the former. Moreover, the curves showing the pattern of operant activity across time appear similar, but not identical. In addition to the decrease of $D_3R$ expression revealed by autoradiography in the present study, SNc-lesioned rats also exhibited a dramatic reduction ($-70\%$) of extracellular DA levels in the dorsal striatum (Favier et al. 2014). These cumulative DA dysfunctions in 6-OHDA rats may account for these differences, indicating that pharmacological blockade of dorsostriatal $D_3R$ may reproduce only a part of the deficits induced by the SNc lesion.

Taken together, these data clearly emphasize a critical, while probably non-exclusive, implication of dorsostriatal $D_3R$ in the pathophysiology of motivational deficits in PD patients related to apathy.

**Fig. 4** (continued) striatum, *DMS* dorsomedial striatum, *NAc* nucleus accumbens. (**d–f**) Photographs of autoradiograms obtained at striatal level for sham and 6-OHDA-lesioned rats, colorized using Autoradio V4.03 Software. D, $D_3R$; E, $D_1R$; F, $D_2R$. AP levels are indicated from bregma. Scale: 1 mm. *AP* Anteroposterior. From Favier et al. (2017)

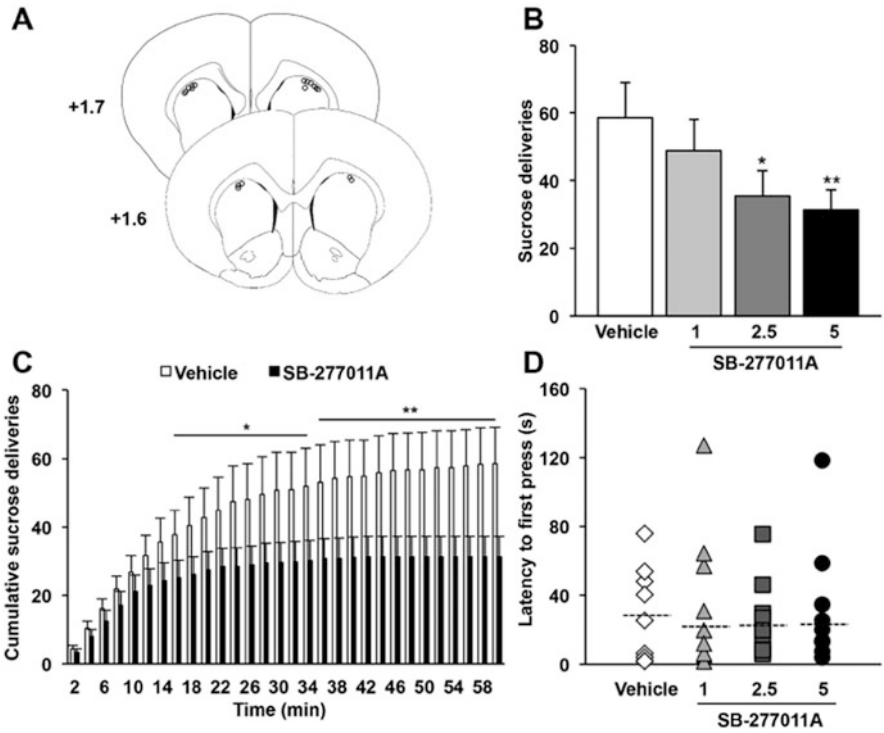


**Fig. 5** Intracerebral infusion into the dorsal striatum of a selective $D_3R$ antagonist decreases operant sucrose self-administration. (**a**) Diagrams showing the locations of each individual guide cannula for the animals included in behavioral experiments ($n = 9$). The circles represent the tips of the microinjectors, visualized on coronal sections counterstained with Cresyl violet. AP levels are indicated in mm from bregma. (**b**) Mean ± SEM number of sucrose deliveries over 60-min sessions. Dorsostriatal infusion of the $D_3R$ antagonist SB-277011A dose-dependently decreased instrumental performance for sucrose self-administration. One-way repeated ANOVA and post-hoc analyses with Student–Newman–Keuls test were used. $*p < 0.05$, $**p < 0.01$, vehicle vs SB-277011A ($n = 9$ per group). (**c**) Mean ± SEM number of cumulative sucrose deliveries within 2-min bins over 60 min sessions. SB-277011A administration (5 μg.μl−1) specifically affected maintaining the instrumental response while no effect was seen during the first part of the procedure. Two-way repeated ANOVA and post-hoc analyses with the method of contrasts were used. $*p < 0.05$, $**p < 0.01$, vehicle vs SB-277011A ($n = 9$ per group). (**d**) Median latencies for the first active lever press (dotted bars) and individual values. SB-277011A administration did not modify the latency for the first active lever press (Friedman test). Each microinjection experiment with vehicle vs SB-277011A was conducted by using a counterbalanced within-subject design. *AP* Anteroposterior. From Favier et al. (2017)

## 11 Distinct Functional Roles of $D_3R$ in the Dorsal Striatum and Nucleus Accumbens

With the notable exception of experimental studies on drugs of abuse and L-Dopa-induced dyskinesia (Jeanblanc et al. 2006; Bezard et al. 2003; Guillin et al. 2001), the role of dorsostriatal $D_3R$ in motivational processes remains poorly investigated because of its low basal level of expression within this area.

We show that infusion of the selective $D_3R$ antagonist in the dorsal striatum specifically alters the maintenance, but not the initiation of operant sucrose self-administration (Fig. 5). This effect is highly reminiscent of the "extinction-like" effect observed after systemic (Wise 2004) or dorsostriatal, but not accumbal, intracerebral infusions of broad-spectrum DA antagonists at moderate doses (Beninger et al. 1993). However, and contrary to the initial postulate of Wise (Wise et al. 1978), a decrease in the hedonic/rewarding effects of sucrose cannot account for this result. Indeed, the effect of the $D_3R$ antagonist on maintenance of operant behaviors was observed even in absence of the reward, and neither consummatory behaviors nor preference for the sucrose solution in a two-bottle choice procedure was affected by the dorsostriatal infusion of the $D_3R$ antagonist. Therefore, our data clearly demonstrate that blocking or reducing $D_3R$ transmission within the dorsal striatum induces a specific deficit in goal-oriented behavior.

According to the concept of incentive salience developed by Berridge and collaborators (Berridge 2007), DA signaling is necessary for transforming the "neutral" perception of a conditioned stimulus, or a goal object at a distance into an attractive incentive capable of eliciting appetitive or instrumental behaviors toward it (wanting). During motivational tasks, incentive salience assignment to reward-related stimuli and actions is maintained or strengthened by the presentation of the reinforcer (i.e., a correct prediction has been made). It is proposed that DA mediates this "reboosting" effect so that the reinforcer and associated cues remain "wanted" at later occasions (Berridge 2007; Berridge and Robinson 1998). Based on this data, we hypothesize that blockade of dorsostriatal $D_3R$ may induce a specific impairment of "reboosting" processes, accounting for the early termination in instrumental activity reported in the present study. Consistently, Howe et al. showed that prolonged tonic DA signals, or "ramps," detected by fast-scan cyclic voltammetry in the dorsal striatum could provide the motivational drive needed to maintain instrumental behavior (Howe et al. 2013). Besides, the dorsal striatum and especially its lateral part have also been shown to mediate the properties of reward-related stimuli to stimulate operant responding (Corbit and Janak 2007). Our findings suggest that, despite a limited level of expression, dorsostriatal $D_3R$ may have a critical functional implication in these DA-mediated processes.

In contrast to the dorsal striatum, infusion of a $D_3R$ antagonist into the NAc did not affect the maintenance of instrumental behaviors, but rather induced a discrete decrease in the rate of responses for sucrose (Fig. 6). The effects related to $D_3R$ blockade in the NAc are consistent with the literature since in operant tasks with low ratio requirement, as in the FR1 procedure used here, only slowing in the rate of

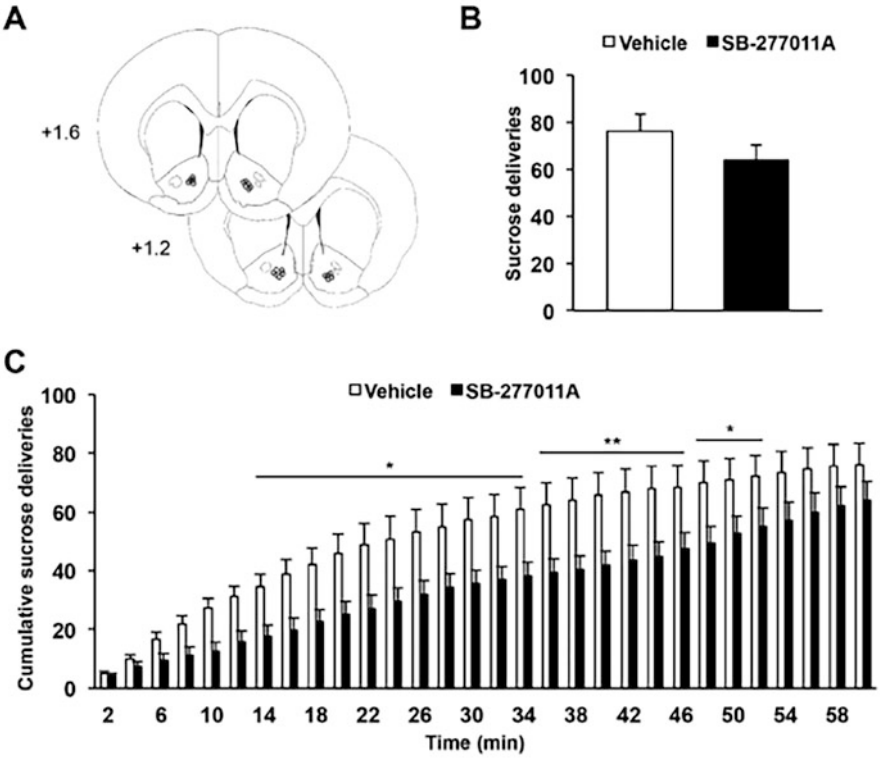


**Fig. 6** Intracerebral infusion of a selective $D_3R$ antagonist into the nucleus accumbens does not reduce operant sucrose self-administration. (**a**) Diagrams showing the locations of each individual guide cannula of animals included in behavioral experiments ($n = 11$). The circles represent the tips of the microinjectors, visualized on coronal sections counterstained with Cresyl violet. AP levels are indicated in mm from bregma. (**b**) Mean ± SEM number of sucrose deliveries over 60 min sessions. (**c**) Mean ± SEM number of cumulative sucrose deliveries within 2 min bins over 60 min operant self-administration sessions. Intra-accumbal infusion of SB-277011A (5 μg.μl–1) did not modify instrumental performance for sucrose self-administration over the entire duration of the test, but instrumental performance of the animals was temporarily decreased in the middle period of the test. Two-way repeated ANOVA and post-hoc analyses with the method of contrasts (cumulative number of sucrose deliveries) or t-test (total number of sucrose deliveries) were used. $^*p < 0.05$, $^{**}p < 0.01$, vehicle vs SB-277011A ($n = 11$ per group). Each microinjection experiment was conducted by using a counterbalanced within-subject design. AP: Anteroposterior. From Favier et al. (2017)

responding and increased tendency to pause are observed after blockade of DA receptors or DA depletion in the NAc (Salamone et al. 2003; Mingote et al. 2005). The differential implication of accumbal and dorsostriatal $D_3R$ in instrumental behaviors and motivational processes deserves further investigation, notably by relying on cue-driven reward-seeking procedures (McGinty et al. 2013; Pecina and Berridge 2013).

## 12 Conclusion

Our work, along with other lesion-based studies (Magnard et al. 2016), selective optogenetic (Ilango et al. 2014; Rossi et al. 2013; Keiflin et al. 2019; Howe and Dombeck 2016; da Silva et al. 2018) and chemogenetic (Goutaudier and Carnicella, unpublished data) modulation of SNc DA neurons, reinforces the role of the nigrostriatal DA system, and specifically the dorsolateral striatum, in motivational processes. The precise role of nigrostriatal DA neurotransmission remains however to be elucidated. A cohort of recent studies suggests that dorsostriatal DA may be more involved in an alerting aspect (response to sensory events that reflect surprise) and instrumental reinforcement, i.e. signaling movement, while that ventrostriatal DA contributes to reward prediction error and reward identity prediction, i.e. signaling reward, as different parts of an actor/critic model (Bromberg-Martin et al. 2010; Howe et al. 2013; Keiflin et al. 2019; Howe and Dombeck 2016; da Silva et al. 2018). Alternatively, the variety of DA signals within the striatum may be interpreted as a modulation of resource allocation decisions. In the dorsolateral striatum the resource would be movement (decide to move or not to move), in the dorsomedial striatum the resources could be cognitive processes (attention and working memory) and in the NAc the resource might be time (engage in a task means that other beneficial ways of spending time must be foregone) (Berke 2018). In addition, our findings emphasize a critical role of dorsostriatal $D_3R$ neurotransmission in the pathophysiology of PD-related neuropsychiatric disorders. Consistently with recent clinical and preclinical data suggesting that stimulation of $D_3R$ may have important beneficial effects in apathy and depression in PD (Leentjens et al. 2009; Carcenac et al. 2015; Carnicella et al. 2014; Pagonabarraga et al. 2015; Favier et al. 2017; Yang et al. 2020; Thobois et al. 2013), our findings clearly designated $D_3R$ as a promising target for the treatment of apathy and other affective disorders in PD patients.

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# Dopamine D3 Receptor, Cognition and Cognitive Dysfunctions in Neuropsychiatric Disorders: From the Bench to the Bedside

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## Contents

1 Introduction ..... 134
2 Dopamine D3 Receptor, Cognition and Cognitive Dysfunctions: Preclinical Evidence ..... 135
3 Dopamine D3 Receptor, Cognition and Cognitive Dysfunctions: Clinical Evidence ..... 137
3.1 Dopamine D3 Receptor and Cognitive Functions in ADHD ..... 138
3.2 Dopamine D3 Receptor and Cognitive Functions in Autism Spectrum Disorder (ASD) ..... 139
3.3 Dopamine D3 Receptor and Cognitive Functions in Schizophrenia ..... 139
3.4 Dopamine D3 Receptor and Cognitive Functions in Alzheimer Disease (AD) ..... 140
3.5 Dopamine D3 Receptor and Cognitive Functions in Bipolar Disorder (BP) ..... 141
4 The Potential Therapeutic Value of Drugs/Ligands Targeting the D3R for the Treatment of Cognitive Dysfunctions ..... 141
4.1 D3R Antagonists in Preclinical Studies ..... 141
4.2 Drugs Targeting D3R in Clinical Studies ..... 142
4.3 Risperidone ..... 144
4.4 Cariprazine ..... 144
5 Conclusions ..... 148
References ..... 148

**Abstract** The dopamine D3 receptor (D3R) plays a prominent role in the modulation of cognition in healthy individuals, as well as in the pathophysiological mechanism underlying the cognitive deficits affecting patients suffering from neuropsychiatric disorders. At a therapeutic level, a growing body of evidence suggests that the D3R blockade enhances cognitive and thus it may be an optimal therapeutic strategy against cognitive dysfunctions. However, this is not always the case because other ligands targeting the D3R, and behaving as partial agonists or biased agonists, may exert their pro-cognitive effect by maintaining adequate level of dopamine in key brain areas tuning cognitive performances. In this chapter, we

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review and discuss preclinical and clinical findings with the aim to remark the crucial role of the D3R in cognition and to strengthen the message that drugs targeting D3R may be excellent cognitive enhancers for the treatment of several neuropsychiatric and neurological disorders.

**Keywords** Cognition · Dopamine 3 receptor · Dopamine 3 receptor antagonists · Pro-cognitive effects · Psychiatric disorders

## 1 Introduction

Cognitive dysfunction is a scarcely controlled but significant feature of neuropsychiatric disorders frequently correlated with a negative impact on quality of life and psychosocial functioning (Bowie and Harvey 2006; Demirtas-Tatlidede et al. 2013; Green 2007; Green et al. 2000, 2004; Millan et al. 2012). Currently available treatments likewise many candidates' pro-cognitive compounds show limited or no effects in ameliorating cognitive alteration (Kaduszkiewicz et al. 2005; McShane et al. 2006; Saddichha and Pandey 2008). Thus, effective treatments for cognitive dysfunction still remain an unmet medical/clinical need. The development of new ligands or the repositioning of already approved drugs for the treatment of cognitive dysfunction should be a fundamental goal to improve the quality of life of neuropsychiatric patients.

Dopamine (DA) modulates several physiological functions including cognition through two classes of DA receptors, the D1-like receptors (D1R and D5R) and the D2-like receptors (D2R, D3R, and D4R), which are G-protein-coupled receptors coupled to Gs and Gi protein, respectively (Leggio et al. 2011, 2015, 2016; Torrisi et al. 2019). Dopaminergic neurons, mainly located in the midbrain, originate four major dopaminergic pathways (Hillarp et al. 1966). Dopaminergic neurons of the ventral tegmental area (VTA) give rise to the mesocortical pathway by innervating the prefrontal cortex (PFC). They also give rise to the mesolimbic pathway sending dopaminergic projections to the ventral striatum. The nigrostriatal pathway consists of dopaminergic projections from the substantia nigra to the dorsal striatum. Lastly, dopaminergic neurons located in the arcuate nucleus of the hypothalamus, which sends projections to the median eminence, create the tuberoinfundibular pathway. DA modulation of bidirectionally interconnected cortico-striatal circuitries is fundamental for the expression of cognitive functions. This modulation appears to be rather complex. For instance, the relationship between dopamine and cognition, mainly in the PFC, follows an inverted U-shaped curve, where either high or low DA levels impair performance in cognitive tasks (Cools and D'Esposito 2011; Kroener et al. 2009). This is further complicated by findings showing variable effects of dopaminergic drugs on cognition of human subjects, being related to their baseline levels of cognitive performance (Gibbs and D'Esposito 2005; Kimberg et al. 1997). According to preclinical studies, these baseline-dependent effects of

dopaminergic drugs may depend on baseline levels of DA in the PFC (Lidow et al. 2003; Zahrt et al. 1997), which hosts a large amount of DA receptors (de Almeida et al. 2008). Although the PFC is the brain region most studied in this field, there is evidence suggesting that DA modulates cognition by acting directly within other brain areas, such as the striatum and the hippocampus (Cools 2011; Darvas and Palmiter 2009; Lodge and Grace 2008, 2011). The striatal hyperdopaminergic state, characterizing schizophrenic patients, has been historically linked to positive symptoms, but may also be responsible for the appearance of cognitive impairments (Simpson et al. 2010). This may be strictly linked to the functional and anatomical connection between the striatum and the cortex.

Therapeutic interventions aim at reversing these dopaminergic dysfunctions may be relevant for treating cognitive dysfunctions. In this context, the dopamine D3 receptor (D3R) seems a promising molecular target for the development of novel pro-cognitive treatments (de Krom et al. 2009; Dubertret et al. 1998; Gross and Drescher 2012; Keefe and Harvey 2012; Loiseau and Millan 2009; Watson et al. 2012a).

The objective of this chapter is to review and discuss preclinical and clinical findings showing dopaminergic mechanisms as key players in the cognitive improvement, to underline the role of the D3R in cognition, and to evaluate potential drugs targeting D3R as candidate cognitive enhancers and/or treatments for cognitive dysfunction associated with several neuropsychiatric disorders.

## 2 Dopamine D3 Receptor, Cognition and Cognitive Dysfunctions: Preclinical Evidence

Dopamine modulates cognition by mainly binding to its receptors located in bidirectionally interconnected cortico-striatal circuitries. In this context, a member of the D2-like family, the D3R, has elicited a great interest. Indeed, multiple findings indicate that D3R-mediated signaling plays an overriding role in the expression of cognitive functions and also that perturbations of the D3R-mediated signaling are strictly linked to cognitive dysfunctions. It is important to remark that, while in the past the D3R was believed to have mainly a subcortical role, nowadays, new technological advancements (transgenic reporter mice (Clarkson et al. 2017)) have demonstrated a cortical localization of the D3R, and thus a role of this receptor in the PFC-related cognitive processes. In particular, Clarkson and colleagues reported that in the layer V of the medial PFC (mPFC), D3Rs are localized in a subset of pyramidal neurons projecting toward both cortical and subcortical areas. This subset of pyramidal neurons appears both electrophysiologically and anatomically different from neighboring neurons expressing D1Rs or D2Rs (Clarkson et al. 2017). In line with these findings, both pharmacological and genetic manipulations of prefrontal D3R are able to affect cognition (Black et al. 2002; Glickstein et al. 2002, 2005; Watson et al. 2012b, 2016). It is however noteworthy that the first evidence of a role

of D3R in cognition came from preclinical studies carried out in the early 2000s, in which the use of the D3R knock-out mice led to encouraging results regarding different cognitive domains. Indeed, except for one study in which D3KO mice showed an impaired spatial working memory (Glickstein et al. 2002), the vast majority of those studies and more recent studies as well revealed an enhanced cognition of D3KO mice. Indeed, D3KO mice, compared to their WT littermates, exhibit better attentional set-shifting, aversive/associative learning, social discrimination, spatial and working memory (Cao et al. 2013; Chourbaji et al. 2008; Glickstein et al. 2002, 2005; Leggio et al. 2021; Micale et al. 2010; Watson et al. 2012a; Xing et al. 2010). Over the years, these promising results have been corroborated by pharmacology studies, in which the use of selective ligands targeting the D3R has produced consistent data. In fact, several D3R antagonists, such as S33084 (Loiseau and Millan 2009), S33138 (Millan and Brocco 2008), SB277011 (Loiseau and Millan 2009), (+)S14297 (Millan et al. 2007), nafadotride (Sigala et al. 1997), RGH-1756 (Laszy et al. 2005), U-99194A (Laszy et al. 2005), RG-15 (Gyertyan et al. 2008), and Y-QA31 (Sun et al. 2016b) work as cognitive enhancers. In addition, the pro-cognitive effect triggered by D3R blockade seems to not depend on age. Millan and colleagues indeed reported that S33138 rescues age-related working memory dysfunction, in old rhesus monkeys assessed in a delayed matching-to-sample task. Among all the above experimental drugs, F17464, a potent D3R antagonist, has intriguingly reached the clinical phase as potential antipsychotic (Krogmann et al. 2019). In rodents, F17464 results more effective in improving scopolamine-induced cognitive dysfunctions than other second-generation antipsychotics (Sokoloff and Le Foll 2017). The pro-cognitive effect of F17464 was demonstrated in a randomized, double-blind, placebo-controlled clinical study involving patients with schizophrenia. Indeed, F17464 showed therapeutic efficacy against positive and negative symptoms and, more importantly, against cognitive dysfunctions, without inducing severe side effects, such as weight gain or extrapyramidal side effects (Bitter et al. 2019). Notably, the therapeutic efficacy of F17464 was correlated to the D3R occupancy, because few hours after the administration of F17464, the D3R occupancy measured in brain was sufficient to account for the therapeutic efficacy, including the pro-cognitive effect. Other studies, by contrast, indicated that the D3R agonists PD128907 (Watson et al. 2012a) and 7-OHDPAT (Bernaerts and Tirelli 2003) produce cognitive impairment. This concept however may change according to the kind of ligand used and also according to the disease investigated. In this respect, more recent studies show that SK609, a selective G-protein biased signaling D3R agonist, ameliorates cognitive deficits in both rodent and non-human primate models of Parkinson's disease (Marshall et al. 2019; Schneider et al. 2021). In this scenario, the D3R partial agonists can make the difference. By definition, partial agonists behave either as agonist or antagonist depending on the surrounding neurotransmitter's level. Thus, D3R partial agonists may result effective on a wide range of cognitive dysfunctions prompted by different D3R-mediated signaling perturbations. According to this view, several studies have remarked the potential therapeutic value of D3R partial agonists in treating cognitive dysfunctions. Laszy and colleagues firstly found that the selective partial D3R

agonist BP-897 attenuates the scopolamine-induced learning deficit (Laszy et al. 2005). A more recent study strengthens this concept by showing the novel antipsychotic candidate TPN672, which is also endowed with D3R partial agonist activity, is able to ameliorate multiple phencyclidine-induced cognitive dysfunctions (Wang et al. 2021). In this context, all the preclinical findings showing the pro-cognitive effects of the antipsychotic cariprazine are worthy of remark. Cariprazine is in fact a D3R/D2R partial agonist, which preferentially binds to D3R. Among antipsychotics acting as partial agonists, cariprazine has the highest affinity for D3R (Calabrese et al. 2020; Pich and Collo 2015). This feature may be linked to the pro-cognitive effect of cariprazine reported in several experimental studies. Cariprazine indeed ameliorated PCP-induced impairments of working memory, attention set-shifting, and recognition memory in wild-type mice, but not in D3R knock-out mice (Zimnisky et al. 2013). Other preclinical studies corroborated this concept by reporting cariprazine as effective against experimental cognitive dysfunctions relevant for schizophrenia (Neill et al. 2016; Watson et al. 2016). Among antipsychotics endowed with high affinity for D3R, blonanserin has further been found to induce a pro-cognitive effect in different experimental studies. Also, for this drug, the pro-cognitive effect appears to depend on the D3R-mediated signaling. A rodent study in fact provided evidence for an involvement of D3R in the beneficial effect of blonanserin on the cognitive impairment obtained by PCP administration. In this study, the pro-cognitive effect of blonanserin was counteracted by a pretreatment with a D3R agonist. It was further observed that the blonanserin-induced cortical-striatal acetylcholine, DA, noradrenaline, and striatal DA efflux as well as the concomitant pro-cognitive effect rely selectively on its antagonism on D3R (Hida et al. 2015). Interestingly, the pro-cognitive effect of this drug can be observed across species. Blonanserin was indeed reported to rescue the D3R agonist-induced executive function deficits in marmosets. It is further important to mention the fact the other commercially available drugs targeting the D3R are able to act as cognitive enhancer. In this respect, our study shows that buspirone counteracted MK-801-induced deficit of temporal order recognition memory, selectively via its D3R antagonism (Torrisi et al. 2017). Importantly, our findings are supported by a clinical study showing buspirone, co-administered with second-generation antipsychotic drugs (SGAs), is more effective than SGAs alone in ameliorating cognitive dysfunctions of patients with schizophrenia.

## 3 Dopamine D3 Receptor, Cognition and Cognitive Dysfunctions: Clinical Evidence

In the previous paragraph, we discuss how D3R affects cognition in preclinical models, here, we want to focalize on the role of D3R in human cognition. In the human brain, D3R mRNA is widely distributed (Suzuki et al. 1998). Several studies demonstrated a high mRNA expression of D3R in the islands of Calleja, the ventral

striatum and the dentate gyrus, brain areas involved in the modulation of cognition, reward, and emotional processes (Meador-Woodruff et al. 1994, 1996; Sokoloff et al. 1990; Suzuki et al. 1998). In humans, the D3R gene (DRD3) is located in the chromosome 3q13.3 (Le Coniat et al. 1991). The most extensively studied polymorphism on D3R is the single nucleotide polymorphism (SNP) in the $Ser^{9}Gly$ (rs6280) that consists in a serine to glycine substitution in the N-terminal extracellular domain of the receptor protein at position 9 (Lannfelt et al. 1992). This polymorphism has been implicated in several psychiatric disorders including autism spectrum disorder (ASD), attention/deficit hyperactive disorder (ADHD), schizophrenia, Alzheimer disease (AD) (Correia et al. 2010; Fageera et al. 2018; Mant et al. 1994). Moreover, drugs showing higher affinity for D3R, compared to D2R, ameliorate cognitive deficits in patients with psychiatric disorders (Joyce and Millan 2005; Nakajima et al. 2013). The role of D3R in psychiatric disorders and especially in cognitive functions, previously underestimated, is now on the rise and several studies are investigating the relationship between D3R and cognition.

## *3.1 Dopamine D3 Receptor and Cognitive Functions in ADHD*

ADHD is one of the most heritably disorders which affects 5% of children and 2.5% of adults all over the world (Castellanos and Tannock 2002; Faraone et al. 2005; Rube 2012; Wilens et al. 2004). The core symptoms of ADHD are high impulsivity and locomotor activity and low attention (Rube 2012). Several studies demonstrated a strong relationship between dopamine dysfunctions and ADHD symptomatology. Dopamine plays a crucial role in modulating some human behaviors related to ADHD, including attention and impulsivity (Bari and Robbins 2013; Nieoullon 2002). The DRD3 has been proposed as a candidate gene for the ADHD etiology. Indeed, mice lacking D3R showed high levels of locomotor activity (Accili et al. 1996); in addition, administration of dopaminergic agonist that selectively binds the D3R reduces locomotor activity in rats (Daly and Waddington 1993). Also, human studies demonstrated that D3R is widely localized in brain regions implicated in cognition processes. A recent pharmaco-behavioral and genetic study performed by Fageera and colleagues (Fageera et al. 2018) provides the first evidence of a relationship between the DRD3 and cognitive deficits in patients with ADHD. In this study, the authors evaluated children with ADHD carrying the D3 polymorphism under three different experimental conditions: 1 week of baseline observation, followed by 1 week of methylphenidate (MPH) and 1 week of placebo. Patients with ADHD were finally tested in the Conner's Global Index scale and in the continuous performance test (CPT). Results reported that children carrying the rs3260 polymorphism, with the Ser/Ser variant, performed worse in the CPT, showing lower levels of attention and vigilance. This variant is over transmitted in people that exhibit higher levels of impulsivity and hyperactivity. In addition, children showing the

Ser/Ser variant were less sensitive to the treatment with MPH, probably because they are less sensitive to the dopamine and are more vulnerable to ADHD and cognitive dysfunction (Fageera et al. 2018). Previous studies (Barr et al. 2000; Muglia et al. 2002) failed to find any relationships between DRD3 polymorphism and ADHD. However, these studies did not analyze cognitive performances. Furthermore, they hypothesize, as a limit of the study, that the loss of association between the D3R polymorphism and ADHD may be ascribed to the small sample size used (Barr et al. 2000; Muglia et al. 2002).

## *3.2 Dopamine D3 Receptor and Cognitive Functions in Autism Spectrum Disorder (ASD)*

ASD is a complex neuropsychiatric disorder with an early clinical onset (1.5–2 years), characterized by repetitive behavior, developmental delay, social and cognitive impairments (Bhat et al. 2014). Evidence about the association between DRD3 and autism arises from chromosomal microarray analysis studies, indeed deletion of 3q13.2-q13 increases the risk to develop autism (Staal 2015). In the last few years, several genetic studies highlighted a relationship between the SNP rs167771 and ASD in British, Dutch, and Spanish patients (de Krom et al. 2009; Staal et al. 2012; Toma et al. 2013), however none of these explores the role of this polymorphism in cognitive abilities. To the best of our knowledge, only Correia and colleagues (2010) investigate the role of $Ser^{9}Gly$ polymorphism on cognition in autistic patients. In this study, the authors evaluate the Autism Treatment Evaluation Checklist (ATEC) in patients under risperidone therapy. In contrast with other scales, the ATEC allows to evaluate both behavioral and cognitive abilities: a decrease score in the ATEC is associated with better performances. Although patients carrying the $Ser^{9}Gly$ polymorphism exhibit lower score in ATEC compared with the SerSer patients, even if, in general, treatment with risperidone was able to ameliorate performance of all patients (Correia et al. 2010). This study represents only the first step to investigate the relationship between D3R and cognition in autistic patients and more data are required.

## *3.3 Dopamine D3 Receptor and Cognitive Functions in Schizophrenia*

Schizophrenia (SZ) is a neurodevelopmental psychiatric disorder with a strong genetic component that affects 1% of population worldwide. The main symptoms of schizophrenia are classified into positive (e.g., hallucinations), negative (e.g., social withdrawal), and cognitive symptoms (e.g., deficits of executive functions, memory and leaning impairments) (van Os and Kapur 2009). The association

between the D3R and schizophrenia is hypothesized because in a post mortem study, Gurevich et al. (1997) found a higher expression level of D3R in the brain of untreated patients compared to control and patients treated with antipsychotic (Gurevich et al. 1997). In addition, recent positron emission tomography (PET) studies with the selective D3R agonist [11C]-(+)-PHNO revealed that after the treatment with antipsychotic, schizophrenic patients show increased baseline of endogenous DA levels in the dorsal striatum at D2/3R (Caravaggio et al. 2015; Girgis et al. 2015; Graff-Guerrero et al. 2009). However, the relationship between D3R and cognitive performance in schizophrenic patients has been poorly investigated (Leggio et al. 2021; Nakajima et al. 2013), and data about the interaction between the polymorphism on DRD3 and schizophrenia are still controversial. Szekeres et al. (2004) revealed that patients with $Ser^9Gly$ SNP performed better in the Wisconsin Card Sorting Test (WCST) compared with the SerSer patients; moreover, they were more sensitive to the antipsychotic treatments (Szekeres et al. 2004). In line with these data, Keri and colleagues have investigated the role of $Ser^9Gly$ polymorphism in the habit learning, proving that both schizophrenic patients and healthy people with $Ser^9Gly$ show better performance in the striatal habit learning (Keri et al. 2005). On the other hand, Rybakowski et al. (2005) failed to find any associations between WCST performance and DRD3 polymorphisms. To better understand the role of $Ser^9Gly$ in schizophrenia, a meta-analysis was performed by Qi and colleagues (Qi et al. 2017). The meta-analysis considered 73 studies but also in this case, data suggest that no association exists. To justify the lack of association the authors propose three possible explanations. Firstly, in their analysis they consider study performed in different populations (e.g., Caucasian, Japanese, Latinos) and in the control groups the frequencies of the $Ser^9Gly$ polymorphism are highly variable. Secondly, people living in different countries are exposed to different epigenetic factors, and thirdly, the sample size considered in some of these study is very small (Qi et al. 2017). All together, these data suggest that the D3R might be implicated in dopamine dysregulation that characterized schizophrenia, however more data are necessary to clarify its role in cognitive dysfunctions.

### *3.4 Dopamine D3 Receptor and Cognitive Functions in Alzheimer Disease (AD)*

Cognitive and behavioral dysfunctions are the core symptoms of AD (Deardorff and Grossberg 2019). Sweet and colleagues (Sweet et al. 1998) observed, for the first time, the association between AD and genetic alterations on D3R. However, in this case the authors did not analyze the role of D3R in cognition, but only in psychosis, finding a higher probability to develop psychotic attack in patients homozygous for either DRD3 allele (Sweet et al. 1998). In a post mortem study, conducted in AD patients with diagnosis of dementia, Piggott and colleagues (Piggott et al. 1999)

demonstrated that D3R binding increases in the striatum when compared to healthy controls (Piggott et al. 1999). To the best of our knowledge there are no other studies investigating the interaction between D3R and cognitive dysfunctions in patients with AD; thus, there might be relevant mechanisms related to D3R yet to be unraveled.

### *3.5 Dopamine D3 Receptor and Cognitive Functions in Bipolar Disorder (BP)*

BP is a large group of affective disorders characterized by both episodes of depression (e.g., loss of pleasure and reduced energy) and episodes of mania (e.g., increased energy and reduced need for sleep) (Phillips and Kupfer 2013) interspersed with euthymic periods (Burdick et al. 2007). Cognitive impairments, such as deficits in attention, memory, and learning, are obvious not only during the acute episodes of depression and mania, but persist also during the euthymia (Burdick et al. 2007). The association between D3R and BP is not completely clarified. Indeed, Parsian and colleagues have reported that polymorphism on DRD3 confers vulnerability to develop BP, conversely, Leszczyńska-Rodziewicz and colleagues failed to replicate these data (Leszczynska-Rodziewicz et al. 2005; Parsian et al. 1995). Furthermore, one study performed on patients affected by BP under treatment with pramipexole showed an improvement in cognitive functions (Burdick et al. 2012). However, also for BP, the role of D3R in cognitive impairments remains unexplored, and more data are necessary to better understand this relationship.

## 4 The Potential Therapeutic Value of Drugs/Ligands Targeting the D3R for the Treatment of Cognitive Dysfunctions

### *4.1 D3R Antagonists in Preclinical Studies*

Growing preclinical evidence supports the role of the D3R as a possible target for the development of new medication for the treatment of cognitive dysfunction. In this framework, Watson and collaborators demonstrated that both the D3R selective antagonist S33084 (p$K$i = 9.6 for D3R, >100-fold lower affinity for D2R) and the preferential D3 antagonist S33138 are able to reverse the impairment in the novel object discrimination test in a neurodevelopmental animal model based on the post-weaning isolation in rats. By contrast, the L741,626 compound, a preferential antagonist at D2 vs. D3, was devoid of effect on isolated rats but impaired novel object discrimination in control group (Watson et al. 2012b). The contribution of D3R in the modulation of cognitive functions by the S33084 compound was

confirmed in mice lacking the D3R. Indeed, the S33084 had no effect in a set-shifting paradigm and social discrimination, while it improved the performance in wild-type mice (Watson et al. 2012a). The efficacy of the S33138 compound as a potential pro-cognitive medication is also indicated by other studies, where it improves the accuracy in a variable-delayed response task of attention and working memory in rhesus monkeys treated with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), a compound known to produce cognitive impairments without any motor side effect (Millan et al. 2010).

Other studies show the efficacy of a new compound, YQA-31, a D3R antagonist with an affinity 186-fold higher for D3R vs. D2R (D3 *K*i = 0.28 nM; D2 *K*i = 52.1 nM; (Sun et al. 2016b)). Pro-cognitive effects of YQA-31 were tested in a mouse model of schizophrenia-like behaviors induced by injection of MK-801. YQA-31, at low dose, ameliorates the MK-801-induced cognitive impairment in a novel object recognition paradigm without affecting locomotion or cataleptic side effects (Sun et al. 2016a).

Furthermore, D3R preferring ligands have been shown to be effective in attenuating cognitive impairment in animal models. In particular, learning deficit induced by scopolamine, an anticholinergic amnestic agent, is reversed by the selective D3R antagonists SB-277011 (D2 *K*i = 1,047 nM, D3 *K*i = 11 nM (Audinot et al. 1998)), RGH-1756 (D2 *K*i = 12.2 nM, D3 *K*i = 0.12 nM (Kovacs et al. 2001)), and U-99194A (D2 *K*i = 2,281 nM, D3 *K*i = 223 nM (Reavill et al. 2000)), but not by the selective partial agonist BP-897 (D2 *K*i = 61 nM, D3 *K*i = 0.92 nM (Pilla et al. 1999)).

Thus, preclinical evidence supports the functional role of new, but also old (repurposing/repositioning) molecules, that selectively block the D3R as a potential strategy for improving cognitive performance in psychiatric disorders (Table 1).

## *4.2 Drugs Targeting D3R in Clinical Studies*

Pharmacological intervention for the management of cognitive dysfunction in humans is scarcely supported by clinical data. Moreover, few data are available on the effects of selective D3R antagonists due to the lack of D3R preferring (selective) molecules available in the market. Currently approved drugs with high affinity for the D3R are antipsychotics (Graff-Guerrero et al. 2010; Gross and Drescher 2012; Mizrahi et al. 2012; Mugnaini et al. 2013; Schotte et al. 1996; Torrisi et al. 2017, 2020), and many of these also show high affinity for the D2R, resulting in two main problems: first, limited or no effects on cognitive dysfunction and, second, presence of a plethora of debilitating and occasionally disabling motor and metabolic side effects (Kaar et al. 2020; Leucht et al. 2013).

**Table 1** D3R antagonist efficacy in cognition: preclinical studies

| Drugs/ compounds | Species | Impairing agent | Behavioral assay | Effect | References |
|---|---|---|---|---|---|
| S33138 | Rhesus monkey | Aging (27 years) | Delayed matching-to-sample | + | (Millan and Brocco 2008) |
| | Rat | Post-weaning isolation | Novel object recognition | + | (Watson et al. 2012b) |
| | Rhesus monkey | MPTP treatment | Delayed matching-to-sample | + | (Millan et al. 2010) |
| | Rhesus monkey | MPTP treatment | Attentional set-shifting | + | |
| S33084 | Rat | Post-weaning isolation | Novel object recognition | + | (Watson et al. 2012a) |
| | Mouse | None | Attentional set-shifting | + | |
| | Mouse | None | Social discrimination | + | |
| | Mouse | Genetic depletion of D3R | Attentional set-shifting | No effect | |
| | Mouse | Genetic depletion of D3R | Social discrimination | No effect | |
| Y-QA31 | Mouse | MK-801 | Novel object recognition | + | (Sun et al. 2016b) |
| SB-277011 | Rat | Scopolamine | Water labyrinth | + | (Laszy et al. 2005) |
| | Rat | Scopolamine | Social recognition | + | (Millan et al. 2007) |
| | Mouse | MK-801 | Morris water maze | No effect | (Tanyeri et al. 2015) |
| Cariprazine | Mouse | PCP | Attentional set-shifting | + | (Zimnisky et al. 2013) |
| | Mouse | PCP | Social recognition | + | |
| | Mouse | PCP | Delayed alternation T-maze | + | |
| | Rat | Scopolamine | Water-labyrinth | + | (Gyertyan et al. 2011) |
| Blonanserin | Mouse | Scopolamine | Novel object recognition | + | (Hida et al. 2015) |
| | Mouse | 7-OH-DPAT | Novel object recognition | No effect | |
| | Rats | PCP | Novel object recognition | + | (Horiguchi and Meltzer 2013) |
| F17464 | Rats | Scopolamine | Passive avoidance | + | (Slifstein et al. 2020) |
| Buspirone | Mouse | MK-801 | Temporal order recognition | | (Torrisi et al. 2017) |
| | Mouse | D3R genetic depletion + MK-801 | Temporal order recognition | No effect | |
| Risperidone | Mouse | Genetic mutation on D3R and Dys protein | Temporal order recognition | + | (Leggio et al. 2021) |
| | Mouse | Genetic mutation on D3R and Dys protein | 5-trial habituation-dishabituation social interaction | + | |

### 4.3 Risperidone

Is a well-known antipsychotic approved by FDA in 1994, with a balanced serotonin-dopamine antagonist activity. Risperidone exhibits the highest affinity for 5-HT2a receptor ($Ki = 0.4$ nM) and moderate to low affinity for 5-HT1a and dopamine D2R, D3R, D4R (Leysen et al. 1994). Several clinical trials have evaluated the efficacy of risperidone as a pro-cognitive agent. Two different clinical trials found no effect of risperidone on social cognition (Sergi et al. 2007) or cognitive deficits (Citrome and Volavka 2014; Lindenmayer and Khan 2011) whereas other studies demonstrated a positive effect of 12 weeks treatment with risperidone in improving executive and social cognitive dysfunction in schizophrenic patients (Zhou et al. 2017). These different findings might be due to the treatment duration (4 and 12 weeks, respectively). Further evidence for risperidone as a potential cognitive enhancer results from translational studies. Indeed, Scheggia and colleagues demonstrated that risperidone effectively reverses cognitive deficits in a mice model carrying a genetic variant associated with reduced expression of Dysbindin protein (Scheggia et al. 2018). Consistently, we recently reported the presence of an epistatic interaction between the dysbindin protein and D3R which triggers an improvement in cognitive performance, including executive function and working memory, following chronic risperidone administration, in both schizophrenic patients and mice bearing concomitant hypofunction of both DRD3 (D3R) and DTNBP1 (dysbindin) genes (Leggio et al. 2021).

### 4.4 Cariprazine

Is a recently FDA-approved medication for the treatment of schizophrenia and bipolar disorder. This molecule attracted great interest for its pharmacological profile characterized by a partial agonism at D2R/D3R. Contrary to other antipsychotics, cariprazine is endowed with a higher affinity for D3R ($Ki = 0.085$ nM) vs. D2R ($Ki = 0.69$ nM) while acts as an antagonist at 5-HT2a and 5-HT2b (Citrome 2013). Very recently and interesting, it has been reported that cariprazine binds selectively the D3R localized in the granular cells of islands of Calleja (Prokop et al. 2021).

In preclinical studies, cariprazine effects were evaluated in different animal models of cognitive dysfunction based on the administration of scopolamine or phencyclidine (PCP). In contrast with other second-generation antipsychotics, cariprazine had a dose-dependent effect in counteracting scopolamine-induced impairment in a spatial learning paradigm (Gyertyan et al. 2011). Furthermore, acute administration of cariprazine prevents PCP-induced deficits in social recognition memory, extradimensional set-shifting, and spatial working memory (Zimnisky et al. 2013). Interestingly, no significant effects of cariprazine on cognitive impairments have been found in PCP-treated mice lacking D3R, demonstrating the

fundamental contribution of D3R on cariprazine's effect. These findings are further supported by three different randomized, double-blinded, placebo-controlled clinical trials in patients with manic episode associated with bipolar disorder. Cariprazine treatments, at flexible dose ranging from 3 to 12 mg/die for 3 weeks, significantly improves the Positive and Negative Syndrome Scale (PANSS) and the Cognitive Subscale Score compared to the placebo group (McIntyre et al. 2021). The pro-cognitive effect of cariprazine might be ascribed to its D3R partial agonist activity, even if there is no evidence indicating the involvement of D3R in the onset of cognitive dysfunction in patients with bipolar disorder.

### 4.4.1 Blonanserin

Is an antipsychotic medication only approved in Japan and Korea, which differs from other antipsychotic drugs for its mixed 5-HT2a ($Ki = 0.812$ nM) and D2R ($Ki = 0.142$ nM) antagonist activity. Interestingly, blonanserin also exhibits a good affinity for D3R ($Ki = 0.494$ nM) (Tenjin et al. 2012). The efficacy of blonanserin in the treatment of cognitive dysfunction was demonstrated in both preclinical and clinical studies. Indeed, in rodents, blonanserin administration at the dose of 1 mg/Kg reverses the PCP-induced deficits in a paradigm of novel object recognition (NOR) (Horiguchi and Meltzer 2013). These findings were confirmed by Hida and collaborators, which demonstrated the beneficial effect of blonanserin (3 mg/Kg) on the NOR cognitive impairment induced by PCP administration in mice. In addition, blonanserin effect is inhibited by pretreatment with D3R agonist, 7-OH-DPAT (Hida et al. 2015). These pro-cognitive effects of blonanserin showed in rodents may be ascribed to its potent binding affinity for D3R. Blonanserin in fact extensively occupies D3R (76.8%) compared to other antipsychotic as risperidone (20.2%), olanzapine and aripiprazole (Not determined) (Baba et al. 2015).

In clinical trials pharmacological effects of blonanserin were evaluated in social and cognitive function in schizophrenic patients. A nonrandomized, 8-week, open-label study reported that both blonanserin and risperidone improve cognitive and social functioning, that include the evaluation of verbal memory, working memory, motor speed, verbal fluency, attention and processing speed, and executive functions. In particular, risperidone ameliorated verbal fluency while blonanserin had a beneficial effect also on executive functions in schizophrenic patients after 8 weeks of medication (Hori et al. 2014).

### 4.4.2 Pramipexole

Is a medication used in the treatment of Parkinson's disease, alone or in combination with levodopa. The pharmacological profile is characterized by partial agonism at dopaminergic D2R (affinity $Ki = 3.05$ nM), D3R (affinity $Ki = 0.5$ nM), and D4R (affinity $Ki = 5.1$ nM). Clinical evidence on the efficacy of pramipexole as

pro-cognitive is controversial. Pramipexole, indeed, improves the accuracy of the working memory performance in cognitive-impaired PD patients (Costa et al. 2009) and also has shown potential beneficial effects in a subgroup of bipolar patients (Burdick et al. 2012). Conversely, in a randomized, controlled, clinical study, Pramipexole was not effective as a cognitive enhancer drug in bipolar patients (Van Meter et al. 2021) and impaired reversal shifting learning in PD patients (Cools et al. 2006). Further studies are necessary to better understand the specific role of Pramipexole as a pro-cognitive drug in different brain disorders.

### 4.4.3 Buspirone

Is an azapirone anxiolytic drug traded as a 5-HT1aR partial agonist ($Ki = 21$ nM), is also endowed with a D3R and D4R antagonist activity. Indeed, it has been demonstrated that buspirone binds the D3R ($Ki = 98$ nM with an affinity five-fold higher than D2R ($Ki = 484$ nM) (Bergman et al. 2013). In rodents, buspirone is effective in ameliorating working memory dysfunctions in a mouse model based on NMDA hypofunction induced by MK-801. The role of D3R has been demonstrated by the failure of buspirone in reversing the MK-801-induced memory impairment in D3R KO mice (Torrisi et al. 2017).

### 4.4.4 F17464

Other experimental compounds targeting selectively D3R are currently in clinical development. A potential pro-cognitive drug is F17464 which is endowed with a unique pharmacological profile characterized by a high affinity for D3R ($Ki = 0.17$ nM) and the 5HT1a ($Ki = 0.16$ nM) and a $>$ 50-fold lower affinity for D2R (Cosi et al. 2021). In rodents, F17464 exhibits a dose-dependent pro-cognitive effect in reversing scopolamine-induced cognitive deficit compared to other second-generation antipsychotics that, in contrast, show no effects (Sokoloff and Le Foll 2017). The effectiveness and the safety demonstrated in preclinical studies has been confirmed by a randomized, double-blind, placebo-controlled phase II clinical trial, which demonstrated the pharmacological efficacy of F17464 on positive, negative, and also cognitive impairments in patients with acute exacerbation of schizophrenia. Interestingly, F17464, at the effective dose of 40 mg/day, has no detrimental motor or metabolic effects (Bitter et al. 2019). The promising results achieved in clinical trials might be ascribed to the correlation between the plasma concentration of F17464 and the D3R occupancy. In a brain imaging study by Slifstein, the authors demonstrated that D3R occupancy, 6–9 h post-administration (30 mg/Kg), ranges between 89–98% (Slifstein et al. 2020).

### 4.4.5 ABT-925

The only selective D3 antagonist currently in phase II clinical trials is ABT-925, a D3R selective antagonist ($Ki = 2.9$ nM), however, the study is aimed at evaluating the safety and the efficacy of this compound only on positive and negative symptoms in patients with acute exacerbation of schizophrenia, not on cognitive impairments (Bhathena et al. 2013).

### 4.4.6 GSK598809

Is a promising compound with a selective action on the D3R as antagonist ($Ki = 6.2$ nM), which reversed the attentional deficit in a color-name word in abstinent smokers.

The mechanism that underlies this observation has been ascribed to the pro-cognitive effects related to the blockade of D3R (Mugnaini et al. 2013) (Table 2).

**Table 2** Clinical trials of drugs targeting D3R

| Drug/ compound | Disease | Dose | Results | References |
|---|---|---|---|---|
| Risperidone | Schizophrenia or schizoaffective disorder | 4 mg/die for 8 weeks | No differences in social cognition | (Sergi et al. 2007) |
| | Schizophrenia or schizoaffective disorder | 25 mg or 50 mg every 2 weeks | No differences in social cognition | (Lindenmayer and Khan 2011) |
| Cariprazine | Bipolar disorder (I) | From 3 to 12 mg/die for 3 weeks | Cognitive improvement | (McIntyre et al. 2021) |
| Blonanserin | Schizophrenic patients | Dose was individual adjusted for each patient | Improvement of psychotic symptoms and cognitive function | (Hori et al. 2014) |
| Pramipexole | Parkinson's Disease | 0.7 mg TID | Improvement of cognitive performances | (Costa et al. 2009) |
| | Bipolar disorder (I, II) | From 0.125 mg/die to 4.5 mg/die | No effects in cognitive performances | (Van Meter et al. 2021) |
| F17464 | Schizophrenia | 40 mg/die for 6 weeks | Improvement of symptoms in patients with acute exacerbation of schizophrenia | (Bitter et al. 2019) |
| AB-125 | Schizophrenia | 50 mg or 150 mg/die for 6 weeks | Improvement of positive and negative symptoms, no effects in cognition | (Bhathena et al. 2013) |

## 5 Conclusions

Cognitive dysfunction is a significant feature of several neuropsychiatric disorders. However, effective treatments for cognitive dysfunction still remain an unmet medical/clinical need. The pharmacological treatment of cognitive dysfunction in neuropsychiatric patients in fact is challenging because many factors are involved in the control of cognitive performances.

According to data discussed in this chapter, the D3R, affecting the dopaminergic signaling on GABAergic and/or glutamatergic neurons located in different brain areas, might modulate the individual capacity to recover from cognitive dysfunction related to neuropsychiatric disorders. Thus, using a therapeutic approach based on the genetics of individual patients and identifying novel mechanisms involved in cognitive responses, particularly those dopaminergic-related, may produce a more precise patient stratification and help to guide the choice for more appropriate, personalized drug treatment.

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# Current Perspectives on Selective Dopamine $D_3$ Receptor Antagonists/Partial Agonists as Pharmacotherapeutics for Opioid and Psychostimulant Use Disorders

**Amy Hauck Newman, Zheng-Xiong Xi, and Christian Heidbreder**

## Contents

1 Introduction: Brief Historical Perspective/Epidemiology ..... 158
1.1 The Perfect Storm ..... 158
1.2 Opioid Crisis ..... 159
1.3 Psychostimulant Use Disorder: Cocaine and Methamphetamine ..... 160
1.4 Polysubstance Use Highlighting Opioids/Methamphetamine ..... 160
1.5 Co-Morbidities with Other Neuropsychiatric Disorders ..... 161
2 $D_3$R Neurocircuitry and Relationship to SUD ..... 161
2.1 Rationale of $D_3$R-Based Medication Development for the Treatment of Psychostimulant and Opioid Use Disorders ..... 161
2.2 Relationship to Neural Targets and Therapeutic Potential ..... 166
2.3 $D_3$R Neuroadaptations Due to SUD ..... 170
2.4 Relationship of $D_3$R to Pain ..... 172
2.5 Relationship to Other Comorbid Neuropsychiatric Disorders ..... 173
3 $D_3$R Antagonists and Partial Agonists Currently under Preclinical Investigation for SUD ..... 174
3.1 Past $D_3$R Preferential and Selective Antagonists that Were Tested in Clinical Trials ..... 174
3.2 New and Promising $D_3$R Selective Antagonists/Partial Agonists for SUD ..... 176
3.3 Potential Challenges: Cardiovascular Toxicity in the Presence of Cocaine ..... 179
4 Perspective on Clinical Application as Treatments for SUD ..... 180
4.1 Limitations and Advances in the Translational Value of Animal Models of SUD ..... 180
4.2 Key Translational Medicine Questions Relevant to Clinical Development ..... 181
4.3 Most Suitable Clinical Paradigms for Medication Development Purpose ..... 183
4.4 From Monotherapy to Combination of Medications ..... 185
5 Summary ..... 186
References ..... 187

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**Abstract** Over three decades of evidence indicate that dopamine (DA) $D_3$ receptors ($D_3R$) are involved in the control of drug-seeking behavior and may play an important role in the pathophysiology of substance use disorders (SUD). The expectation that a selective $D_3R$ antagonist/partial agonist would be efficacious for the treatment of SUD is based on the following key observations. First, $D_3R$ are distributed in strategic areas belonging to the mesolimbic DA system such as the ventral striatum, midbrain, and ventral pallidum, which have been associated with behaviors controlled by the presentation of drug-associated cues. Second, repeated exposure to drugs of abuse produces neuroadaptations in the $D_3R$ system. Third, the synthesis and characterization of highly potent and selective $D_3R$ antagonists/partial agonists have further strengthened the role of the $D_3R$ in SUD. Based on extensive preclinical and preliminary clinical evidence, the $D_3R$ shows promise as a target for the development of pharmacotherapies for SUD as reflected by their potential to (1) regulate the motivation to self-administer drugs and (2) disrupt the responsiveness to drug-associated stimuli that play a key role in reinstatement of drug-seeking behavior triggered by re-exposure to the drug itself, drug-associated environmental cues, or stress. The availability of PET ligands to assess clinically relevant receptor occupancy by selective $D_3R$ antagonists/partial agonists, the definition of reliable dosing, and the prospect of using human laboratory models may further guide the design of clinical proof of concept studies. Pivotal clinical trials for more rapid progression of this target toward regulatory approval are urgently required. Finally, the discovery that highly selective $D_3R$ antagonists, such as *R*-VK4-116 and *R*-VK4-40, do not adversely affect peripheral biometrics or cardiovascular effects alone or in the presence of oxycodone or cocaine suggests that this class of drugs has great potential in safely treating psychostimulant and/or opioid use disorders.

**Keywords** D3 receptor antagonist · D3 receptor partial agonist · Dopamine · Opioids · Psychostimulants · Substance use disorders

## 1 Introduction: Brief Historical Perspective/Epidemiology

> Meteorologists see perfect in strange things, and the meshing of three completely independent weather systems to form a hundred-year event is one of them. My God, thought Case, this is the perfect storm.
>
> — Sebastian Junger, The Perfect Storm: A True Story of Men Against the Sea

### *1.1 The Perfect Storm*

The COVID-19 pandemic has brought our world to its knees in a way that most of us never imagined. The SARS-CoV-2 virus has managed to infect and mutate, becoming more virulent over time, resulting in death and destruction of economies,

livelihoods, and a way of life that no one could have predicted. While massive resources and biomedical research focused on vaccines and medications to save lives, two other crises were brewing. The opioid epidemic was just starting to see the beginning of a downward trend, at least in terms of death by overdose (Ahmad et al. 2021). And although the use of psychostimulants such as cocaine and methamphetamine was still a relevant health assailant, it had not yet reached the point of crisis. Nevertheless, the devastation, isolation, hopelessness, and fatigue brought on by the COVID-19 pandemic have exacerbated substance misuse, joining forces to reverse the upward trend of life longevity in the USA (Manchikanti et al. 2021) and resulting in >90,000 drug overdose deaths, an increase of >30% in 2020 over the year before (Ahmad et al. 2021; Volkow 2021). The decrease in health services, limited access to medical care, and increased access to highly potent opioids such as fentanyl, etonitazene, and their illicit analogues have been complemented by an increased supply of methamphetamine, the combinations of which were more deadly than either one alone or sometimes ingested without the user's knowledge (Narayan and Balkrishnan 2021).

The challenges that the COVID-19 epidemic introduced to mental health cannot be underestimated. Isolation-related anxiety and depression are among the disorders that have increased and been exacerbated. Closely coupled to these is the management of pain, which has also been impaired by lack of access to medical care and is the leading reason patients take prescription opioids that for some can lead to dependence or addiction (Kibaly et al. 2021; Taquet et al. 2021a, b). Sheltering in place and restrictions in travel have impacted patients' ability to obtain proper medical care and necessary medications. Patients in chronic pain become depressed and the vicious cycle is unrelenting, unless acutely mitigated by the use of illicit drugs – a solution that has devastating consequences.

People with substance use disorders (SUD) are at heightened risk for other life-threatening comorbidities including cardiovascular disease, mucociliary dysfunction, compromised immunity as well as multiple social factors that prevent proper treatment (Manchikanti et al. 2021). And indeed, those whose prescription opioid taking accelerates to illicit drug use and addiction, only enhances their chances of becoming infected with SARS-CoV-2 and succumbing to the virus, overdose, or both. Sadly, as with other crises, underserved populations receive the disproportionate impact of this trifecta of tragedy (Narayan and Balkrishnan 2021).

## *1.2 Opioid Crisis*

By 2019, the opioid epidemic in the USA was noted as a health crisis that was continuing to escalate (Lyden and Binswanger 2019). Although illicit opioids such as heroin had been contributing to opioid-related deaths for decades before, the increase in prescribed opioids for the management of pain, and especially the over prescription of extended-release formulations of oxycodone (e.g., oxycontin) significantly escalated opioid dependence and addiction in the USA. As oxycontin was

first marketed as less addictive than other opioid narcotics, a dramatic increase in its use for pain management ultimately resulted in escalated opioid overdoses in the last decade (Azadfard et al. 2021; Kibaly et al. 2021; Walker 2018). According to the Centers for Disease Control and Prevention (CDC), 96,779 people died from drug overdose in the 12-month period ending March 2021. Approximately 72,805 of these deaths were attributed to all opioids, and 61,230 were attributed to synthetic opioids such as fentanyl (Ahmad et al. 2021).

Studies that attempt to quantify the burden of opioid-related mortality conclude that premature deaths caused by opioid overdose has and undoubtedly will continue to impose an enormous health and economic burden on the USA (Gomes et al. 2018). In 2016, years of life lost (YLL) exceeded those attributed to hypertension, HIV, and pneumonia (Gomes et al. 2018). Sadly, 25–34 years of age was the demographic with the highest opioid overdose death rate. Young adults who had the potential to contribute so much to our society and may have left children behind – yet another tragic reality.

## *1.3 Psychostimulant Use Disorder: Cocaine and Methamphetamine*

As if the COVID-19 pandemic and the opioid crisis were not enough to keep researchers and health care providers, legislators, and parents up at night, a new wave of drug abuse is now rolling through our cities and rural areas alike. Although cocaine continues to be a drug of high abuse potential and related death by overdose, methamphetamine has roared into our streets and communities (Compton et al. 2021; Fogger 2019; Jones et al. 2020). Methamphetamine is easily synthesized in home laboratories, has a longer half-life than cocaine, and is more easily accessible, likely contributing to its added popularity, which has increased during the COVID-19 pandemic.

## *1.4 Polysubstance Use Highlighting Opioids/Methamphetamine*

The "old practice" of combining heroin with cocaine known as "speedball" has been replaced with the combination of methamphetamine and heroin or fentanyl, called "goofball" (Glick et al. 2021) with grave consequences. Some users of this combination of drugs claim that the addition of methamphetamine to the opioid reduces unpleasant sluggishness/lethargy and the opioid decreases the unpleasant intensity of methamphetamine (Ciccarone 2021; Glick et al. 2021). Clearly polysubstance use is prevalent and highly complex, leading to an increase in morbidity and poses further challenges for prevention and treatment. Although a decline in overdose deaths

appeared in 2017–2018, the CDC reports an increased mortality that is alarming, driven by a dramatic increase in opioid-related deaths and now a "fourth wave" of high mortality involving cocaine and primarily methamphetamine (Ciccarone 2021).

### *1.5 Co-Morbidities with Other Neuropsychiatric Disorders*

In addition to polysubstance use, prevailing public health problems that have been exacerbated by the COVID-19 pandemic are psychiatric disorders, including anxiety, major depressive disorder, and bipolar disorder. These disorders are complex and often difficult to treat. Equally alarming is the comorbidity between these disorders and SUD, a public health concern that emerged long before COVID-19 but has undoubtedly increased (Angarita et al. 2021a; Hellem et al. 2015; Murthy et al. 2019).

## 2 $D_3$R Neurocircuitry and Relationship to SUD

### *2.1 Rationale of $D_3$R-Based Medication Development for the Treatment of Psychostimulant and Opioid Use Disorders*

#### 2.1.1 Dopamine Hypothesis of Drug Reward

It is well documented that the mesolimbic and nigrostriatal dopamine (DA) systems are critically involved in psychostimulant and opioid reward (Galaj and Xi 2021; Lammel et al. 2014) (Fig. 1). These systems originate from DA neurons in the ventral tegmental area (VTA) and substantia nigra pars compacta (SNc) in the midbrain and project to the nucleus accumbens (NAc), dorsal striatum (DST), ventral pallidum (VP), prefrontal cortex (PFC) and insula, as well as the amygdala (Amy). A great deal of evidence supports the importance of both the DA projection pathways in SUD. First, almost all addictive drugs, including cocaine, opioids, nicotine, and ethanol, increase extracellular DA in the NAc and DST (Koob and Bloom 1988; Self and Nestler 1995). Second, almost all addictive drugs can be self-administered by animals either intravenously or locally into the VTA or NAc, which can be blocked or attenuated by either chemical lesions of DA terminals or by pharmacological blockade of DA receptors (Bressan and Crippa 2005; Gardner 2000). And third, electrical or optical stimulation of brain DA loci maintains intracranial self-administration, which can be enhanced by drugs of abuse and attenuated by DA receptor antagonists (Wise 1996).

Psychostimulants and opioids activate the mesolimbic and nigrostriatal DA systems by different molecular and cellular mechanisms. Cocaine elevates extracellular DA levels in the DA projection areas mainly by blockade of DA reuptake,

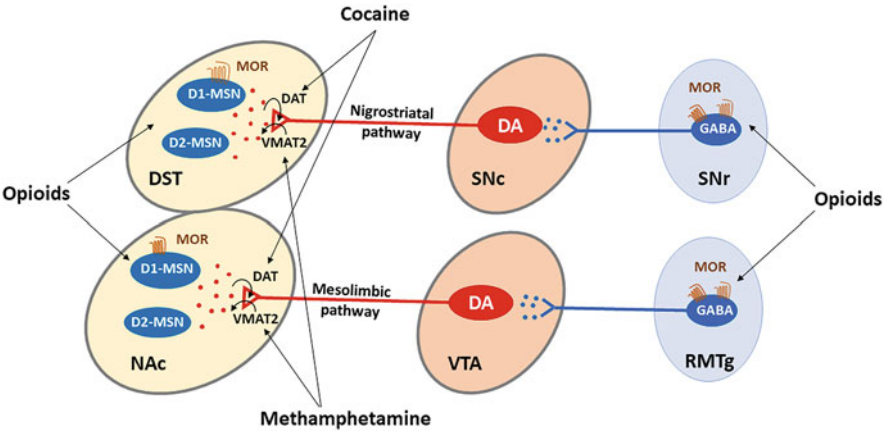


**Fig. 1** Schematic diagram of the mesolimbic and nigrostriatal reward pathways, illustrating the action sites (targets) of psychostimulants (cocaine, methamphetamine) and opioids in the brain. The mesolimbic DA circuit (RMTg → VTA → NAc) originates in the midbrain ventral tegmental area (VTA) and projects predominantly to the nucleus accumbens (NAc) and other forebrain regions (not shown). VTA DA neurons receive GABAergic inputs from local VTA GABA neurons and other brain regions including the NAc, ventral pallidum (VP), and rostromedial tegmental nucleus (RMTg), particularly from the RMTg. Psychostimulants elevate extracellular NAc DA by blocking DA transporters (DAT) (by cocaine) or reversing VMAT2 (by methamphetamine) on DA axon terminals in the NAc and dorsal striatum (DST). The nigrostriatal DA circuit (SNr → SNc → DST) originates from DA neurons in the substantia nigra pars compacta (SNc) and projects to the DST. SNc DA neurons receive dense GABAergic inputs from multiple brain regions including the SNr and RMTg, but mainly from SNr. Mu opioid receptors (MOR) are highly expressed in GABA neurons, particularly in the RMTg and SNr. Opioids bind to MORs and inhibit GABA neuron activity and GABA release, which subsequently disinhibits DA neurons in the VTA and SNc

while amphetamine or methamphetamine mainly promotes DA release from DA terminals by reversal of vesicular monoamine transporter 2 (VMAT2), which promotes DA exit from vesicles into cytoplasm and causes DA release from cytoplasm to extracellular space by reversal of membrane dopamine transporter (DAT) (Elkashef et al. 2008; Freyberg et al. 2016; Shen et al. 2021) (Fig. 1). These increases in synaptic or extracellular DA in the forebrain reward loci – especially in the NAc – are thought to underlie the euphoria associated with psychostimulant use (Wise 2005).

In contrast to psychostimulant reward, the neural mechanisms underlying opioid reward and abuse are still not fully understood. A classical hypothesis is that opioids initially bind to mu opioid receptors (MOR) located on GABAergic interneurons within the VTA and functionally inhibit GABAergic neuronal activity, which subsequently disinhibits neighboring DA neurons within the VTA (Galaj and Xi 2021; Xi and Stein 2002). This canonical two-neuron hypothesis, which was upheld for over half a century, has been challenged by recent findings suggesting that high density MORs are expressed in GABAergic neurons mainly in the rostromedial

tegmental nucleus (RMTg, also called the tail of the VTA) and substantia nigra pars reticulata (SNr) in the midbrain (Galaj et al. 2020a; Galaj and Xi 2021; Matsui et al. 2014; Matsui and Williams 2011). It has been shown that DA neurons in the VTA and SNc receive intensive GABAergic inputs mainly from the RMTg and the SNr, respectively (Galaj et al. 2020a; Matsui and Williams 2011), suggesting that DA neurons in the VTA and SNc may be activated mainly by activation of MORs in GABAergic neurons in both the RMTg and SNr via a disinhibition mechanism. Thus, a two-pathway hypothesis (e.g., RTMg → VTA → NAc, SNr → SNc → DST) has been proposed to explain opioid reward and abuse (Fig. 1) (Galaj et al. 2020a; Galaj and Xi 2021).

Based on this DA hypothesis, one strategy to manipulate the downstream DA transmission in the brain reward circuitry is to target (block) DA receptors ($D_1$, $D_2$, $D_3$, $D_4$) for the treatment of SUD and another is to target the DAT specifically for the treatment of psychostimulant use disorder (PSUD) (Newman et al. 2021). The $D_3R$ is a major focus in the former strategy (Galaj et al. 2020b), which will be addressed extensively below, while developing various DAT inhibitors, particularly atypical DAT inhibitors, is the major focus in the latter strategy, which has recently been reviewed extensively elsewhere (Hersey et al. 2021; Tanda et al. 2021).

### 2.1.2 Unique Profiles of $D_3R$

There are five G protein-coupled DA receptor subtypes identified, which are classified into $D_1$-like ($D_1$, $D_5$) and $D_2$-like ($D_2$, $D_3$, $D_4$) groups based on their homology, pharmacology, and intracellular signaling properties (Beaulieu et al. 2015; Beaulieu and Gainetdinov 2011; Martel and Gatti McArthur 2020; Missale et al. 1998). The $D_1$ and $D_5$ receptors share 80% homology of their seven transmembrane domains, while the $D_2$ receptors share 75% homology of their protein structure with $D_3R$ and only 53% homology with $D_4$ receptors. The main structural differences among DA receptors are differences in size of the third intracellular loop connecting transmembrane domains and of the carboxyl-terminal intracellular segment. $D_1$-like receptors stimulate intracellular cAMP signaling pathway through $G\alpha_s$ G-proteins, whereas $D_2$-like receptors inhibit DA signaling through $G\alpha_{i/o}$ G-proteins.

#### High $D_3R$ Binding Affinity to DA

Each DA receptor binds endogenous ligand DA with affinities in the nM range. The $D_2$-like receptor subtypes bind DA with higher affinities than the $D_1$-like family with $D_3R$ binding DA with the highest affinity (Missale et al. 1998). Therefore, $D_3R$ has been described as being a major receptor underlying DA transmission in the brain reward system. Given that basal levels of extracellular DA (5–10 nM) and synaptic DA (~50 nM) (He and Shippenberg 2000; Ross 1991), it is expected that a fraction of $D_3R$ is constitutively activated, thus playing an essential role in both tonic and phasic DA signaling.

## Restricted $D_3R$ Distribution

The human $D_3R$ was first cloned in 1990 (Giros et al. 1990), which was followed by the cloning and characterization of the rat $D_3R$ (Sokoloff et al. 1990). Since then, various radiolabeled ligands such as [$^3$H]7-OH-DPAT), [$^3$H]PD-128907, and [$^{125}$I] epidepride were developed (Hall et al. 1996; Herroelen et al. 1994; Murray et al. 1994). A variety of techniques such as quantitative autoradiography and in situ mRNA hybridization have been used to map the distribution of $D_3R$ in the brain and periphery.

Using a polyclonal $D_3R$ antibody, Diaz et al. detected dense $D_3R$-immunostaining mainly in the islands of Calleja and mammillary bodies, moderate to low signals in the shell of NAc, frontoparietal cortex, SNc, VTA, and lobules 9 and 10 of the cerebellum, but very low or no signal in other rat brain regions such as DST (Diaz et al. 2000; Lammers et al. 2000). However, due to the concerns of DA receptor antibody specificity, autoradiography and PET imaging have become the major techniques to map $D_3R$ distributions in the brain (Diaz et al. 2000; Lammers et al. 2000). Consistent with the findings by immunostaining, an autoradiogram study with [$^{125}$I]7-OH-PIPAT also showed the restricted distributions of $D_3R$ in the rat brain with the highest level of $D_3R$ expression in the islands of Calleja, ventromedial shell of NAc, VP, and SN (Stanwood et al. 2000). Such a restricted distribution of $D_3R$ expression was also found in other species, such as mouse, guinea pig, and rabbit (Diaz et al. 1994, 1995; Levant 1998). Among these four species the mouse shows high density $D_3R$ expression in hippocampus and low expression in the frontal cortex (Levant 1998).

Subsequent PET imaging studies showed that [$^{11}$C](+)-PHNO, a mixed $D_2R/D_3R$ agonist (Narendran et al. 2006; Seeman et al. 2005) produces preferential uptake in the ventral pallidum and globus pallidus of humans and baboons in contrast to radiolabeled $D_2R$ antagonists (such as [$^{11}$C]raclopride) or other $D_2R$ agonists (such as [$^{11}$C]NPA) that show preferential uptake in the dorsal striatum (Gallezot et al. 2012; Ginovart et al. 2007; Graff-Guerrero et al. 2008; Kiss et al. 2011; Narendran et al. 2009; Rabiner and Laruelle 2010; Rabiner et al. 2009). The specific binding of [$^{11}$C](+)-PHNO in the globus pallidus of baboons was inhibited by the partial $D_3R$ agonist BP-897 (*N*-[4-[4-(2-methoxyphenyl)-1-piperazinyl]butyl]naphthalene-2-carboxamide) suggesting that the $D_3R$ contribution to the specific binding signal of [$^{11}$C](+)-PHNO is higher than that of [$^{11}$C]raclopride (Doot et al. 2019).

The distribution of the $D_3R$ gene in rats and mice is well established. Figure 2 shows the overall brain distribution of $D_1$, $D_2$, and $D_3$ transcripts in mice using in situ hybridization (ISH) assays. $D_1R$ mRNA is highly expressed in the basal ganglia, including the DST, NAc, and olfactory tubercle (Monsma et al. 1990). $D_2R$ mRNA displays similar regional distribution as the one of $D_1R$ mRNA (Gerfen et al. 1990). In addition, $D_2R$ mRNAs were found in dopaminergic cell bodies within the SNc and VTA (Bunzow et al. 1988). In contrast, the highest level of $D_3R$ mRNA was seen in the islands of Calleja, the NAc, hippocampus (Hipp), and insular cortex in rats (Fig. 3) (Bouthenet et al. 1991; Landwehrmeyer et al. 1993a; Sokoloff et al. 1990). The levels of $D_4R$ and $D_5R$ mRNA in the striatum are very low (Meador-Woodruff et al. 1992; O'Malley et al. 1992).

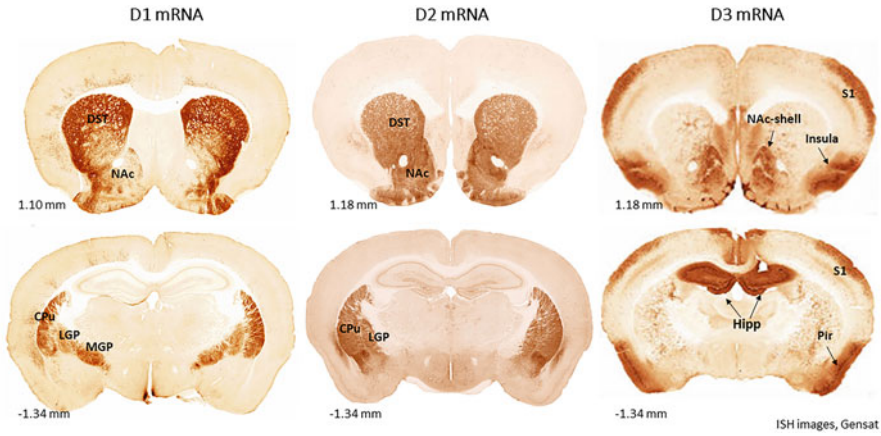


**Fig. 2** $D_3$ mRNA expression in rat brain as assessed by ISH at the level of the NAc (up panels) and thalamus (lower panel). *DST* dorsal striatum, *NAc* nucleus accumbens, *S1* primary sensory cortex, *CPu* caudate putamen, *LGP* lateral globus pallidus, *MGP* medial globus pallidus. From the public (NIH) database at: https://www.ncbi.nlm.nih.gov/probe/docs/projgensat/

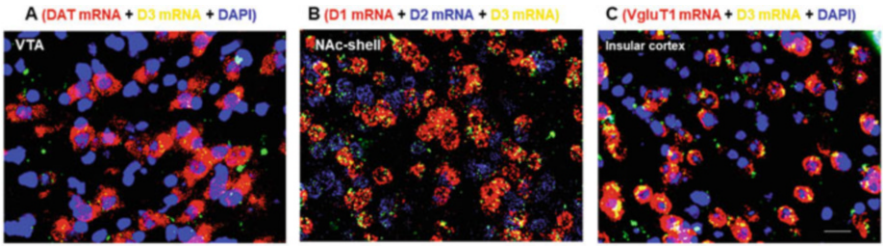


**Fig. 3** RNAscope in situ hybridization results, illustrating that low density $D_3R$ mRNA is expressed in a subpopulation of dopaminergic neurons in the VTA (**a**, red—DAT-positive dopamine neurons; yellow—D3 mRNA signal; blue—DAPI-labeled nuclei), while high density $D_3R$ mRNA is expressed in NAc $D_1$-MSNs (red) (**b**, red—D1-MSNs; yellow—D3 mRNA signal; blue—DAPI-labeled nuclei) and insular glutamate neurons (red) in mice (**c**, red—VgluT1-positive glutamate neurons; yellow—D3 mRNA signal; blue—DAPI-labeled nuclei) (Xi ZX et al., unpublished data)

In the *post-mortem* human brain, $D_3R$ mRNA expression was found on principal cells of the prefrontal cortex (PFC) and abundant in basal ganglia, but low level of expression was also evident in cingulate cortex and subcortical regions (including thalamus, Amy, locus coeruleus, raphe nuclei, etc.) (Gurevich and Joyce 1999; Landwehrmeyer et al. 1993b; Larson and Ariano 1995; Suzuki et al. 1998). In contrast to the rat, in human no $D_3R$ mRNA was detected in the VTA (Gurevich and Joyce 1999). Combination of [$^{11}$C]-(+)-PHNO PET imaging results with brain $D_3R$ and $D_2R$ mRNA expression demonstrated highest level of [$^{11}$C]-(+)-PHNO binding in the VP, globus pallidus, NAc. There is strong correlation between [$^{11}$C]-(+)-PHNO binding and $D_3R$ mRNA, but not $D_2R$ mRNA, expression (Komorowski et al. 2020). In addition, using [$^3$H]-PD128907, high densities of $D_3R$ binding were

also observed in the superficial layers of the dorsal horn at cervical and lumbar levels followed by the pars centralis and dorsal horn (Levant and McCarson 2001).

Cellular Distribution of $D_3R$

To understand which neural substrates underlie $D_3R$ function, it is critical to understand which types of cells express $D_3R$. Using a polyclonal $D_3R$ antibody, Diaz et al. detected $D_3R$-immunostaining in all tyrosine hydroxylase (TH)-positive DA neurons in the VTA, SNc, and A8 retrorubral fields, suggesting that $D_3R$ may act as a functional autoreceptor regulating DA neuron activity and DA release from their projection terminals (Diaz et al. 2000). Using double-staining ISH methods to examine $D_3$ mRNA expression in the NAc (Le Moine and Bloch 1996), the $D_3R$ mRNA is detected in a subpopulation of $D_1$- or $D_2$-expressing medium-spiny neurons (MSN)s, and also, in substance P- or enkephalin-expressing neurons, implying that DA may act on each population of postsynaptic neurons in the NAc, producing DA-dependent effects. Given that commercially available DA receptor antibodies (including anti-$D_3$ antibodies) display poor receptor specificity (Bodei et al. 2009) and classical ISH images display poor mRNA signal resolution at cellular levels, the above findings regarding the $D_3R$ cellular distribution could not be conclusive. Recently, using more specific and sensitive fluorescent $D_3R$ reporter mice, Clarkson and colleagues identified $D_3R$ signal in a small population of pyramidal neurons in the layer 5 of the PFC (Clarkson et al. 2017). We have recently used a highly sensitive and specific RNAscope ISH assays to detect the cellular distribution of $D_3R$ mRNA. We found that $D_3R$ mRNA is expressed only in a subpopulation of dopamine neurons in the VTA, while high density $D_3R$ mRNA is detected in dopamine $D_1R$-expressing medium-spiny neurons ($D_1$-MSNs) in the NAc-shell and vesicular glutamate transporter 1 (VgluT1)-positive glutamate neurons in the insular cortex of mice (Fig. 3), indicating that such new advanced techniques are highly valuable in identifying the cellular distributions of $D_3R$ genes or protein in different brain regions and tissues.

## *2.2 Relationship to Neural Targets and Therapeutic Potential*

The exact loci and neural substrates that $D_3R$ antagonists/partial agonists target in the brain are not fully understood. Based on the restricted regional and cellular distributions of $D_3R$ described above, it is reasonable to predict that both presynaptic and postsynaptic $D_3R$ mechanisms may underlie the therapeutic effects of $D_3R$ antagonists/partial agonists in animal models of drug addiction (Fig. 4).

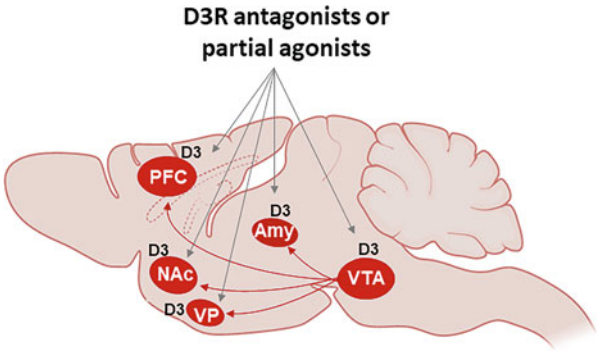


**Fig. 4** Schematic diagram of the mesolimbic DA projection system in rat brain, illustrating where high densities of $D_3R$ binding or mRNA are found or upregulated by chronic use of psychostimulants or opioids, which may constitute important targets that $D_3R$ antagonists or partial agonists act

### 2.2.1 Presynaptic $D_3R$ Mechanism

As stated above, both systemic administration of psychostimulants and opioids produce an initial increase in extracellular DA in the NAc and DST, whereas prolonged withdrawal or abstinence seems to trigger a "hypodopaminergic state" in the mesolimbic DA system, which is closely associated with craving and relapse to drug seeking (Blum et al. 2021a, b; Luscher and Pascoli 2021; Salin et al. 2021; Samaha et al. 2021; Sanna et al. 2021). One may therefore hypothesize that normalization of decreased DA transmission in the reward circuits may decrease drug craving and relapse to drug-seeking behavior. Growing evidence indicates that activation of $D_3R$ by the agonist PD-128907 inhibits DA release in the NAc and PFC possibly via presynaptic $D_3$ autoreceptors on DA terminals (Millan et al. 2010), while $D_3R$ antagonists/partial agonists produce an increase in extracellular DA levels in the NAc, PFC, or ventral hippocampus possibly by presynaptic $D_3$ autoreceptor disinhibition (Gobert et al. 1996; Huang et al. 2019; Lacroix et al. 2003; Millan et al. 2000). Thus, we propose that blockade of presynaptic $D_3R$ may in part normalize (restore) the hypodopaminergic state, and therefore, contribute to the therapeutic effects of $D_3R$ antagonists in preventing relapse to drug seeking after abstinence.

In addition, previous studies have shown that $D_1R$ or $D_2R$ agonism improves various aspects of cognitive performance in rodents as well as primates (Cai and Arnsten 1997; Marino and Levy 2019; Nakako et al. 2013). Accordingly, elevated extracellular DA after $D_3R$ antagonism may in turn stimulate $D_1R$ and $D_2R$ in both the NAc and PFC (Clarkson et al. 2017), producing pro-cognitive and pro-social behavioral changes. Thus, indirect $D_1R$ or $D_2R$ activation following presynaptic $D_3R$ antagonism may also in part contribute to $D_3R$ antagonists' effects on cognition and motivation for drug-seeking behavior.

### 2.2.2 Postsynaptic $D_3R$ Mechanisms

In addition to the presynaptic $D_3R$ mechanism, blockade of postsynaptic $D_3R$ in the brain reward circuits may also underlie $D_3R$ antagonists' action in reducing drug-taking and drug-seeking behavior.

#### NAc $D_3R$ Mechanism

Recent optogenetic studies indicate that activation of $D_1$-MSNs in the NAc is associated with positive reinforcement, while activation of $D_2$-MSNs is mostly associated with aversion (Kravitz et al. 2012; Lobo et al. 2010). Accordingly, it was hypothesized that the acute rewarding effects of psychostimulants or opioids are most likely mediated by activation of $D_1$-MSNs via Gs-coupled $D_1R$ and inhibition of $D_2$-MSNs via Gi-coupled $D_2R$ (Hikida et al. 2013; Kravitz et al. 2012; Smith et al. 2013; Yawata et al. 2012). As stated above (Fig. 3), $D_3R$ appears to be co-expressed mainly in $D_1$-MSNs and less in $D_2$-MSNs in the NAc-shell. Thus, we hypothesize that blockade of $D_3R$ in $D_1$-MSNs would cause $D_1$-MSN disinhibition and increase their excitability, which may normalize the hypodopaminergic state observed in chronic drug users, and therefore, decrease craving and motivation for drug-seeking behavior. In contrast, blockade of $D_3R$ in $D_2$-MSNs would also disinhibit $D_2$-MSNs and increase their excitability, and therefore, potentiate $D_2$-MSN-mediated aversive effects. However, $D_3R$ expression in $D_2$-MSNs is very low (Fig. 3), and therefore, $D_3R$ antagonist action in $D_2$-MSNs should be minimal. Thus, the final net effect of $D_3R$ antagonism on brain reward function would be mediated mainly by blockade of $D_3R$ on postsynaptic $D_1$-MSNs. Furthermore, blockade of $D_3R$ directly counteracts DA action after acute drug administration.

#### VP $D_3R$ Mechanism

The VP is a key hub within the reward system that mediates drug-taking and drug-seeking behaviors (Creed et al. 2016; Heinsbroek et al. 2020). Previous studies have shown that drugs of abuse enhance DA release within the VP and produce reinforcing effects (Panagis and Spyraki 1996). As stated above, high density $D_3R$ is expressed in the VP. Thus, blockade of VP $D_3R$ may also in part explain how $D_3R$ antagonists attenuate the rewarding effects produced by psychostimulants or opioids under certain experimental conditions. In addition, Pribiag et al. (2021) recently reported that 2 weeks of forced abstinence from cocaine self-administration upregulates $D_3R$ expression in VP GABAergic neurons, which project to the lateral habenula (LHb). Activation of $D_3R$ in VP GABAergic neurons underlie contextual cue-induced cocaine-seeking behavior in rats via a VP-LHb circuit (Campbell and Lobo 2021; Pribiag et al. 2021). In the LHb, glutamatergic neurons project to the RMTg, where GABAergic neurons project to DA neurons in the VTA and

functionally modulate DA neuron activity (Jhou et al. 2009). These findings suggest that $D_3R$ in VP GABA neurons may regulate VTA DA neuron activity via a VP-LHb-RMTg-VTA circuit, and therefore, modulate cocaine-seeking behavior. Accordingly, blockade of VP $D_3R$ may also explain how $D_3R$ antagonists attenuate drug- or cue-induced drug-seeking behavior.

### PFC $D_3R$ Mechanism

An early study indicated low levels of $D_3R$ are expressed in the PFC (Larson and Ariano 1995), suggesting possible involvement of cortical $D_3R$ in the cognitive effects of $D_3R$ ligands (Nakajima et al. 2013). This is supported by a recent finding that a unique population of PFC principal neuron in layer 5 expresses $D_3R$ (Clarkson et al. 2017). Notably, such $D_3R$-expressing cortical neurons lack expression of $D_1$ or $D_2$ receptor and activation of $D_3R$ in PFC neurons inhibits low-voltage-activated $Ca_V3.2$ calcium channels at the axon initial segment, causing a reduction in action potential (AP) firing. Importantly, the $D_3R$-expressing PFC neurons send axonal projections to the contralateral cortex, NAc, and basolateral amygdala (BLA), thereby possibly modulating drug-taking and drug-seeking behavior via PFC-NAc and PFC-BLA circuits (Clarkson et al. 2017).

### Insula $D_3R$ Mechanism

The insula is another node involved in the networks underlying SUD (Naqvi and Bechara 2009). The general notion emerging from recent studies is that drug craving and cue-induced urges could be complex interoceptive emotions that are processed in the insular cortex, particularly in its anterior part. Several studies in humans and experimental animals indicated insula lesions diminished drug-seeking behaviors (Contreras et al. 2007; Naqvi et al. 2007), an effect that was even more pronounced by combined damage of the insula and putamen (Gaznick et al. 2014), suggesting an abnormal connectivity of these two regions in SUD. This is further supported by a recent finding that alcoholism is associated with a loss of insula gray matter (Senatorov et al. 2015), and decreased functional connectivity between the NAc and insula was observed in alcohol-dependent rats (Scuppa et al. 2020) and aversion-resistant alcohol intake in rodents (Seif et al. 2013; Sullivan et al. 2013). The mechanisms and significance of this action remain unclear. Given that a hypodopaminergic state within the brain reward circuitry is a hallmark of an addicted state and that $D_3R$ mRNA is detected in presynaptic DA neurons in the VTA and postsynaptic glutamate neurons in the insular cortex (Fig. 3C), we predict that presynaptic $D_3R$ antagonism in the insula may also contribute to the normalization of the hypodopaminergic status, and therefore, improve the insula-NAc functional connectivity. Similarly, blockade of postsynaptic $D_3R$ in the insula would also counteract the action produced by elevated DA after acute cocaine or opioid administration.

Amygdala (Amy) $D_3R$ Mechanism

In addition to the above brain regions, the Amy is also involved in drug-taking and drug-seeking behavior. The Amy receives dopaminergic innervation (Asan 1997) and has high $D_3R$ expression (Herroelen et al. 1994; Murray et al. 1994; Suzuki et al. 1998; Tupala et al. 2001). Cocaine injections or exposure to cocaine-associated cues activates the Amy in animals and humans as assessed by neuroimaging and c-fos expression studies (Grant et al. 1996; Neisewander et al. 2000) and increase $D_3R$ expression in the Amy (Guerrero-Bautista et al. 2021). Amy lesions or microinjections of $D_3R$ receptor antagonists inhibit cocaine self-administration and contextual cue-induced cocaine seeking (McGregor and Roberts 1993; Xi et al. 2013). Microinjections of psychostimulants into the central amygdala (CeA), but not the BLA, produce a conditioned place preference, whereas selective lesions of the BLA do not affect cocaine self-administration (Meil and See 1997; Yun and Fields 2003), suggesting dissociable roles for the CeA and BLA in cocaine-related behavior (Li et al. 2008; Lu et al. 2005; O'Dell et al. 1999). The $D_3R$ expression and function in the CeA vs. BLA in psychostimulants or opioid action remain to be determined.

## *2.3 $D_3R$ Neuroadaptations Due to SUD*

A growing body of evidence suggests that aberrant $D_3R$ signaling contributes to several brain disorders. Consequently, $D_3R$ has emerged as a potential therapeutic target in the treatment of major neurological and neuropsychiatric disorders such as schizophrenia, Parkinson's disease, and SUD. However, the mechanisms underlying $D_3R$ signaling are poorly understood, either in healthy or diseased brain. Therefore, unraveling the unknown downstream signaling pathways activated by $D_3R$ in both the healthy and the diseased brain is likely to reveal new therapeutic strategies toward DA-associated disorders.

### 2.3.1 Development Changes in $D_3R$ Expression

Brain $D_3R$ mRNA is detected early in development and continually expressed during the postnatal period (Araki et al. 2007; Gurevich et al. 1999; Levant 1997). ISH assays indicate that $D_3R$ mRNA expression is restricted, almost entirely to the ventricular neuroepithelium during the whole prenatal ontogeny and that the neuronal expression of the $D_3R$ appears during the first postnatal week (after the DA innervation) (Diaz et al. 1997; Stanwood et al. 1997), suggesting that the increase in $D_3R$ mRNA expression in adults is likely to reflect functional changes in the dopaminergic innervation of the ventral striatum (Shafer and Levant 1998). This is supported by the findings that a lesion of DA neurons, impairment of axonal transport, or reduction of DA neuron firing causes a reduction in $D_3R$ gene expression (Levesque et al. 1995) and repeated treatment with levodopa rescued $D_3R$

mRNA expression in the NAc and induced an ectopic expression within the dorsal striatum (Bordet et al. 1997).

### 2.3.2 Tolerance and Desensitization after $D_3R$ Activation

DA induces only a marginal fraction of $D_3R$ to translocate from cell surface to intracellular vesicles, in stark contrast to $D_2R$ (Kim et al. 2001; Min et al. 2013), suggesting that $D_3R$ undergoes limited agonist-induced internalization. However, recent studies indicate that $D_3R$ agonists are able to induce $D_3R$ desensitization and internalization (Xu et al. 2019) via multiple intracellular signal mechanisms, including protein kinase C (PKC)- and $Ca^{2+}$/calmodulin-dependent protein kinase II (CaMKII). Desensitization may occur at homodimeric and heterodimeric $D_3R$. For example, $D_1R$-$D_3R$ heterodimers can be internalized in response to the paired stimulation of both $D_1R$ and $D_3R$ via a β-arrestin-dependent mechanism in human embryonic kidney 293 cells (Fiorentini et al. 2008; Westrich et al. 2010). PKC-mediated phosphorylation of $D_3R$ can also induce clathrin-mediated $D_3R$ endocytosis and lysosomal $D_3R$ degradation (Zhang et al. 2016b). CaMKII-mediated $D_3R$ desensitization is intracellular $Ca^{++}$-dependent, and therefore, is associated with neuronal activity (Liu et al. 2009). Palmitoylation is another post-translational modification that can regulate $D_3R$ activity. Palmitoylation is essential for cell surface expression, PKC-mediated endocytosis, and agonist-induced tolerance of $D_3R$ (Zhang et al. 2016a). Compared with $D_2R$, $D_3R$ undergoes a more extensive palmitoylation on its cysteine residues at the carboxyl terminus tail.

### 2.3.3 Neuroadaptations after Exposure to Drugs of Abuse

In vitro and in vivo studies in experimental animals suggest that drugs of abuse may cause $D_3R$ signaling abnormalities. In vitro, cocaine increases dendritic arborization and soma area in cultured dopaminergic neurons from mouse via $D_3R$-dependent activation of ERK and Akt (Collo et al. 2012). In rats, nicotine upregulates $D_3R$, but reduces $D_3$nf mRNA levels in the NAc, and therefore, increasing the $D_3R/D_3$nf ratio (Smith et al. 2015). In humans, chronic drug use induces long-lasting neuroadaptations in $D_3R$ expression, although some of the findings are conflicting (Richtand 2006). PET imaging studies with the $D_3R$-preferring radioligand [$^{11}$C](+)PHNO have shown higher number of available $D_3R$ in the SN, hypothalamus, and Amy of patients who are addicted to cocaine, compared with healthy controls (Matuskey et al. 2014). Notably, SN $D_3R$ levels correlated with years of cocaine use. Consistent with this finding, a six-fold increase in $D_3R$ mRNA levels was found in the NAc of cocaine overdose victims, as compared with age-matched and drug-free control subjects (Segal et al. 1997). Similarly, increased [$^{11}$C](+)PHNO binding is also observed in the SN of methamphetamine users (Boileau et al. 2016), and in the hypothalamus of alcohol-dependent patients (Erritzoe et al. 2014). Furthermore,

the functionally enhanced $D_3R$-Gly-9 variant was associated with the development of early-onset heroin dependence in a Chinese population (Kuo et al. 2014).

The neural mechanisms underlying $D_3R$ upregulation after chronic drug abuse are unclear. As stated above, almost all drugs of abuse increase extracellular DA and subjects with chronic drug use display hypodopaminergic states in the mesolimbic system (Leyton and Vezina 2014; Luscher and Pascoli 2021; Ron and Jurd 2005; Samaha et al. 2021). These findings suggest that the changes in $D_3R$ signaling (desensitization vs. upregulation) could be adaptative or compensatory responses to changes in extracellular DA. This is supported by the finding that DA depletion induces compensatory increases in the number and the affinity of $D_3R$ to endogenous DA or exogenous DA receptor ligands (Avalos-Fuentes et al. 2015; Prieto et al. 2011). A better understanding of how drugs of abuse alter $D_3R$ activity may uncover pathophysiologic mechanisms underlying SUD and lead to discovery of novel molecular targets for pharmacotherapeutic treatment.

## *2.4 Relationship of $D_3R$ to Pain*

Previous studies have explored the role of DA receptors in opioid analgesia and tolerance. The majority focused on the $D_2R$ and showed that nonspecific $D_2$-like receptor ligands (agonists or antagonists) are able to prevent morphine tolerance (Dai et al. 2016; Gomaa et al. 1989; Ozdemir et al. 2013). To dissect the role of different $D_2$-like receptor subtypes in this action, the $D_3$-preferring agonists 7-OH-DPAT and pramipexole were also tested. It was found that both the compounds can prevent tolerance to opioids (Cook et al. 2000; Rodgers et al. 2020; Zarrindast et al. 2002), suggesting that $D_3R$ mechanisms may be also involved in opioid analgesia. This is supported by our recent finding that both the highly selective $D_3R$ antagonists/partial agonists (VK4-116 and VK4-40) attenuate oxycodone self-administration and reinstatement to drug seeking, but without compromising oxycodone's antinociceptive effects in rats (Jordan et al. 2019b; You et al. 2019). In fact, a potentiation effect on oxycodone analgesia was observed at higher doses.

However, the neural mechanisms underlying this $D_3R$ modulation of opioid analgesia are poorly understood. Early studies indicate that intra-NAc or VTA microinjections of a DA receptor antagonist blocks noxious stimuli-induced antinociception (Altier and Stewart 1998; Gear et al. 1999), suggesting that the mesolimbic DA system could be one of the major loci that $D_3R$ antagonists modulate pain and opioid analgesia (Schmidt et al. 2002). In addition, the spinal cord could be another important location underlying DA and opioid interactions as DA ($D_1$, $D_3$) and MOR receptors are detected in the dorsal horn (Abbadie et al. 2001; Levant and McCarson 2001). This is further supported by the finding that genetic deletion of $D_3R$ in $D_3$-mutant mice altered pain-associated responses and morphine-induced antinociception at the spinal cord (Brewer et al. 2014; Clemens and Hochman 2004; Keeler et al. 2012). Furthermore, considerable evidence suggests an interaction between the $D_1R$ and $D_3R$ or between $D_3R$ and MOR receptors. The $D_3R$ has

been shown to colocalize with $D_1R$ or form $D_1$-$D_3$ heterodimers in the striatum (Fiorentini et al. 2008, 2010), which has been reported to modulate opioid analgesia and reward (Rodgers et al. 2019). In addition, $D_3R$ and MOR are colocalized with $D_1R$ in NAc $D_1$-MSNs (Galaj et al. 2020a), suggesting the possible presence of functional $D_3$-MOR heterodimers. Given that both $D_3R$ and MOR modulate intracellular adenylate cyclase and cAMP levels, it is suggested that the $D_3R$ and MOR interaction may occur at intracellular cAMP/PKA level (Zarrindast et al. 2002; Zhang et al. 2008, 2012).

## *2.5 Relationship to Other Comorbid Neuropsychiatric Disorders*

There is significant comorbidity between neuropsychiatric and SUD, which may be particularly evident in women (Chander and McCaul 2003). Persons living with affective and anxiety disorders are more likely to use alcohol or drugs of abuse. Recognition for both psychiatric and SUD comorbidity is important for improving treatment outcomes for these co-occurring conditions.

SUD and major depressive disorder (MDD) are prevalent and frequently co-occur (Volkow 2004). Comorbidity between bipolar disorder (BPD) and SUD is also highly prevalent (Post and Kalivas 2013; Salloum and Brown 2017). Lifetime prevalence estimates of depression are 30 ~ 50% among persons with cocaine use disorder (CUD) (Conway et al. 2006). The presence of depressive symptoms is associated with poorer outcomes in CUD (Leventhal et al. 2006; Raby et al. 2014). Anhedonia is a core symptom of MDD and characterized by reduced experiencing of pleasure. Anhedonia has been linked to DA dysfunction in the mesolimbic system (Der-Avakian and Markou 2012). In rodents, lower DA concentrations in the NAc have been associated with fewer attempts to work for rewards (Manduca et al. 2016). In humans, decreased DA response to psychostimulants, decreased availability of striatal $D_{2/3}$ receptors, and increased availability of DA transporters have been observed and associated with a "reward deficiency" state in patients with MDD (Koob 2013). This hypodopaminergic state may in part explain such negative symptoms experienced during abstinence as dysphoria, anhedonia, and craving, which may lead to higher reward pursuits and motivation for using illicit drugs or precipitating relapse. Accordingly, prescription stimulants such as dextroamphetamine have been proposed to address such reward deficiency in a way similar as methadone for OUD (Angarita et al. 2021b) and antidepressants have been used for the treatment depression and SUD comorbidity (Zhou et al. 2015). However, a major concern with stimulants, such as amphetamine, is their abuse potential. An alternative strategy to minimize this potential risk involves the development and use of atypical DAT inhibitors (Newman et al. 2021). In addition, the $D_3R$ antagonists/partial agonist could be promising for the treatment of the CUD and MDD comorbidity (Keck et al. 2015; Newman et al. 2012) since blocking presynaptic $D_3R$ may

facilitate DA release and normalize the hypodopaminergic status, while activation of postsynaptic DA receptors by DA or $D_3R$ partial agonists may not only mitigate withdrawal effects during abstinence but also improve dysphoria and anhedonia in patients with SUD and MDD comorbidity.

Anxiety disorders (AD) are characterized by excessive fear, anxiety, and related behavioral disturbances (Craske et al. 2017). Epidemiological studies revealed striking rates of co-occurring anxiety and SUD (Compton et al. 2007; Rogers et al. 2021). It is well documented that Amy directly modulates anxiety (Kalin et al. 2004; Lesscher et al. 2008). Early research emphasized a role of DA in the pathophysiology of anxiety (Taylor et al. 1982), which recently has been reinvigorated (Dedic et al. 2018; Kienast et al. 2008) as the Amy provides the main input to midbrain DA neurons (Fudge and Haber 2000). A recent study investigated the relationships between AD and brain $D_{2/3}$ functional activity and functional connectivity. It was found that higher DA release in the Amy was associated with lower trait anxiety and lower cingulate–amygdala functional connectivity, suggesting that a negative relationship between DA functional activity and anxiety levels and a hypodopaminergic state may also exist in AD (Berry et al. 2019). Accordingly, we hypothesize here that $D_3R$ antagonists/partial agonists may also be useful for the treatment of SUD and AD comorbidity since blockade of presynaptic $D_3R$ may increase DA release and activation of postsynaptic $D_3R$ by DA or partial $D_3R$ agonist may normalize decreased DA transmission, thereby producing anxiolytic effects.

## 3 $D_3R$ Antagonists and Partial Agonists Currently under Preclinical Investigation for SUD

### *3.1 Past $D_3R$ Preferential and Selective Antagonists that Were Tested in Clinical Trials*

To the best of our knowledge, there have been only a few selective $D_3R$ antagonists (GSK598809) or preferential $D_3R$ partial agonists (buspirone, cariprazine) that have been available clinically and only buspirone has been directly tested in a clinical study for CUD (Bergman et al. 2013).

#### 3.1.1 GSK598809

GSK598809 (Fig. 5) is a selective $D_3R$ antagonist with ~120-fold selectivity for $D_3R$ ($K_i = 6.2$ nM) over $D_2R$ ($K_i = 740$ nM) (Micheli et al. 2010; Searle et al. 2010). In a clinical study focusing on craving in smokers, a single dose of GSK598809 produced 72% to 89% $D_3R$ occupancy and transiently alleviated craving for cigarette smoking after overnight abstinence (Mugnaini et al. 2013). In addition, GSK598809 effectively reduced appetitive responses to food cues in overweight and obese

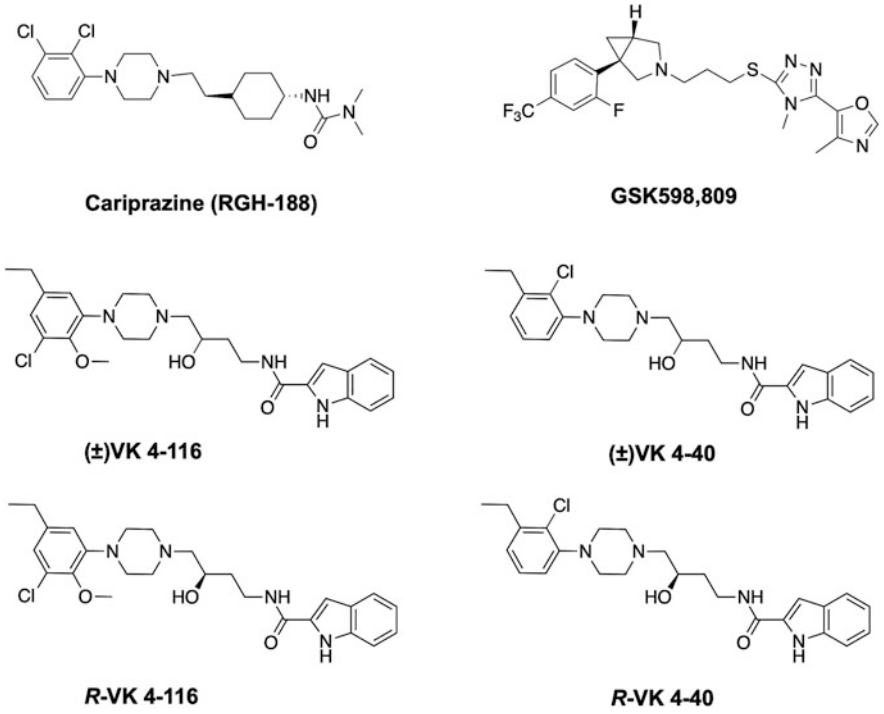


**Fig. 5** Chemical structures of lead $D_3R$ antagonists/partial agonists

individuals (Mogg et al. 2012; Nathan et al. 2012). In nonclinical studies in dogs and rats, GSK598809 was reported to increase blood pressure especially in the presence of cocaine (Appel et al. 2015), which dampened enthusiasm for conducting a clinical trial in patients with CUD.

### 3.1.2 Buspirone

Buspirone is an FDA-approved medication for the treatment of anxiety. Its therapeutic effects are believed to be mediated mainly by its partial agonist action at 5-$HT_{1A}$ receptors $K_{i\text{-High}}$ (19.2 nM) and $K_{i\text{-Low}}$ (111 nM) (Noël et al. 2014). However, buspirone also binds to $D_3R$ ($K_i = 98$ nM) (Bergman et al. 2013; Kula et al. 1994) and it was therefore proposed but failed to be effective for a clinical population with CUD (Bergman et al. 2013; Newman et al. 2012). Paradoxically, clinical studies indicate that buspirone is effective for the treatment of anxiety in individuals with alcohol use disorder (Malec et al. 1996) but not in those with OUD (McRae et al. 2004). Buspirone is also ineffective in prevention of relapse for cigarette smoking (Schneider et al. 1996) or in reductions of drug (cocaine, cannabis) and alcohol consumption (Malec et al. 1996; McRae-Clark et al. 2015;

Winhusen et al. 2014). The mechanisms underlying these negative findings are unclear; however, this may be related to its non-selectivity and low occupancy at the $D_3R$ in human brain (Le Foll et al. 2016) at doses used clinically. So far, there has been no clinical trial to evaluate the effectiveness of buspirone in controlling opioid intake and relapse. However, buspirone has been shown to reduce withdrawal symptoms in heroin addicted individuals (Buydens-Branchey et al. 2005; Rose et al. 2003).

### 3.1.3 Cariprazine

Cariprazine (Fig. 5; RGH-188) is a $D_3R$-preferring partial agonist (Citrome 2013; Gyertyan et al. 2007; Kiss et al. 2010), showing approximately ten-fold higher affinity for human $D_3R$ ($pK_i = 10.07$; $K_i = 0.085$ nM) over human $D_{2L}$ ($pK_i = 9.16$; $K_i = 0.49$ nM) and $D_{2S}$ receptors ($pK_i = 9.31$; $K_i = 0.69$ nM). In addition, it is an antagonist with high affinity at human 5-$HT_{2B}$ receptors ($pK_i = 9.24$; $K_i = 0.58$ nM). Cariprazine has been recently approved for the treatment of schizophrenia and manic or mixed episodes associated with bipolar I disorder, by the FDA. Preclinical studies indicate that cariprazine is able to reduce the rewarding effect of cocaine and relapse to cocaine-seeking behavior with half maximal effective dose ($ED_{50}$ values of 0.2 mg/kg) (Gyertyan et al. 2007; Roman et al. 2013). In addition, a recent case report indicates that cariprazine is able to improve both psychotic and addictive symptoms in subjects with persistent methamphetamine use (Ricci et al. 2022). Notably, a patient reported an abrupt decrease in substance craving and use and an improvement in positive and negative psychotic symptoms. These findings suggest that cariprazine deserves further research as an antipsychotic candidate for the treatment of SUD with bipolar disorder. Indeed, a new clinical trial has recently begun to assess the effectiveness of cariprazine for treatment of comorbid CUD and OUD (Kampman 2021).

## *3.2 New and Promising $D_3R$ Selective Antagonists/Partial Agonists for SUD*

Although developing highly selective $D_3R$ antagonists/partial agonists with improved bioavailability and pharmacokinetics profiles is challenging (Heidbreder and Newman 2010; Keck et al. 2015; Leggio et al. 2016; Pich and Collo 2015), significant progress in medicinal chemistry has been made. High $D_3R$ selectivity maybe essential to minimize $D_2R$-mediated extrapyramidal and motor side effects that would undoubtedly reduce compliance. Improved bioavailability and pharmacokinetics (PK) profiles are also critical to future translational studies toward the development of novel treatment modalities. Several $D_3R$ antagonists/partial agonists have been developed and tested in experimental animal models and have been

recently reviewed systematically elsewhere (Galaj et al. 2020b; Keck et al. 2015; Newman et al. 2021). Although there are several groups who are continuing to pursue this class of agents toward application to SUD (Ewing et al. 2021; Lv et al. 2019; Thomsen et al. 2017), herein we highlight just a few promising $D_3R$ antagonists/partial agonists for the treatment of OUD and possibly PSUD based on their favorable receptor binding and PK profiles and their pharmacological efficacy in reducing drug-taking and drug-seeking behavior (Fig. 5).

### 3.2.1 (±)-VK4-116 and its R-Enantiomer

Racemic (±)-VK4-116 is a highly selective $D_3R$ antagonist with ~1700-fold binding selectivity for $D_3R$ ($K_i = 6.84$ nM) over $D_2R$ ($K_i = 11{,}400$ nM) and is also highly selective across >70 receptors, enzymes, and transporters (Kumar et al. 2016, NIDA Treatment Discovery Program). It also showed very high metabolic stability and half-life ($t_{1/2} = 250$, 116 and 102 min in rat, human, and monkey liver microsomes, respectively) (Kumar et al. 2016). (±)-VK4-116 displayed excellent brain penetration, after oral administration (You et al. 2019) and thus was identified as a lead compound with translational potential.

Preclinical studies in rodents with (±)-VK4-116 showed promising results. For example, pretreatment with (±)-VK4-116 dose-dependently reduced the acquisition of oxycodone-induced CPP, oxycodone self-administration under FR2 and PR reinforcement schedules in rats (You et al. 2019). In addition, pretreatment with (±)-VK4-116 decreased the escalation of oxycodone self-administration in male and female rats with extended access to drug (de Guglielmo et al. 2019), facilitated extinction of drug seeking, and reduced oxycodone-primed reinstatement of drug seeking in rats (You et al. 2019). It also reduced oxycodone-induced hyperactivity and repeated oxycodone-induced locomotor sensitization in mice (Kumar et al. 2016). Furthermore, pretreatment with (±)VK4-116 dose-dependently reduced naloxone-precipitated conditioned place aversion in rats (You et al. 2019) and withdrawal-induced hyperalgesia and irritability-like behaviors (de Guglielmo et al. 2019), suggesting that (±)-VK4-116 has the ability to attenuate opioid withdrawal symptoms, a critical aspect for therapeutic utility (Koob 2021). Notably, (±)-VK4-116 has been shown to potentiate the analgesic effects of oxycodone, as assessed in a hot plate assay (You et al. 2019). This unique characteristic of (±)-VK4-116 not only supports its potential utility in the treatment of opioid use disorders (OUD) but also suggests its coadministration with prescription opioids in pain management as lower doses of prescription opioids could be used to mitigate pain when combined with (±)-VK4-116, and thus reduce the risk of abuse and the development of dependence. Of note, *R*-VK4-116 (Fig. 5) is also a highly $D_3R$-selective antagonist (Shaik et al. 2019) and is currently under development for treatment of OUD.

### 3.2.2 (±)-VK4-40 and its Enantiomers

Racemic (±)-VK4-40 (Fig. 5) is another newly developed and low efficacy $D_3R$ partial agonist with high affinity for $D_3R$ ($K_i = 0.36$ nM) over $D_2R$ ($K_i = 151$ nM) and ~ 400-fold selectivity (Kumar et al. 2016). The *R*-enantiomer (*R*-VK4-40) is a $D_3R$ antagonist, whereas the *S*-enantiomer is a partial agonist, like the racemate. *R*-VK4–40 displays high affinity for $D_3R$ ($K_i = 0.29$ nM) over $D_2R$ ($K_i = 75.8$ nM) and 261-fold selectivity for $D_3R$ over $D_2R$ (Shaik et al. 2019). The *S*-enantiomer is equally $D_3R$-selective. (±)-VK4–40 was shown not only to attenuate cocaine-primed reinstatement and cocaine-enhanced brain-stimulation reward maintained by optical stimulation of VTA DA neurons, but also to reduce cocaine self-administration across multiple cocaine doses under an FR2 schedule (Jordan et al. 2020), suggesting that (±)-VK4-40 is a potential $D_3R$ partial agonist candidate for the treatment for PSUD.

*R*-VK4–40 is metabolically stable in the presence of NADPH with 86% remaining level in the plasma over 1 h and showed excellent brain penetration after oral administration in rats (Jordan et al. 2019b). In animal models of OUD, *R*-VK4-40 dose-dependently inhibited oxycodone self-administration maintained under FR1 and PR schedules of reinforcement in rats and attenuated oxycodone-enhanced ICSS maintained by optical activation of VTA DA neurons in mice (Jordan et al. 2019b), suggesting that *R*-VK4-40 can reduce the rewarding effects of opioids. Notably, *S*-VK4-40 displayed similar pharmacological efficacy, as *R*-VK4-40, in attenuation of cocaine-enhanced brain-stimulation reward in the optical intracranial self-stimulation (ICSS) assays (Galaj et al. 2020b; Newman et al. 2021). Pretreatment with *R*-VK4–40 did not compromise the analgesic effects of oxycodone and in fact, it increased latencies to emission of thermal nociceptive response, shifting the oxycodone-dose response curve upward (Jordan et al. 2019b), suggesting an additive analgesic effect to oxycodone. *R*-VK4-40 alone also produced analgesic effects without affecting locomotor activity or performance on the rotarod test (Jordan et al. 2019b). The neural mechanisms underlying *R*-VK4-40-induced analgesic effects are yet to be determined. A possible interaction between $D_3R$ and MOR may occur in the dorsal horn of the spinal cord (Abbadie et al. 2002; Levant and McCarson 2001; Ray and Wadhwa 2004), which may in part underlie the potentiation of opioid analgesia after $D_3R$ antagonism (Jordan et al. 2019b).

### 3.2.3 Dual-Target Mu Opioid Receptor (MOR): $D_3R$ Partial Agonists

The recognition of $D_3R$ antagonism/partial agonism as an alternative and nonopioid approach for treatment of OUD without compromising opioid analgesia, combined with the possible presence of $D_3R$-MOR heterodimers prompted us to develop a novel class of dual-target ligands with MOR partial agonist and $D_3R$ antagonist/partial agonist profiles (Bonifazi et al. 2021). The idea was that these molecules would, on the one hand, block $D_3R$, mitigating the reinforcing effects of opioids as

reported previously (de Guglielmo et al. 2019; Jordan et al. 2019a; Kumar et al. 2016; You et al. 2017, 2019), while, on the other hand, partially activate MORs, producing additive or synergistic effects on opioid analgesia as $D_3R$ antagonism potentiates opioid analgesia. This drug design may lead to the development of safer dual target drugs, bridging the most promising pharmacological effects of two classes of molecules/targets previously developed independently.

### *3.3 Potential Challenges: Cardiovascular Toxicity in the Presence of Cocaine*

Although $D_3R$ has long been a focus of medication development for addiction, translational potential of $D_3R$-targeted ligands to clinical settings has, to date, been limited. One potential safety concern relates to cardiovascular effects after systemic administration. This is based on the finding that the $D_3R$, in addition to their CNS expression, are also found in the kidney, regulating blood pressure. It was reported that blockade of peripheral $D_3R$ may cause sodium retention and possibly hypertension by antagonizing the inhibitory effects of DA on sodium transport (Zeng et al. 2004, 2008). Such effects were observed in mice with genetic deletion of $D_3R$ that developed elevated systolic blood pressure and diastolic hypertension (Jose et al. 1997). In addition, two older $D_3R$ antagonists SB277011A and GSK598809 were reported to produce an increase in blood pressure in dogs and rats, particularly in the presence of cocaine (Appel et al. 2015).

To further address this issue, we have recently examined the cardiovascular effects of the novel $D_3R$ compounds *R*-VK4-116 and *R*-VK4-40 in comparison with SB-277011A and L-741,626 (a selective $D_2R$ antagonist) as controls. In this study, we found that neither *R*-VK4-116 nor *R*-VK4-40 exhibited adverse cardiovascular effects (Jordan et al. 2019b), while both SB277011A and L-741,626 did. In rats implanted with telemetric devices, cocaine or oxycodone produced a small increase in blood pressure, heart rate, body temperature, and locomotor activity, while *R*-VK4-116 produced a reduction in body temperature when administered alone (Jordan et al. 2019b). However, pretreatment with *R*-VK4-116 significantly reduced oxycodone-induced increases in body temperature and blood pressure. Similarly, cocaine-induced increases in blood pressure and heart rate were also attenuated by *R*-VK4-116 (Jordan et al. 2019b). Moreover, *R*-VK4-40 also lacks these adverse cardiovascular effects. *R*-VK4-40 alone reduced blood pressure and heart rate in rats, while pretreatment with *R*-VK4-40 attenuated oxycodone-induced increases in blood pressure and oxycodone or cocaine-induced increases in heart rate and body temperature (Jordan et al. 2019b). Greater selectivity for $D_3R$ over other receptors (e.g., $D_1$, $D_2$, or 5-HT receptors) could be an important reason why *R*-VK4-116 and *R*-VK4-40 do not share the cardiovascular effects of other older $D_3R$ antagonists (SB-277011A and GSK598809) since many other receptors also regulate cardiovascular function (Alves et al. 2019; Cuevas et al. 2013; Yang et al. 2021).

Nevertheless, these unique characteristics make both *R*-VK4-116 and *R*-VK4-40 attractive lead candidates in translational medicine for OUD and PSUD.

# 4 Perspective on Clinical Application as Treatments for SUD

## *4.1 Limitations and Advances in the Translational Value of Animal Models of SUD*

Based upon their favorable preclinical safety profiles and overwhelming evidence of efficacy in animal models of reinstatement to drug-seeking behavior, selective $D_3R$ antagonists would be expected to reduce relapse to drug-, cue-, and stress-driven consumption post-abstinence and to produce some pro-cognitive effects. Before discussing potential clinical applications of selective $D_3R$ antagonists, one must first recognize the inherent limitations of preclinical models, hence limitations in the translational value they carry to clinical research.

To mimic real-world situations, drug delivery should be active (i.e., the subject must have full control over drug delivery), dose-response effects should be systematically observed, and drug exposure should be chronic or sub-chronic rather than acute. Several animal models are based on passive drug administration, systematic dose-response studies are inconsistent, and relatively low exposure to the drug is still observed. Furthermore, evaluations of potential pharmacotherapies for SUD in animal models most often use acute medication pretreatment paradigms. The predictive validity of those models would improve if they were to adopt protocols that include longer periods of medication treatment.

Most reinstatement models include extinction training. Although the latter isolates the influence of the conditioned stimuli on reinstatement from that of the context, response habit or stress, it reduces the face validity of the model given that humans rarely undergo extinction. Thus, models that assess drug seeking after a drug-free abstinence period as opposed to instrumental extinction training may better capture the nature of cue-induced relapse in humans. In the case of abstinence models, the fact that subjects do not undergo extinction training improves the face validity of this model but restricts data interpretation as drug-seeking may actually reflect response habit, novelty-induced stress, exploratory behavior, and/or innate motivation in addition to context-induced incentive motivation for drug.

Despite limitations, recent advances in nonclinical paradigms also show promise in modeling specific DSM-5 criteria for SUD. First, the concept of addiction as a progressive transition from a positive to a negative reinforcement process that drives the motivated behavior somehow reaffirms the importance of withdrawal in addiction. In that respect, measuring the degree of dysphoria produced by drug withdrawal is highly relevant. Second, the escalation in drug intake observed after long-access training and drug intake escalation mimic increased consumption over time. Third,

the increased final ratios observed in progressive ratio paradigms appear to model the increased time and energy expended to obtain the drug. Fourth, the translational value of behavioral economics models to address the notion of discounting of delayed rewards may provide a readout of impulsivity and its related corollary of loss of control. Animal studies investigating the link between abnormal information processing in the mesocorticolimbic system and changes in responding for delayed or intermittent reinforcement are thus extremely valuable. Similarly, procedures examining choice responding under concurrent schedules of reinforcement may provide valuable insight into drug-seeking because the impact of competing reinforcers, and the work required to obtain each, can be measured simultaneously. Finally, significant work remains to be done to explore the mechanisms involved in animal models of craving and relapse and how to relate these mechanisms to vulnerability to SUD.

## *4.2 Key Translational Medicine Questions Relevant to Clinical Development*

The translational value of nonclinical paradigms should be based upon a good understanding of what needs to be achieved for the target patient population and how pharmacodynamic (PD) data can be reliably linked to pharmacological kinetics (PK). This can only be done by answering the following questions: What exactly is the therapeutic indication for the $D_3R$ drug candidate (target product profile)? What is the proposed treatment response profile? What is the proposed clinical route and frequency of dosing? What is the expected efficacious concentration in a physiological fluid (i.e., concentration-effect relationship)? How long should that concentration be maintained to obtain the desired pharmacological response? What, if any, are the biological markers to monitor toxicity and/or therapeutic effects? Do changes in route or delivery rate alter the course of effect? Is response to treatment time-dependent (e.g., onset mechanism, disease progression)? If a valid PK/PD strategy is in place and if a strong PK/PD relationship is characterized, then efficacy and tolerability can be reliably predicted from the PK data and relevant scenarios can be simulated for decision-making or clinical purposes.

The availability of PET ligands as discussed in another chapter significantly strengthens this strategy by providing PK/PD combined with receptor occupancy (RO) estimates. In this case, the investigational $D_3R$ drug can be radiolabeled and its anatomical distribution and binding in the target tissue can be traced. Alternatively, one may assess the extent to which an unlabeled investigational $D_3R$ drug inhibits specific binding of a PET ligand with known receptor affinity. In the latter case, receptor occupancy at the target receptor can be quantified, thereby enabling a deep understanding of the relationship between dose, plasma concentration, occupancy, and pharmacodynamic or clinical effects of the investigational drug. This information, in turn, leads to invaluable information to optimally design clinical Phase 1 and

Phase 2 proof of concept studies. For example, a randomized, double-blind, placebo-controlled, balanced two-way crossover study established a relationship between the occupancy of $D_3R$ in the brain using ex-vivo $^{[125I]}$7OH-PIPAT autoradiography in the rat and [$^{11}$C](+)-PHNO PET in human, the pharmacokinetic exposure to GSK598809, the ability of GSK598809 to reduce nicotine-seeking behavior using a conditioned place preference paradigm in rats, and the effect of GSK598809 on cigarette craving in smokers (Mugnaini et al. 2013). In this study, a single dose of GSK598809, giving 72–89% levels of $D_3R$ occupancy, transiently alleviated craving in smokers after overnight abstinence. GSK598809 also partially reversed the attentional bias of abstinent smokers as assessed by the Stroop test, a model of selective attention and cognitive flexibility.

The combination of PET and resting-state functional magnetic resonance imaging (fMRI) is another example of translational medicine efforts to support the development of new molecules targeting the $D_3R$. [$^{11}$C](+)-PHNO binding combined with fMRI showed that high midbrain $D_3R$ availability is associated with reduced functional connectivity between the orbitofrontal cortex and neuronal networks implicated in cognitive control and salience processing (Cole et al. 2012). Furthermore, using a rat model of chronic intermittent exposure (CIE) to alcohol (i.e., daily cycles of alcohol intoxication and withdrawal over weeks or months to mimic a pattern of alcohol use typically seen in populations with alcohol use disorder), it was shown that a history of alcohol use produced weaker functional connectivity between the insular and the cingulate cortex, but stronger connectivity between the insula and components of the mesolimbic DA system. The selective $D_3R$ antagonist, SB-277011A, however, was shown to normalize the aberrant connectivity induced by CIE to alcohol (Scuppa et al. 2020).

Altogether, these examples emphasize the importance of a thorough PK/PD/RO strategy to determine reliable dosing in humans, and/or to design combined Phase IIb/III trials allowing for more rapid progression of the medication toward regulatory approval. Specifically, they suggest that $D_3R$ are upregulated in persons living with SUD, an effect that is opposite to that found for $D_2R$. Second, they show that greater dopaminergic transmission at the $D_3R$ may contribute to motivation to use drugs of abuse. Third, they suggest that drug craving and relapse to drug-seeking behavior can be partly explained by disrupted connectivity within highly integrated neuronal networks that are relying on optimal $D_3R$ availability. One may therefore logically suggest that by modulating specific nodes in those networks, selective $D_3R$ antagonists have the potential to "normalize" functional connectivity to significantly reduce reinstatement of drug-seeking and drug-taking behaviors.

### 4.3 Most Suitable Clinical Paradigms for Medication Development Purpose

Based on the efficacy of selective $D_3R$ antagonists in a wide range of animal models of SUD and preliminary clinical Phase I data, three hypotheses could be tested in the clinic: (1) selective $D_3R$ antagonists enhance the ability to stop using the substance; (2) selective $D_3R$ antagonists have value in treating withdrawal symptoms; (3) selective $D_3R$ antagonists prevent relapse to drug-seeking and drug-taking after abstinence has been achieved (or relapse to heavy use after a reduction in use). Clinical endpoints depend upon which of these efficacy criteria are chosen and can therefore be quit rates, reduction in withdrawal symptoms, or relapse (conversely abstinence) rates over time.

There is no animal model for self-motivated stopping, little is known about the neurochemical substrates of readiness for change (stopping), and there are no data to suggest that selective $D_3R$ antagonists would enhance readiness to stop substance use. There is, however, some evidence to suggest that selective $D_3R$ antagonists would be effective for treating withdrawal symptoms. For example, (±)VK4-116 (You et al. 2018) and SB-277011A were shown to reduce conditioned place aversion (CPA) produced by naloxone-precipitated withdrawal from acute opioid administration (Rice et al. 2012) and SB-277011A also attenuated the expression of fear conditioning (Swain et al. 2008).

Based upon their efficacy in animal models of reinstatement to drug-seeking behavior, selective $D_3R$ antagonists might be considered optimal medications for the prevention of relapse in the newly abstinent substance-dependent individual across all SUD. If relapse prevention is the expected target endpoint of selective $D_3R$ antagonists, a clinical proof of concept study could be a design in which a withdrawal phase precedes randomization to either placebo vs. the new $D_3R$ antagonist in a blinded parallel design that would last 6–12 weeks. However, individuals who successfully quit during the withdrawal phase may decline to enter the randomization phase, and/or the rate of successful quitting (achieving abstinence) may be so small that large numbers of subjects must be enrolled for a relatively small number of subjects in the two arms of the randomization phase. These operational challenges translate into costly and unusually long trials for a proof of concept.

In contrast, human laboratory trials can model several aspects of SUD that are most relevant to selective $D_3R$ antagonists including cue-induced craving in abstinent individuals, choice or reward paradigms, progressive ratio paradigms, and assessments of how much a subject is willing to work for a given substance in the abstinent state. These paradigms are ideal for demonstrating clinical proof of concept since they require small sample sizes, rely primarily on crossover rather than parallel designs, and are relatively short. Although these human laboratory models have been studied with various substances, their predictive validity to demonstrate clinical efficacy of a new chemical entity remains to be established.

Craving has been described as a core feature of SUD, including those associated with opioids, alcohol, nicotine, cannabis, cocaine, and other psychoactive substances

(Kakko et al. 2019). The importance of craving as both a symptom and driver of SUD has elevated the relevance of its reduction as a critical treatment target and has renewed research focus on its role in addiction treatment and relapse (Kleykamp et al. 2019). This need was recently reinforced by the US Food and Drug Administration (FDA) in a statement on the necessity for new approaches to treat OUD (Opioid-Use-Disorder 2020; Statement-from-FDA-Commissioner 2018). There is substantial evidence showing increased craving and signs of physiological arousal to drug-related vs. neutral cues in drug users. Cue-induced craving can be studied in the human laboratory and/or in combination with imaging assessments. For example, reproducible findings have been observed in cue-induced craving in newly abstinent alcoholics (Myrick et al. 2008; Wrase et al. 2008) and in abstinent smokers (Brody et al. 2007; Due et al. 2002). The effect of a new medication on these reproducible cue-induced fMRI signals could be relatively easily determined in either single or repeat dose, parallel or crossover design, using a small number of subjects and completing the trial in a relatively short time period. Recent preliminary evidence (Regier et al. 2021) also suggests that a sustained response to repeated cocaine cues within a single passive-viewing fMRI task, featuring novel evocative (cocaine, sexual, aversive) and neutral comparator cues which were repeated later, is a potential predictor of drug-use outcomes. One may therefore suggest that pharmacological interventions that would restore a normal (i.e., decreased) response to the repeated presentation of drug-associated cues in this paradigm may predict a reduction in future drug use. This hypothesis, however, warrants future studies with potential new investigational drug candidates such as selective $D_3R$ antagonists.

Other surrogate markers might include abstinence-induced cognitive changes, such as interference on the Stroop task. For example, abstinent smokers may show altered reaction time to cigarette cues vs. neutral cues in the Stroop task, known as attentional bias induced by cues. If a compound, such as a selective $D_3R$ antagonist, is effective in preventing cue-induced relapse it would also be expected to prevent abstinence-induced cognitive changes, many of which are cue-induced. Medication effects have been demonstrated in this paradigm (Franken 2003) using either a single or repeat dose crossover study design (Patterson et al. 2009).

Ultimately, a more suitable model for SUD might be the one typically used for major depressive disorder (MDD). That model proposes acute treatment of 4–-9 months post-clinical response for the first MDD episode, but even longer treatment for 2 or more episodes (Qaseem et al. 2008). Such a treatment paradigm is one for which selective $D_3R$ antagonists would be uniquely suited, perhaps providing long-term relapse prevention for the highly recurrent and relapsing disorders of substance dependence.

As extensively reviewed, $D_3R$ is highly expressed in several brain regions such as the NAc, Amy, and prefrontal cortex (including the insula) that are critically involved in reward, anxiety, and cognitive functions. A hypodopaminergic state may exist in persons living with SUD or comorbidity with MDD or AD. Thus, we propose that selective $D_3R$ antagonists or partial agonists may be ideal for the treatment of SUD, perhaps particularly for those with MDD or AD comorbidity (Fig. 6). On the one hand, blockade of presynaptic $D_3R$ in these brain regions may

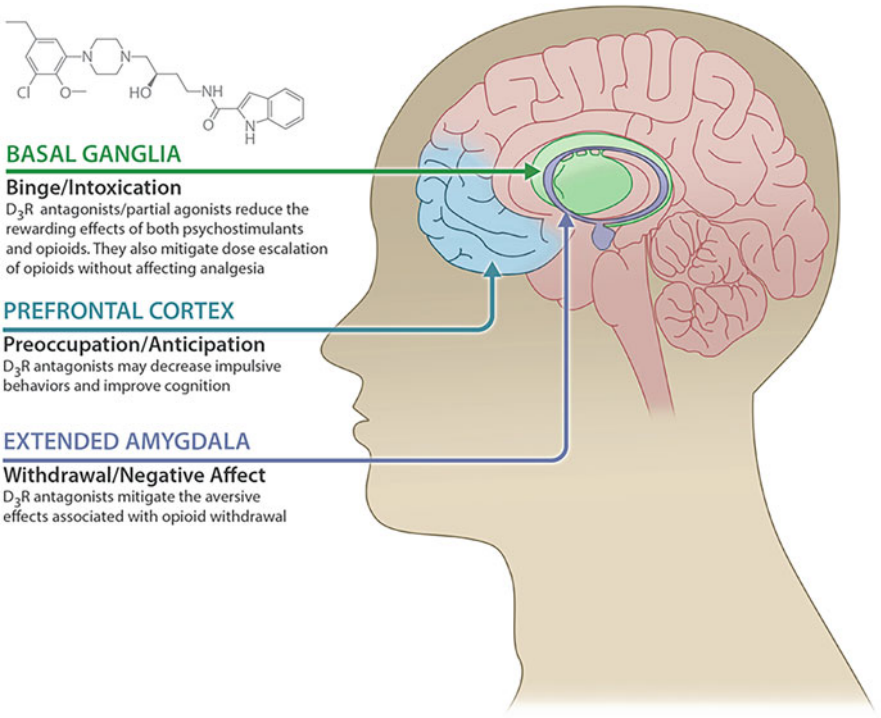


**Fig. 6** Schematic diagram illustrating the major brain regions that $D_3$R antagonists or partial agonists may target, and the major pharmacological action produced by $D_3$R antagonists or partial agonists based on recent findings from preclinical and clinical studies

normalize the hypodopaminergic state, therefore relieving craving motivation for drug seeking, and improve withdrawal/negative affect and cognitive function. Conversely, blockade of postsynaptic $D_3$R in these brain regions may reduce the rewarding effects produced by acute use of psychostimulants and/or opioids.

## *4.4 From Monotherapy to Combination of Medications*

The population of persons living with SUD has evolved considerably over time. Recent analyses suggest that among fatal opioid overdoses 78% involved another opioid, 21.6% involved cocaine, 11.1% involved alcohol, and 5.4% involved a psychostimulant other than cocaine (Jones et al. 2018). Polysubstance use of tobacco, psychostimulants, cannabis, or alcohol has also been observed in opioid-related emergency department visits (Liu and Vivolo-Kantor 2020), and the likelihood of these visits has been associated with the degree of severity of other SUD (John et al. 2019; Zale et al. 2015). Recent reports also indicate that

methamphetamine use is associated with a discontinuation of buprenorphine treatment in people with an OUD (Tsui et al. 2020).

Polysubstance use is therefore a considerable challenge for translational medicine and medication development. The majority of research on SUD has indeed focused on single drugs in isolation, with a multiple drug use history often considered an exclusion criterion for pivotal clinical trials. Real-world settings, however, indicate that polysubstance use is associated with poorer treatment retention, higher rates of relapse, and a three-fold higher mortality rate compared to mono-substance use (Williamson et al. 2006). This is to say that pharmacotherapy may also require multipronged rather than monotherapeutic strategies. Therefore, studies examining the efficacy of pharmacotherapy alone vs. combined medication and psychosocial counselling are required to better understand the role each treatment modality may have. Preliminary data indicate that buprenorphine + naloxone, used in combination with an extended-release injectable formulation of naltrexone may be associated with reductions in cocaine use among people who met DSM-4 criteria for cocaine dependence and past or current opioid dependence or abuse (Ling et al. 2016). Similarly, adults with methamphetamine use disorder who received extended-release injectable naltrexone plus oral extended-release bupropion over a 12-week period seemed to show a reduction in use as well (Trivedi et al. 2021). The use of long-acting injectable formulations of well-established medications for OUD in combination with new investigational drug candidates such as a $D_3R$ antagonist/partial agonist may open new avenues to prevent reinstatement of drug-seeking and drug-taking behaviors. In addition, the $D_3R$ antagonist/partial agonist may allow the reduction in dose of the canonical monotherapies, such as methadone or buprenorphine, and thus reduce side effects (e.g., constipation) and potential overdose.

## 5 Summary

The prevalence and horrific loss of life from SUD has recently been highlighted by the opioid crisis. COVID-19 has further exacerbated this societal problem. Social isolation, devastation brought on by massive loss of life, and fatigue of lives disrupted have all contributed to an increase in SUD which has then translated into >90,000 drug overdose deaths in the past year, in the USA alone. The rapidity with which vaccines and medications have been developed to treat COVID-19 demonstrates that when a crisis is taken seriously, biomedical research can in fact be translated into clinically useful treatments quickly. And yet, this same fervor has never been directed toward SUD. The sad outcome is limited or indeed no medications available to treat PSUD. Although medications to treat OUD are clinically approved, they are not always effective (Strain et al. 2021) nor universally available, especially in this time of restricted access. The need for moving new medications forward through the pipeline is far overdue. Although admittedly complicated, SUD is a serious and life-ending disorder for many. The time for advancing medications

such as the $D_3R$ antagonists/partial agonists as monotherapies or as part of a therapeutic regimen is now. Even if these medications are only effective for a subpopulation of patients, e.g., those who suffer from comorbidities with SUD, lives will be saved, and a perfect storm may be survived.

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# The Role of Dopamine D3 Receptors in Tobacco Use Disorder: A Synthesis of the Preclinical and Clinical Literature


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## Contents

1 Introduction ..... 204
2 Tobacco Use Disorder ..... 205
3 Pharmacotherapy for Smoking Cessation ..... 205
4 Dopaminergic System and Tobacco Use Disorder ..... 206

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5 Dopamine Receptor D3 ..... 207
6 *DRD3* Genetic Polymorphisms and Nicotine Dependence ..... 208
7 Dopamine Receptor D3 Density ..... 209
8 Reinforcement ..... 210
9 Conditioned Stimuli ..... 212
10 Conditioned Place Preference ..... 213
11 Reinstatement ..... 214
12 Drug Discrimination ..... 214
13 Sensitization ..... 215
14 Executive Function ..... 215
15 Withdrawal Signs ..... 217
16 Summary of Translational Synthesis ..... 218
References ..... 221

**Abstract** Tobacco smoking is a significant cause of preventable morbidity and mortality globally. Current pharmacological approaches to treat tobacco use disorder (TUD) are only partly effective and novel approaches are needed. Dopamine has a well-established role in substance use disorders, including TUD, and there has been a long-standing interest in developing agents that target the dopaminergic system to treat substance use disorders. Dopamine has 5 receptor subtypes (DRD1 to DRD5). Given the localization and safety profile of the dopamine receptor D3 (DRD3), it is of therapeutic potential for TUD. In this chapter, the preclinical and clinical literature investigating the role of DRD3 in processes relevant to TUD will be reviewed, including in nicotine reinforcement, drug reinstatement, conditioned stimuli and cue-reactivity, executive function, and withdrawal. Similarities and differences in findings from the animal and human work will be synthesized and findings will be discussed in relation to the therapeutic potential of targeting DRD3 in TUD.

**Keywords** Dopamine · Dopamine receptor D3 · Nicotine dependence · Smoking cessation · Tobacco use disorder

## 1 Introduction

This chapter provides an overview of the role of the dopamine receptor D3 (DRD3) in processes relevant to tobacco use disorder (TUD). To begin, we define TUD and introduce the problem with the existing pharmacological treatments, we summarize the importance of the dopaminergic system in TUD, and we outline what the DRD3 is and why it may be an important target for novel pharmacological treatments for TUD. We then review existing preclinical and clinical studies relevant to the role of DRD3 in TUD. The resulting translational synthesis presented facilitates discussion of the future therapeutic potential of DRD3 as a novel target for tobacco smoking cessation as well as identifying future avenues for research in this field.

## 2 Tobacco Use Disorder

TUD is a Diagnostic and Statistical Manual of Mental Disorders (DSM-5) substance use disorder characterized by a problematic tobacco use pattern. TUD symptoms may include compulsive use which may manifest as use despite negative consequences, unsuccessful attempts to control use, and strong persistent craving or urge to use. Symptoms may also include the development of tolerance (i.e., requiring more tobacco to achieve the desired effect or a diminished effect with continued use of the same amount) or the development of dependence and the presence of a withdrawal syndrome (American Psychiatric Association 2013). The principal addictive component found in tobacco products is nicotine (Benowitz 2010).

Tobacco smoking is a global public health problem. There are over 1 billion smokers worldwide (World Health Organization 2019), the prevalence of daily smoking was estimated at 15% in 2015 (Peacock et al. 2018) and in 2018 nearly 75% of the 34 million smokers in the USA were estimated to be daily smokers (Creamer et al. 2019). This level of tobacco smoking is associated with high rates of morbidity and mortality. For instance, it has been estimated that tobacco use is associated with thousands of billions of dollars in health care costs and losses in productivity (Goodchild et al. 2018; Makate et al. 2019) and over eight million deaths annually (World Health Organization 2019). Health outcomes and the risk of dying from smoking-related diseases are improved by smoking cessation (Jha et al. 2013) but unfortunately TUD is a chronic relapsing condition characterized by repeated cycles of quitting and relapse (Chaiton et al. 2016; Leshner 1997).

## 3 Pharmacotherapy for Smoking Cessation

There are currently three established first-line medications that the U.S. Food and Drug Administration (FDA) has approved for smoking cessation: nicotine replacement therapy (NRT), bupropion (Zyban), and varenicline (Chantix). NRT acts via agonist action at nicotinic acetylcholine receptors mimicking the nicotine normally delivered via tobacco use, bupropion is a norepinephrine and dopamine reuptake inhibitor as well as having antagonist properties at nicotinic acetylcholine receptors, and varenicline binds highly selectively to $\alpha4\beta2$ containing nicotinic acetylcholine receptors where it acts as a partial agonist (for a full review of the pharmacological mechanisms of action of NRT, bupropion, and varenicline, see Aubin et al. (2014)). All three pharmacotherapies improve abstinence rates compared to placebo with meta-analytic evidence using abstinence data from more than 101,000 participants across 267 studies suggesting that the efficacy of NRT and bupropion is similar while the efficacy of varenicline is superior to both NRT and bupropion alone (Cahill et al. 2013).

Modeling of data from over 40 smoking cessation trials suggests that 12-month abstinence rates with these three evidence-based medications is 23% or less (Jackson

et al. 2019). While this does represent a significant improvement over unaided quit attempts, where as few as 3–5% of attempts may be successful (Hughes et al. 2004), there is clearly room to improve abstinence rates further. In addition, evidence suggests diminishing benefits from the use of smoking cessation pharmacotherapy over the first 12 months (Agboola et al. 2015; Rosen et al. 2018). For instance, varenicline appears to be better at assisting smokers into initial abstinence rather than maintaining abstinence over the longer term (Agboola et al. 2015). In summary, relapse remains the most likely outcome of any cessation attempt even when using an evidence-based FDA-approved medication and existing smoking cessation pharmacotherapy has focused on modulating activity at the nicotinic acetylcholine receptor. There is therefore a strong clinical and public health need to discover and implement novel smoking cessation pharmacotherapy with improved efficacy capable of supporting the maintenance of long-term abstinence.

## 4 Dopaminergic System and Tobacco Use Disorder

The catecholamine neurotransmitter dopamine and the dopaminergic mesocorticolimbic circuitry (specifically the mesolimbic pathway, which projects from the ventral tegmental area (VTA) to the nucleus accumbens (NAc) in the ventral striatum, and the mesocortical pathway, which projects from the VTA to the prefrontal cortex) have long been implicated in substance use disorders (Feltenstein and See 2008). For instance, nicotine induces dopamine release in non-human primates (Marenco et al. 2004) and in humans, cigarette smoking induces dopamine release in these midbrain and cortical dopaminergic regions (Brody et al. 2004; Le Foll et al. 2014a, b; Wing et al. 2015). The ability of nicotine to increase midbrain dopamine is thought to underlie its reinforcing and motivational effects with the magnitude of dopamine release following smoking challenge associated with motivation to smoke (puff rate) and a reduction in both craving and withdrawal symptoms (Le Foll et al. 2014a, b).

In addition to its critical role in heightened nicotine reinforcement, dopamine or neuroadaptation within the dopaminergic system has been studied in association with several other addiction-relevant processes. For example, the conditioned learning of drug-related cues and the attribution of incentive salience that is thought to be an important motivational driver of use (drug "wanting") as well as underlying cue-induced urge to use (drug "craving") involve dopamine and the mesolimbic dopaminergic circuitry (Berridge 2007). Dopaminergic tone in the NAc has also been found to correlate with somatic and affective symptoms of a mecamylamine precipitated withdrawal syndrome in nicotine-dependent rats (Hildebrand et al. 1998; Natividad et al. 2010; Zhang et al. 2012) suggesting midbrain dopaminergic involvement in nicotine withdrawal. In addition, dopaminergic neuroadaptation in mesocortical projection regions resulting in reduced activity in the cingulate gyrus and dorsolateral prefrontal cortex have also been reported in those with substance use disorders and are thought to account for impairments in inhibitory control and

executive function that characterize those with substance use disorders (Volkow et al. 2009). Indeed, both hypo- and hyperdopaminergic states have been postulated to account for various substance use disorder phenomena depending on the absence or presence of drug-related cues (Leyton and Vezina 2014) and the dopamine hypothesis of drug addiction (Melis et al. 2005) implicates a long-lasting hypodopaminergic state throughout the addiction cycle including persistence of this state in withdrawal. As dopamine and neuroadaptation within the dopaminergic system are involved in several processes considered to drive compulsive drug use and relapse, this neurotransmitter system represents a valid target for novel pharmacotherapies for smoking cessation.

## 5 Dopamine Receptor D3

Five dopamine receptors named in the order of their date of cloning and forming two major receptor sub-classes, based upon their pharmacology and sequence homology, have been identified through which the actions of dopamine are mediated. DRD1-like receptors (DRD1 and DRD5) are G-protein-coupled receptors (GCPRs) which activate adenylyl cyclase (AC) and stimulate production of cyclic adenosine monophosphate (cAMP). Conversely, DRD2-like receptors (DRD2, DRD3, and DRD4) are GCPRs that inhibit AC activity and the production of cAMP (Jaber et al. 1996). DRD3 shares approximately 50% homology with DRD2 (Sibley and Monsma 1992) and since it was first described in 1990 (Sokoloff et al. 1990) there has been much interest in characterizing functions that may distinguish DRD3 from DRD2. DRD2 has been a historical pharmacological target of interest, particularly for schizophrenia and Parkinson's disease. Modulation of DRD3 is of particular interest in substance use disorders due to its localization in addiction-relevant areas of the brain (Le Foll et al. 2000, 2005a). The greatest density of DRD3 is found in limbic regions, known to be associated with reward, motivation, and emotion (Gurevich and Joyce 1999; Murray et al. 1994), including addiction-relevant processes briefly described above. For instance, DRD3 has been found to be localized to the islands of Calleja, mammillary bodies, the NAc shell, the frontoparietal cortex, the substantia nigra/ventral tegmental area, and cerebellar lobules 9 and 10 (Diaz et al. 2000). Midbrain DRD3 is localized to tyrosine hydroxylase containing neurons suggesting a pre-synaptic, autoreceptor function at these sites (Diaz et al. 2000).

The restricted localization of DRD3 along with the increased selectivity of behavioral effects observed with DRD3 modulating agents in comparison with those believed to occur with DRD2 agents (for further discussion on this, see Le Foll et al. (2014b)) suggests that treatments targeting the DRD3 may have fewer side effects. For example, Parkinson's-like side effects that are often seen with DRD2 antagonists were not observed with the DRD3 antagonist, SB-277011-A (Reavill et al. 2000). Despite the theoretical interest in modulating the DRD3 for the treatment of TUD and for substance use disorders more generally, there have been surprisingly few studies examining the role these receptors play in processes relevant

to TUD, or the effects of DRD3 modulating pharmacological agents in nicotine-dependent animals or in humans with TUD. One reason for this has been the historical lack of selective DRD3 agents. In this chapter, we review studies conducted using animal models of nicotine dependence and the existing human studies in TUD in order to provide a translational synthesis of the role of the DRD3 in TUD and to uncover the therapeutic potential of pharmacologically modulating this receptor as a novel smoking cessation strategy.

## 6 *DRD3* Genetic Polymorphisms and Nicotine Dependence

Candidate gene studies focusing on the dopaminergic system have demonstrated that the *dopamine receptor D3* (*DRD3*) gene is significantly associated with nicotine dependence severity in European Americans and Han Chinese, with weaker associations found in African Americans (Huang et al. 2008; Wei et al. 2012). One study investigating 13 single nucleotide polymorphisms (SNPs) within the *DRD3* gene in 2,037 participants suggested that the rs6280 SNP, a functional polymorphism corresponding to a serine to glycine substitution at position 9 in the extracellular N-terminal domain of the DRD3 (Ser9Gly) resulting in higher dopamine affinity and amplified intracellular signaling, was likely driving the association between the *DRD3* gene and nicotine dependence (Huang et al. 2008). The glycine allele at this Ser9Gly polymorphism is associated with both frequency (time to first cigarette) and quantity (heaviness of smoking) of smoking indices and in addition to this, one study also found an interaction between polymorphisms of the gene encoding the DRD2 and the *DRD3* gene impacting nicotine withdrawal severity, specifically the "trouble concentrating" symptom (Vandenbergh et al. 2007). Other genetic studies have implicated a role for DRD3 in smokers with mental health disorders that are known to be associated with increased prevalence of TUD and difficulty quitting smoking. For instance, the rs1025398 polymorphism within the *DRD3* gene has been found to be associated with quantity of tobacco smoked in schizophrenia patients (Novak et al. 2010) and the rs2399496 polymorphism, a *DRD3*-associated polymorphism located approximately 1.5 kb downstream of the *DRD3* gene, is associated with depression and nicotine dependence comorbidity (Korhonen et al. 2014). The same study also found a rs2399496 genotype–nicotine dependence interaction whereby there was an almost sixfold increase in depression risk for individuals with nicotine dependence and two copies of the minor allele of the rs2399496 polymorphism compared to those without nicotine dependence and with two copies of the major allele (Korhonen et al. 2014). Taken together, the candidate gene evidence presented here provides correlational support for the involvement of DRD3 in TUD. However, polymorphism within the *DRD3* gene was not associated with either short- or long-term quitting (Ton et al. 2007) and genome-wide association studies have tended not to find an association between the *DRD3* gene and nicotine dependence or other smoking traits (e.g., Quach et al. (2020)), which weakens evidence supporting a role for DRD3 in TUD and as a target

for treatment. Nevertheless, given the positive findings described above in those with mental health disorders, future studies that ascertain if *DRD3* genetic variance is associated with difficulty quitting, particularly among vulnerable populations with mental health disorders, may lead to more personalized treatment approaches in these groups.

## 7 Dopamine Receptor D3 Density

Preclinical evidence suggests there may be upregulation of DRD3 with repeated administration of substances of abuse. This contrasts with the findings for DRD2 that typically display lower expression in response to repeated exposure to drugs of abuse (Martinez et al. 2004; Volkow et al. 1996, 2004). For instance, upregulation of DRD3 expression has been documented in response to repeated administration of cocaine and alcohol (Neisewander et al. 2004; Vengeliene et al. 2006). However, this is not without exception and repeated exposure to amphetamine has been found to be associated with downregulation of DRD3 (Chiang et al. 2003). In line with the majority of preclinical findings, studies with repeated administration of nicotine in rats have also shown upregulation of DRD3 expression (Le Foll et al. 2003a, b). However, downregulation of DRD3 has also been reported resulting from stimulation of α4β2 nicotinic acetylcholine receptors (Acharya and Kim 2019), while a further study suggests there may be sex differences in DRD3 levels following repeated nicotine administration, with female rats exhibiting decreased levels of DRD3 compared to males (Harrod et al. 2004).

In humans, positron emission tomography (PET), a molecular imaging technique that uses radioactive labeling to visualize receptor density amongst other things, can be used to assess dopamine receptor levels in the intact, living brain. Evidence from human PET studies has largely corroborated preclinical findings in that increased DRD3 levels have been reported in stimulant users (Boileau et al. 2012, 2015, 2016) and in the hypothalamus, but not the striatum, of those with alcohol use disorder (Erritzoe et al. 2014) compared to healthy controls. In addition, greater expression of DRD3 has been found in post-mortem brain studies following cocaine overdose (Mash 1997; Segal et al. 1997; Staley and Mash 1996). However, in human tobacco-related studies no difference in DRD3 levels was found in striatal autopsy samples of elderly smokers compared to former smokers and non-smokers (Court et al. 1998). In another study, the lymphocytes of smokers had 30% lower DRD3 mRNA expression compared to non-smoker controls, with no such reduction observed in former smokers. In addition, this study also showed that DRD3 mRNA expression negatively correlated with heaviness of smoking (Czermak et al. 2004).

PET studies in smokers using the radiotracers [$^{11}$C]-raclopride or [$^{18}$F]-fallypride, which bind non-selectively to DRD2 and DRD3, have tended to find lower levels of striatal DRD2/DRD3 in smokers compared to non-smokers (Albrecht et al. 2013; Fehr et al. 2008; Wiers et al. 2017) and suggest that male but not female smokers may exhibit DRD2/DRD3 downregulation (Brown et al. 2012). However, the lack of

studies with DRD3 selective radiotracers makes interpretation of these findings difficult. Development of [$^{11}$C]-(+)-PHNO is improving our understanding of DRD3 in TUD but there have been few studies using this radiotracer. While [$^{11}$C]-(+)-PHNO is also non-selective, it has been described as DRD3-preferring (Narendran et al. 2006) and methods have been developed to differentiate between DRD2 and DRD3 binding based upon local DRD2 and DRD3 densities at specific regions of interest, therefore allowing for a more sensitive and selective assessment of DRD3 binding than was previously possible (see Le Foll et al. 2014a, b). In addition, [$^{11}$C]-(+)-PHNO may be more sensitive to measuring smoking-induced dopamine release than [$^{11}$C]-raclopride (Gallezot et al. 2014). Indeed, acute smoking challenge after overnight abstinence reduces [$^{11}$C]-(+)-PHNO binding in both DRD2- and DRD3-rich (e.g., ventral pallidum) areas suggesting that smoking induces dopamine release in DRD3-rich regions (Le Foll et al. 2014a, b). Taken together, TUD-relevant preclinical and human studies investigating DRD3 density have provided mixed findings in terms of whether there is up- or downregulation of these receptors following repeated administration of nicotine. However, since DRD3-regions experience smoking-induced dopamine release, it is possible these receptors mediate at least some addictive behaviors that maintain smoking.

## 8 Reinforcement

One established means of assessing the reinforcing properties of substances of abuse is to measure the propensity with which animals will self-administer them (Weeks and Collins 1964). To achieve this, animals are surgically implanted with an intravenous catheter that extends into the jugular vein to allow for rapid bolus injections of the drug. Animals are then trained to press a lever to receive intravenous infusions of drug. The operant chamber that houses the animal generally has two levers: presses on one lead to infusions of the drug while the second lever is an inactive lever. Presses on the inactive lever have no programmed consequences but serve as a measure of changes in non-selective motor activity. In one study, it was found that the DRD3 antagonist SB-27011-A (0, 3, 10 mg/kg, i.p.) had no effect on responding on either lever under a fixed-ratio schedule of reinforcement under which every second response on the active lever produced an infusion of nicotine (Andreoli et al. 2003). Thus, it appears that DRD3 may not influence nicotine reinforcement. Similarly, the DRD3 partial agonist BP897 (0.3, 1, 3 mg/kg, i.p.) had no effect on responding on either the active or inactive lever for nicotine under a fixed-ratio 5 (every 5 lever presses was reinforced) schedule of reinforcement (Khaled et al. 2010).

A related study investigated the effects of SB-277011A (3, 10, 30, 56 mg/kg) on responding for nicotine under a progressive ratio (PR) of reinforcement. Under a PR schedule of reinforcement animals are required to make progressively more responses for every subsequent infusion of drug. At some point, the animal will no longer work for drug, and this represents the “break point.” PR schedules are thought

to provide a measure of the rewarding properties of a drug (Richardson and Roberts 1996). In one study (Ross et al. 2007), SB-277011-A decreased the number of reinforcers earned and responses for nicotine under a PR schedule, but only at the highest dose. SB-277011-A had no effect on responding for food under a PR schedule suggesting that the effects on responding for nicotine were specific to the drug and not due to other non-selective effects. However, it should be noted that 56 mg/kg of SB-277011-A is a very high dose of drug which may not be entirely selective for DRD3. Indeed, this dose also decreased locomotor activity, which is generally thought to be due to actions at the DRD2 and not the DRD3 (Reavill et al. 2000).

Indirect evidence for a role of DRD3 in reward is provided by one study of the effects of SB-277011-A (3, 6 or 12 mg/kg) on nicotine-enhanced brain stimulation reward. In the brain stimulation reward procedure, an animal is trained to respond on a lever for stimulation directly into the reward centers of the brain. Nicotine and other stimulants potentiate the responding of animals for brain stimulation reward and are thought to reflect the rewarding properties of the stimulants. Pre-treatment with SB-27011-A dose-dependently attenuated nicotine-enhanced brain stimulation reward (Pak et al. 2006). This suggests that DRD3 may participate in some capacity in the rewarding properties of nicotine, even if DRD3 antagonists do not directly impact on the ability of nicotine to support responding for nicotine on its own.

In humans, a common method for assessing the relative reinforcing effects of drugs of abuse is the forced-choice task (Jones and Comer 2013). This task operationalizes how rewarding a participant finds the drug of choice relative to other drug or non-drug options by quantifying the number of times it is selected. For example, in one study, smokers genotyped for the Ser9Gly polymorphism in the *DRD3* gene sampled nicotine-containing and denicotinized cigarettes before making a number of forced choices between the two cigarettes in a double-blind procedure. Smokers selected nicotine-containing cigarettes more than they did denicotinized versions suggesting they found the nicotine in the cigarettes reinforcing. However, the Ser9Gly polymorphism had no impact on the frequency of nicotine choices (Chukwueke et al. 2020).

Behavioral economic procedures have also been used to assess the reinforcing value of cigarettes in smokers. For example, the Cigarette Purchase Task (CPT) is a validated measure (Mackillop et al. 2016) that operationalizes the reinforcing value of cigarettes in monetary terms (or cigarette demand). One study examined the effects of pramipexole, a DRD3-preferring (but non-selective DRD2/DRD3) agonist on the CPT and a choice procedure where smokers could earn cigarettes, chocolate, or music. Dependent smokers had greater demand for cigarettes on the CPT and selected cigarettes more than an alternative reward compared with occasional smokers. However, pramipexole had no effect on demand for cigarettes or on the number of cigarette choices (Lawn et al. 2018). Taken together, while the number of human studies examining the potential role of DRD3 in nicotine reinforcement is limited, studies in smokers lend support to the preclinical self-administration findings suggesting that DRD3 are not directly implicated in nicotine reinforcement.

## 9 Conditioned Stimuli

Conditioned stimuli are environmental stimuli paired with substances of dependence that can induce powerful urges for the drugs by themselves. The results of preclinical studies suggest that responding for nicotine is notably influenced by the presence of conditioned stimuli (Caggiula et al. 2002a, b). In this regard, a number of studies have found that DRD3 antagonists reduce conditioned activity when rats are exposed to an environment paired with nicotine. That is, rodents are naturally inquisitive animals and changes in locomotor activity induced by substances of dependence are believed to activate a natural reward-seeking response in rats (Wise and Bozarth 1987). In one study, SB-277011-A (3, 6, or 12 mg/kg, i.p.) reduced nicotine-induced cue-induced conditioned locomotion (Pak et al. 2006). Another study found that both SB-277011-A and BP 897 reduced conditioned hyperactivity in a nicotine-paired context (Le Foll et al. 2003a, b). There was no effect of these treatments in saline control rats, suggesting that the effects were not on motor activity per se. The DRD3 partial agonist BP 897 also did not affect novelty-induced locomotion, further supporting the conclusion that these treatments do not affect non-selective motor activation.

By comparison to the effects of DRD3 antagonists and partial agonists on conditioned locomotion, SB-277011-A had no effect on responding for a conditioned stimulus. After training to respond for nicotine under a fixed-ratio schedule of reinforcement in the presence of a conditioned stimulus, the drug was withheld and responding for the stimulus on its own was measured. SB-277011-A (0, 3, 10 mg/mg, i.p.) did not affect this responding (Andreoli et al. 2003). Similarly, it had no effect on latencies to respond for the CS. Thus, there appear to be some discrepancies in the role of DRD3 ligands in stimulus-maintained behavior. One explanation for these differences may reflect the fact that conditioned locomotion is under the control of a passively presented stimuli, where responding for the stimulus is an active form of stimulus presentation. Studies have shown that dopamine is increased after presentation of passive stimuli but not actively earned stimuli (Di Ciano et al. 1998a, b; Ito et al. 2000, 2002). DRD3 may be important specifically in behaviors under the control of passively presented stimuli.

In humans, reactivity to smoking-related cues is typically indexed as change from baseline physiological arousal or urge/craving to smoke once these conditioned cues have been presented, and relative to neutral cues. In one such study, the subjective cue-induced craving from smokers genotyped for the Ser9Gly polymorphism in the *DRD3* gene was examined before, during, and after exposure to smoking and neutral cues. Smoking-related cues elicited greater craving in smokers compared to neutral cues. Further, those smokers that were glycine carriers exhibited an attenuated cue-induced craving compared to smokers that were not glycine carriers (Chukwueke et al. 2020). This study implicates DRD3 in reactivity to conditioned smoking cues. However, the direction of the findings is somewhat surprising given that the glycine allele at the Ser9Gly polymorphism has previously been associated with both frequency and heaviness of smoking (Vandenbergh et al. 2007).

Potentially, frequency and heaviness of smoking in glycine carriers may be mediated by means other than cue-induced craving but these cue-reactivity findings will need to be replicated.

Dependent smokers tend to orient their attention toward smoking-related stimuli (i.e., they exhibit an attentional bias to conditioned smoking cues). In one study, the effects of the DRD3-preffering agonist pramipexole on visual fixations toward smoking and money stimuli were examined in smokers in a double-blind placebo-controlled crossover design. Pramipexole reduced the initial attention orienting bias toward smoking-related stimuli compared to money and reduced the urge to smoke following the visual fixation task suggesting that pramipexole can reduce the salience of smoking-related cues (Freeman et al. 2015). These human studies, taken together with preclinical findings, suggest that DRD3 is likely to play an important role in the expression of conditioned cue-induced behavior in smokers.

## 10 Conditioned Place Preference

Another means of testing the conditioned rewarding properties of drugs is through the conditioned place preference (CPP) model (Tzschentke 1998). In this paradigm, rats learn to associate different environments with unique outcomes (drug or non-drug). One side of the box is paired with a drug of reward and the other with placebo or another control. Since the two sides of the box vary on sensory qualities, the animal learns that one side is associated with reward. On test day, the animal is placed in the middle of the two sides of the box and the time spent on either side is measured. Animals tend to spend more time in the side of the box paired with reward. In one study, the DRD3 partial agonist BP 897 (0.1, 0.3, 1 mg/kg) or the DRD3 antagonist ST198 (3, 30, 100 mg/kg) blocked the expression of CPP when rats were injected with these agents prior to the test session (Le Foll et al. 2005b). In another study (Pak et al. 2006), pre-treatment with SB-277011-A (3, 6, or 12 mg/kg) dose-dependently attenuated nicotine CPP. When tested on its own, SB-277011-A had no effects on its own and did not induce a place aversion when paired with one side of a CPP box. Thus, it appears that the effects of DRD3 antagonists on CPP are not related to any aversive properties of these ligands, but rather, they are due to an impact of these antagonists and partial agonists on CPP. These findings are supported by the results of other studies that found that a number of DRD3 antagonists (Micheli et al. 2007), as well as 1 and 3 mg/kg of GSK598809 (Mugnaini et al. 2013), dose-dependently reduced nicotine CPP. This effect was apparent when administered 0.5 h before the test, but was attenuated with a 4 h pre-treatment interval, and there was no effect with an 8 h pre-treatment interval (Mugnaini et al. 2013). It should be noted that CPP is an example of behavior controlled by the presentation of passive stimuli and these findings are therefore consistent with those reviewed above (Le Foll et al. 2003a, b; Pak et al. 2006), which found effects of DRD3 antagonists and partial agonists on conditioned locomotion.

## 11 Reinstatement

Substance use disorder has been characterized as a chronic relapsing disorder (Leshner 1997). Thus, potential treatments for substance use disorder have often focused on the ability of the potential intervention to prevent relapse to drug use. Relapse to drug use is known to be induced by a number of environmental factors including contexts, conditioned stimuli, stress, and exposure to the drug itself. This type of relapse is modeled by the reinstatement paradigm (Epstein and Preston 2003). In this model, animals are trained to respond for a drug to a certain criterion before access to the drug is suspended. After discontinuation of the drug, responding for the drug decreases or extinguishes. The "relapse" occurs when responding on the drug-appropriate lever in reinstated by exposure to stress, contexts, conditioned stimuli, or injections of the drug itself.

In one study, SB-277011-A (0, 3, 10 mg/kg, i.p.) decreased nicotine-induced reinstatement, suggesting that DRD3 antagonists may attenuate this type of relapse (Andreoli et al. 2003). No effects were seen on responding on the inactive lever, suggesting that the effects were selective for nicotine and did not represent changes in motor activity or other activating effects. Similarly, SB-277011-A reduced cue-induced reinstatement (Khaled et al. 2010) without effect on the inactive lever and also attenuated context-induced reinstatement (Sabioni et al. 2016). It should be noted that the DRD3 partial agonist (Pilla et al. 1999) BP 897 (0.3, 1, 3 mg/kg, i.p.) had no effect on cue-induced reinstatement (Khaled et al. 2010). Thus, DRD3 antagonists (SB-277011-A) and partial agonists may have differential efficacy in treating relapse.

Taken together with findings described in the conditioned stimuli section (above), evidence implicates DRD3 in addictive processes that involve the processing of conditioned cues (such as reinstatement of drug seeking in the presence of cues as discussed here, and cue-induced craving, and attentional orienting to drug-related stimuli discussed above).

## 12 Drug Discrimination

Drug discrimination is a paradigm that tests the similarity in interoceptive or subjective effects produced by exposure to different drugs (Solinas et al. 2006). In this model, the animal is trained to respond on two levers. Responding on one lever is reinforced in the presence of a drug such as nicotine, and the other in the presence of a control, such as saline. When trained, the nicotine is replaced with a test substance such as the potent and selective DRD3 antagonist SB-277011-A and responding on the two levers is measured. If the animal responds more on the drug-appropriate lever, then it can be concluded that the test agent has interoceptive/subjective properties that are similar to the original drug.

DRD3 agents do not appear to impact the drug discriminative effects of nicotine. In one study, a DRD3 partial agonist and DRD3 antagonist did not substitute for nicotine in a test of drug discrimination (Le Foll et al. 2005a, b). When given prior to responding for various doses of nicotine, neither drug produced a shift in the dose-response curve, suggesting that DRD3 agents do not impact the subjective effects of nicotine. However, further studies are required to determine if these findings are specific for these DRD3 compounds or whether they constitute a class effect.

## 13 Sensitization

Behavioral sensitization to nicotine appears following repeated administration. It is the process in which this repeated administration produces a progressively greater behavioral response and has been suggested to model some aspects of drug use in humans (Sax and Strakowski 2001). In the sensitization procedure, rats are injected with a rewarding drug repeatedly for several days prior to a no-drug period of a few weeks. When challenged with the drug after a period of withdrawal, the locomotor response to the drug is typically greater than that observed during the initial exposure. In one study (L. N. Smith et al. 2015), the DRD3 antagonist GR 103691 was given either daily during the initial exposure to nicotine or during the test session after 3 weeks of withdrawal. Injections during the initial phase are believed to test the effects of GR 103691 on the induction of sensitization, while pre-treatment on the test day reflects the effect of the treatment on the expression of sensitization. In this study, GR 103691 blocked the induction but not expression of sensitization. Thus, this study provides some evidence for a role of DRD3 in the acquisition of sensitized responding to reinforcing drugs. It should be noted, however, that the effects of GR 103691 on the induction of sensitization were only found in adolescent rats and not in adult rats. This might suggest that DRD3 plays a particular role in the acquisition of behavioral sensitization when nicotine use or smoking onset occurs during specific developmental periods, however, more studies are required to confirm this.

## 14 Executive Function

It has been proposed that cognitive enhancement, particularly enhancement of executive functions such as working memory, response inhibition, and cognitive flexibility, may be a treatment target for addictions (Sofuoglu et al. 2013). However, existing pharmacotherapy for substance use disorders has limited impact on executive function (Butler and Le Foll 2019). Executive dysfunction is a hallmark feature of addictions that is exacerbated during early abstinence and is associated with relapse. For example, nicotine withdrawal-related deficits in working memory and

response inhibition predict smoking relapse (Patterson et al. 2010; Powell et al. 2010).

Preclinical evidence suggests that DRD3 antagonists may improve cognitive performance including on tasks of executive function (Nakajima et al. 2013). For example, the DRD3 antagonist S33138 improved 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced or aged-related deficits in cognitive flexibility performance on an attentional set-shifting task and working memory performance in a delayed matching-to-sample task in rhesus monkeys (Millan et al. 2010). In concordance with these findings, DRD3 knock-out mice performed better on an attentional set-shifting task (Glickstein et al. 2005) and ameliorated age-related deficits on the Morris water maze task of spatial working memory (Xing et al. 2010) compared to wild-type mice. However in contrast, spatial working memory deficits in DRD3 knock-out mice have also been reported (Glickstein et al. 2002) and the DRD3 preferring antagonist nafadotride had no effect on a reversal learning task of cognitive flexibility in rats (Boulougouris et al. 2009). Together these studies provide tentative support for DRD3 antagonists improving aspects of executive function, particularly where baseline impairments are present. It is possible that DRD3 antagonism during early abstinence may ameliorate withdrawal-related executive dysfunction however, studies are required to confirm this speculation.

In humans, the Ser9Gly polymorphism of the *DRD3* gene was associated with perseverative errors on the Wisconsin Card Sorting Task, a measure of cognitive flexibility in a Chinese sample (Lane et al. 2008) also implicating DRD3 in executive function performance. However, the non-selective DRD2/DRD3 antagonist haloperidol reduced response inhibition (No-Go accuracy) in a Go/No-Go task (Luijten et al. 2013). However, it is important to consider here that the actions of haloperidol at DRD2 and not DRD3 may account for the deficits in response inhibition found in this study given that evidence from the schizophrenia literature suggests that DRD2 antagonism may impair cognitive performance in contrast to the potential effectiveness of DRD3 antagonists at reducing cognitive dysfunction (Millan and Brocco 2008).

Deficits in impulsivity are a core neurocognitive feature of substance use disorders including TUD (Lee et al. 2019; J. L. Smith et al. 2014). However, impulsivity is a multifaceted construct that is commonly operationalized in terms of two distinct sub-dimensions: impulsive action (response inhibition, i.e. having difficulty inhibiting a prepotent response) and impulsive choice (i.e., having difficulty delaying gratification, for further discussion of the non-unitary nature of impulsivity, see Broos et al. (2012)). The DRD3 preferring agonist pramipexole had no effect on temporal discounting of monetary reward in smokers (Freeman et al. 2013) suggesting DRD3 may not be implicated in impulsive choice. However, further research might consider if DRD3 is implicated in temporal discounting of cigarettes. Future studies should also examine the association between DRD3 density and the impact of selective DRD3 modulation on tasks of impulsive action in smokers. This is particularly important given that response inhibition predicts smoking relapse (Powell et al. 2010) and because impulsive action, but not impulsive choice, can predict drug-induced dopamine release in the NAc and the attribution of salience to

conditioned stimuli (Zeeb et al. 2016). Indeed, previous PET imaging studies have found significant positive associations between [$^{11}$C]-(+)-PHNO binding and impulsiveness in cocaine-dependent participants and in pathological gamblers (Boileau et al. 2013; Payer et al. 2014). These studies implicate DRD3 in impulsive action and self-report measures of impulsivity and suggest there may be a transdiagnostic association between DRD3 and impulsive action across substance and behavioral addictions.

## 15 Withdrawal Signs

Nicotine withdrawal symptoms including irritability, anxiety, difficulty concentrating, restlessness, increased appetite, depressed mood, and sleep problems may be experienced after quitting or when reducing tobacco use. This withdrawal syndrome often occurs 4–24 h following cessation and peaks on approximately the third day of abstinence, gradually reducing over the proceeding 3–4 weeks (McLaughlin et al. 2015). Withdrawal symptoms are associated with smoking relapse supporting a negative reinforcement interpretation (Robinson et al. 2019) whereby negative or aversive states motivate tobacco smoking resumption. Therefore, reducing severity of the withdrawal syndrome may be an important aspect of smoking cessation treatment.

As discussed above, DRD3 antagonists may be a novel target for acute abstinence-induced impairments in executive function. Similarly, DRD3 antagonists may reduce tobacco craving in early abstinence. In one study, smokers administered a single dose of GSK598809, a selective DRD3 antagonist, resulting in submaximal (72–89%) DRD3 occupancy reduced craving following overnight abstinence (Mugnaini et al. 2013). In contrast, there is also some evidence that agonist activity at DRD3 can alleviate other abstinence-induced nicotine withdrawal signs. For example, one preclinical study found that pramipexole, a DRD3-preferring (but non-selective DRD2/DRD3) agonist, reduced some of the somatic withdrawal signs (teeth chattering/chews and shakes) present during acute nicotine withdrawal in rats (Ohmura et al. 2011). In another study in smokers, the effects of pramipexole on reward responsivity were investigated. Reduced reward responsivity has been observed during acute abstinence compared to satiation and a single dose of pramipexole after 2 h of abstinence enhanced reward responsivity compared to placebo in a double-blind crossover study (Freeman et al. 2013).

Taken together, these studies suggest that DRD3 modulation can reduce acute nicotine withdrawal signs. However, there have been only a limited number of studies investigating the potential of DRD3-selective agents to attenuate withdrawal symptom severity. Future preclinical and human research should determine which withdrawal signs from the entire constellation of withdrawal syndrome symptoms DRD3 modulation can improve, whether this modulation is beneficial at alleviating withdrawal signs at longer durations of withdrawal, and whether symptom attenuation impacts relapse/quit success.

## 16 Summary of Translational Synthesis

Tables 1 and 2 summarize the findings of the preclinical and clinical studies reviewed here. Overall, findings of the reviewed studies provide some evidence for a treatment potential of DRD3 agents in TUD. However, there are some inconsistencies and further studies are warranted to establish further if there is benefit to this pharmacological approach. Evidence suggests that DRD3 may be particularly important for diminishing the impact of cue-controlled behavior. In preclinical studies, both DRD3 antagonists and partial agonists decreased nicotine-induced

**Table 1** Summary of the preclinical/animal studies reviewed

| Paradigm/index | DRD3 manipulation | Finding |
|---|---|---|
| **Reinforcement** | | |
| Self-administration under fixed-ratio schedule | DRD3 antagonist<br>DRD3 partial agonist | –<br>– |
| Self-administration under progressive-ratio schedule | DRD3 antagonist | ↓ |
| Nicotine-enhanced brain stimulation reward | DRD3 antagonist | ↓ |
| Nicotine conditioned place preference | DRD3 antagonist<br>DRD3 partial agonist | ↓↓<br>↓ |
| **Reinstatement** | | |
| Nicotine-induced reinstatement | DRD3 antagonist | ↓ |
| Cue-induced reinstatement | DRD3 antagonist<br>DRD3 partial agonist | ↓<br>– |
| Context-induced reinstatement | DRD3 antagonist | ↓ |
| **Conditioned stimuli** | | |
| Conditioned locomotion | DRD3 antagonist<br>DRD3 partial agonist | ↓↓<br>↓ |
| Responding for a conditioned stimulus (conditioned reinforcement) | DRD3 antagonist | – |
| **Other** | | |
| Drug discrimination | DRD3 antagonist<br>DRD3 partial agonist | –<br>– |
| Induction of behavioral sensitization | DRD3 antagonist | ↓ |
| **Executive function** | | |
| Drug- and age-induced deficits in attentional set-shifting and working memory | DRD3 antagonist | ↓ |
| Reversal learning | DRD3 preferring antagonist | – |
| Attentional set-shifting | DRD3 KO mice | ↑ |
| Working memory | DRD3 KO mice | ↑↓ |
| **Withdrawal signs** | | |
| Somatic signs (teeth chattering/chews/shakes) | DRD3 preferring agonist | ↓ |

*Abbreviations*: – = No effect, ↓ = Limited evidence of reduction, ↓↓ = Strong evidence of reduction, ↑ = Limited evidence of increase, ↑↑ = Strong evidence of increase, ↑↓ = mixed evidence

**Table 2** Summary of the clinical/human studies reviewed

| Paradigm/index | Pharmacological agent/genetic polymorphism | Finding |
|---|---|---|
| **Reinforcement** | | |
| Nicotine choice under forced choice | Ser9Gly | – |
| Nicotine choice vs alternative reinforcer | DRD3-preferring agonist | – |
| Cigarette demand on the cigarette purchase task | DRD3-preferring agonist | – |
| **Conditioned stimuli** | | |
| Cue-induced craving | Ser9Gly (Gly carriers)<br>DRD3-preferring agonist | ↓<br>↓ |
| Attentional orienting to smoking cues | DRD3-preferring agonist | ↓ |
| **Executive function/cognitive control** | | |
| Perseverative errors on the Wisconsin card sorting task | Ser9Gly (heterozygous genotype) | ↑ |
| Response inhibition in a go/no-go task | Non-selective DRD2/3 antagonist | ↓ |
| Temporal discounting | D3 preferring agonist | – |
| Relationship between impulsivity and DRD3 receptor density | PET radiotracer [$^{11}$C]-(+)-PHNO | ↑* |
| **Acute withdrawal signs** | | |
| Craving after overnight abstinence | DRD3 antagonist | ↓ |
| Acute abstinence-induced reduction in reward responsivity | DRD3-prefering agonist | ↑ |

*Abbreviations*: – = No effect, ↓ = Limited evidence of reduction, ↓↓ = Strong evidence of reduction, ↑ = Limited evidence of increase (* = positive association), ↑↑ = Strong evidence of increase, ↑↓ = mixed evidence

conditioned activity and CPP. However, the effects seem selective to passively presented cues such as contexts in these experimental animals. DRD3 antagonists also blocked cue-induced and context-induced reinstatement (as well as nicotine-induced reinstatement) suggesting utility in preventing relapse that is triggered by tobacco-related cues. These preclinical findings suggest that DRD3 agents may be helpful in controlling the "craving" and urges induced by passive exposure to drugs paired with nicotine and may also help attenuate relapse to nicotine use, although further human data testing this hypothesis is needed.

While there is some degree of translational agreement that DRD3 modulation is implicated in cue-controlled behavior, there are translational inconsistencies regarding the direction of these effects when different DRD3 agents are used. For instance, antagonists and partial agonists appear to be beneficial in preclinical models while only the DRD3-preferring agonist pramipexole has been shown to both reduce initial attentional orienting to smoking cues (suggesting a role in cue salience) and reduce craving from overnight abstinence. Further, while some genetic evidence implicates the DRD3 in cue-reactivity, findings were in the opposite direction to what would be hypothesized. Further, genetic evidence has been mixed with genome-wide association studies tending not to implicate the DRD3 loci in TUD and one study explicitly finding no association between polymorphism in the *DRD3* gene and short- or

long-term quitting. Alongside the mixed findings regarding DRD3 expression, these inconsistencies attenuate our confidence in the hypothesis that DRD3 modulation is a promising pharmacological target for smoking cessation. There have however been very few pharmacological studies conducted in humans, with the majority focusing on pramipexole. Further studies with antagonists or partial agonists are now warranted given the promising preclinical findings in relation to cue-controlled behavior.

There were no effects on responding for nicotine or on the discriminative properties of nicotine, suggesting that these ligands do not impact the rewarding or subjective properties of nicotine. Here, there is translational agreement as some human genetic and pharmacological studies also suggest a lack of involvement of DRD3 in nicotine reinforcement in smokers.

DRD3 antagonists may have cognitive enhancing properties particularly where baseline impairments exist and so may offer potential to attenuate executive dysfunction that is exacerbated by withdrawal, but further studies are needed in this area. For instance, it may be particularly interesting to see if selective DRD3 antagonists impact tasks of response inhibition in withdrawn smokers. DRD3 agents also appear to reduce withdrawal signs but again studies are limited in number and have been mixed, with both DRD3 antagonists and DRD3 agonists shown to reduce different withdrawal signs.

Taken together, translational evidence suggests that further studies are warranted with the most compelling evidence suggesting that DRD3 is an important mediator of cue salience and cue-controlled behaviors. Indeed, previous work investigating the impact of a DRD3 agonist and a DRD3 antagonist on maladaptive decision making has shown that the presence or absence of salient cues within the task determines whether DRD3 agents impact choice (Barrus and Winstanley 2016). Additionally, the DRD3 antagonist GSK598809 reduces attentional bias to palatable food cues in overweight and obese participants suggesting that the proposed role of the DRD3 in mediating the effects of cues is not restricted to TUD but may apply more generally to any salient or appetitive cues (Nathan et al. 2012).

Existing pharmacotherapy appears to be better at assisting people into abstinence rather than helping them to maintain longer duration abstinence (Agboola et al. 2015). The finding that DRD3 may be especially important for cue-mediated behavior may indicate that DRD3 agents may have greater success with sustaining abstinence because cues have such a persistent and enduring effect on human craving and relapse (e.g., Bedi et al. (2011)). Further, there may also be implications for TUD treatment in those with psychiatric comorbidities, for example depression. There is increased smoking prevalence in those with depression, and depressed smokers often have greater levels of dependence and have more difficulty quitting. Positive associations have been reported between depression severity and activation of brain regions involved in attributing smoking cue salience as well as between current depression symptoms and tobacco cue-reactivity (Kushnir et al. 2013; Weinberger et al. 2012). Therefore, DRD3 agents may be particularly effective at attenuating cue salience and cue-mediated behavior, which may improve relapse rates, in this group. Further clinical studies with DRD3 modulating agents are

warranted to establish if targeting this receptor in chronic relapsing and difficult to treat groups may improve abstinence rates compared to existing pharmacotherapy.

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# D3 Receptors and Restless Legs Syndrome

**Stefan Clemens**

## Contents

1 Introduction ..... 230
2 Prevalence of RLS ..... 231
3 Periodic Limb Movements During Sleep in RLS ..... 231
4 Pharmacological Treatment Options for RLS: Dopaminergics ..... 232
5 L-Dopa, the First Dopaminergic Drug to Treat RLS ..... 232
6 The Caveat of Dopamine D3 Receptor Agonists in the Treatment of RLS ..... 235
7 Dopamine Receptor Heteromers and D3-D1 Receptor Interactions ..... 237
8 Beyond Dopamine D3 and D1 Receptors as Individual Entities ..... 240
9 Is There a Future for D3 Receptor Modulators in RLS? ..... 241
References ..... 242

**Abstract** Restless Legs Syndrome (RLS) is a sensorimotor disorder that severely affects sleep. It is characterized by an urge to move the legs that is often accompanied by periodic limb movements during sleep (PLMS). RLS has a high prevalence in the population and is usually a life-long condition. While its origins remain unclear, RLS is initially highly responsive to treatment with dopaminergics that target the D3 receptor. However, over time patients often develop a gradual tolerance that can lead to the emergence of adverse effects and the augmentation of the symptoms. While the basal ganglia and the striatum control leg movements, the lumbar spinal cord is the gateway for the sensory processing of the symptoms and critical for the associated leg movements. D3 receptors are highly expressed in nucleus accumbens (NAc) of the striatum and the sensory-processing areas of the spinal dorsal horn. In contrast, D1 receptors are strongly expressed throughout the entire striatum and in the ventral horn of the spinal cord. Long-term treatment with D3 receptor full agonists is associated with an upregulation of the D1 receptor subtype, and D3 and D1 receptors can form functional heteromers, in which the D3R controls the D1R function. It is conceivable that the switch from beneficial

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treatment to augmentation observed in RLS patients after prolonged D3R agonist exposure may be the result of unmasked D1-like receptor actions.

**Keywords** D1 receptor · Dopamine · Dopamine receptors · Receptor interactions · Treatment efficacy

## 1 Introduction

Restless Legs Syndrome (RLS) is a prevalent sensorimotor disorder that is characterized in part by an urge to move the legs that is often accompanied by periodic limb movements during sleep (PLMS). The disorder was first characterized in 1672 and 1685 (Willis 1672, 1685). The term "restless legs" was introduced in the literature in 1945 (Ekbom 1945) and redefined in 1960 (Ekbom 1960). RLS is now defined by the following five essential criteria: (1) An urge to move the legs usually but not always accompanied by, or felt to be caused by, uncomfortable and unpleasant sensations in the legs; (2) The urge to move the legs and any accompanying unpleasant sensations begin or worsen during periods of rest or inactivity such as lying down or sitting; (3) The urge to move the legs and any accompanying unpleasant sensations are partially or totally relieved by movement, such as walking or stretching, at least as long as the activity continues; (4) The urge to move the legs and any accompanying unpleasant sensations during rest or inactivity only occur or are worse in the evening or night than during the day; (5) The occurrence of the above features is not solely accounted for as symptoms primary to another medical or a behavioral condition (e.g., myalgia, venous stasis, leg edema, arthritis, leg cramps, positional discomfort, habitual foot tapping) (Allen et al. 2014). The sensations generally establish themselves in the lower limbs first and are felt mostly during quiet wakefulness and/or when attempting to go to sleep (Odin 2004). These limb paresthesias in RLS have been defined as a "focal akathisia," to distinguish them from whole body akathisia and "inner" psychic restlessness associated with antipsychotic drugs. Unlike akathisia however, paresthesias in RLS are local, commonly idiopathic, and have a circadian peak in expression in the evening and at night (Hening 2002; Meilak et al. 2004; Clemens et al. 2006). Consequently, RLS has a negative impact on sleep, resulting in a wide range of significant associated comorbidities. The essential key criteria to diagnose RLS were originally developed by the International Restless Legs Syndrome Study group (IRLSSG) in 2003 (Allen et al. 2003) and updated in 2014 (Allen et al. 2014) (see Table 1).

In addition, to quantify these subjective and patient-specific parameters, a questionnaire was developed to assess the RLS symptoms in five categories of severity (Walters et al. 2003).

**Table 1** Essential diagnostic criteria of RLS (all must be met) (Allen et al. 2014)

| | |
|---|---|
| 1 | An urge to move the legs usually but not always accompanied by, or felt to be caused by, uncomfortable and unpleasant sensations in the legs |
| 2 | The urge to move the legs and any accompanying unpleasant sensations begin or worsen during periods of rest or inactivity such as lying down or sitting |
| 3 | The urge to move the legs and any accompanying unpleasant sensations are partially or totally relieved by movement, such as walking or stretching, at least as long as the activity continues |
| 4 | The urge to move the legs and any accompanying unpleasant sensations during rest or inactivity only occur or are worse in the evening or night than during the day |
| 5 | The occurrence of the above feature is not solely accounted for as symptoms primary to another medical or behavioral condition (e.g. myalgia, venous stasis, leg edema, arthritis, leg cramps, positional discomfort, habitual foot tapping) |

## 2 Prevalence of RLS

RLS has a wide range of prevalence in different adult populations, ranging from as low as 2% (Earley et al. 2000) to 10% or higher in both Europe and North America (Odin et al. 2002; Trenkwalder et al. 2005; Picchietti et al. 2007; Earley et al. 2011; Didriksen et al. 2017). Thus RLS is more prevalent than Parkinson's disease, which has prevalence of only 0.04% in 40–49-year-olds to <2% in over 80-year-olds (Pringsheim et al. 2014). In addition, the prevalence of the RLS increases with age and women are up to two times more likely to develop the disease than men (Allen et al. 2005). Pregnancy, particularly during the third trimester, is an associated risk factor for RLS, and increased parity is an additional predisposing factor to developing RLS later in life. The onset of RLS symptoms varies widely and daily symptoms often do not emerge before adulthood; however, pediatric RLS and RLS-like symptoms can occur in patients as young as 2 years of age (Bruni et al. 2015) and the prevalence of RLS in school-aged children and adolescents can reach up to 2–4% (Picchietti et al. 2013). To correctly identify RLS in this young population, the diagnostic criteria for RLS in children were simplified and integrated with adult RLS criteria (Picchietti et al. 2013).

## 3 Periodic Limb Movements During Sleep in RLS

RLS is often accompanied by periodic limb movements during sleep (PLMS) that are distinct from periodic limb movements during wakefulness (PLMW). These PLMS movements can serve as an additional outcome measure to assess the severity of the disorder and the efficacy of pharmacological treatments (Wetter and Pollmacher 1997; Garcia-Borreguero et al. 2004; Ferri et al. 2006; Vetrugno et al. 2006; Zucconi et al. 2006; Manconi et al. 2007, 2011; Allen et al. 2014; Ferri et al. 2017). However, PLMS are not unique to RLS, and care must be taken to distinguish PLMS in RLS from PLMS that occur in other sleep disorders or other medical

conditions, since a low percentage of the healthy population can also express PLMS in the absence of RLS (Figorilli et al. 2021).

## 4 Pharmacological Treatment Options for RLS: Dopaminergics

The cause of RLS remains unclear (Chokroverty 2014), and treatment for the disorder relies on a broad variety of drugs. Early reports suggested a relationship between anemia and the emergence of the symptoms, and initial attempts to treat RLS symptoms pharmacologically focused with varying effects on vasodilating drugs, blood transfusions, or iron therapy (Murray 1967).

## 5 L-Dopa, the First Dopaminergic Drug to Treat RLS

The high prevalence of PLMS in RLS patients suggests that the basal ganglia might play a role in the overall symptomatology. Intriguingly, while there is no evidence of a direct link between Parkinson's disease (PD) and RLS (Ondo et al. 2002), PD was associated with low dopamine metabolite levels in the brain (Bernheimer et al. 1973; Price et al. 1978), and treatment of PD was successful with L-dopa medication or a combination of L-dopa plus benserazide (inhibitor of the peripheral decarboxylase) (Birkmayer and Hornykiewicz 1962). In a subsequent study, the first of its kind in RLS patients, this combination of L-dopa/benserazide also showed beneficial responses in treating the symptoms of RLS (Akpinar 1982). In that proof-of-concept trial with five patients enrolled, L-dopa/benserazide completely resolved the symptoms in all patients and these effects were mimicked by the D2 receptor agonist bromocriptine, but worsened by pimozide, a dopamine receptor antagonist with high binding affinities to D2, D3, and D4 receptor subtypes (Silvestre and Prous 2005; Pearlstein et al. 2003). The efficacy of L-dopa and D2-like receptor agonists to significantly improve the primary symptoms of RLS has been confirmed multiple times since (e.g., Hening et al. 2004; Garcia-Borreguero et al. 2013), but the use of L-dopa has been discontinued for treating RLS, due to its strong side effects.

In support of a dopaminergic role on RLS are findings from studies that have identified circadian variations in plasma dopamine levels in healthy human volunteers and in human brain post-mortem studies, each with a trough of dopamine levels in the late evening or at night (Carlsson et al. 1980; Sowers and Vlachakis 1984). Similar data were also obtained in the rodent brain (Schade et al. 1995). As RLS symptoms emerge in the evening and at night, it is tantalizing to speculate that a paucity of dopamine levels at these times might cross a RLS symptom threshold and thus contribute to the development of the symptoms (Earley et al. 2014).

**Table 2** D3 receptor agonists used to treat RLS

| D3 receptor agonist | Full/partial agonist | Half-life (h) | Risk of augmentation |
|---|---|---|---|
| Cabergoline | Partial | 60–70 | Low |
| Ropinirole | Full | 5–6 | High |
| Pramipexole | Full | 8–12 | High |
| Rotigotine | Full | 5–7 | Medium |

Since the 1990s, dopaminergic therapies have been considered the first-line treatment for adults with RLS, both for sleep disturbance and for daytime symptoms, although in the first trials all dopaminergic drugs were administered once daily at nighttime (Trenkwalder et al. 2015). The efficacy of dopamine agonists, such as pramipexole, ropinirole, and rotigotine, have been further confirmed in evidence-based reports (Winkelman et al. 2016, 2018). An overview of currently used dopaminergic therapies is presented in Table 2.

Together, these data suggest a role for the DA system in RLS, and in particular the D2-like and the D3 receptor system, although it remains unclear if the underlying pathology is based on a hypo- (Connor et al. 2009; Montplaisir et al. 1986; Akpinar 1987; Montplaisir et al. 1991) or hyper-dopaminergic state (Earley et al. 2013, 2014, 2017; Khan et al. 2017). In RLS patients, dopaminergic abnormality has been characterized as an overly activated dopaminergic system at the presynaptic level (Earley et al. 2011).

Dopamine receptors can be found both presynaptically (Bonaventura et al. 2017; Barrett and Lokhandwala 1982; Kondo et al. 1986; Jackisch et al. 1994; Gajendiran et al. 1996; Lindgren et al. 2003; Wu et al. 2006) and postsynaptically (Huang et al. 1992; Navarro et al. 1992; Olianas et al. 2012; Centonze et al. 2003; Yang 2000; Shin et al. 2003; Waters et al. 1993; Swant et al. 2008). The presence of these receptors on both sides of the synapse provides a wealth of opportunity for the nervous system to fine-tune dopamine-mediated responses, but it also makes it difficult to ascribe specific modulatory actions to any given receptor subtype or location.

There are several dopamine pathways intrinsic to the brain: the mesocortical pathway, ascending from the ventral tegmental area (VTA); the mesolimbic pathway, also originating from the VTA; the tuberoinfundibular pathway, originating from the arcuate nucleus in the hypothalamus, and the nigrostriatal pathway, which originates from the substantia nigra (SN) (Chakravarthy et al. 2018). As the primary symptoms RLS are sensory in nature ("urge to move the legs") and as the ascending projections from the spinal cord do not project to any of these circuits in the brain, it is unlikely that the fast symptomatic relief of D3 receptor agonists can solely be attributed to any of these four pathways. However, as the basal ganglia provide pre-motor information to the spinal cord, the nigrostriatal projections and the NAc may play an instrumental role in setting the tone for the descending motor commands and the emergence of PLMS movements (Fig. 1).

There exists a fifth group of dopamine neurons in the brain, located in the dorso-posterior aspect of the hypothalamus (A11), that has projections to the frontal cortex

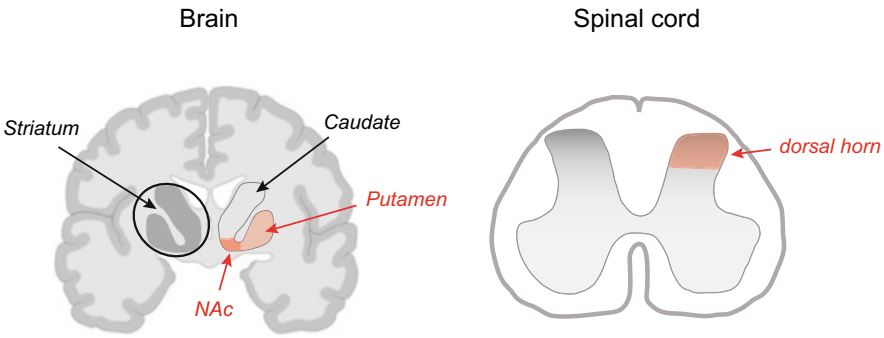


**Fig. 1** Comparison of D3 receptor hotspots in RLS-related tissues of striatum and spinal cord. In the striatum, D3 receptors are predominantly found in the nucleus accumbens (NAc) and, to a lesser degree, in the putamen. In contrast, the D3 receptor is virtually absent from the caudate. All three striatal tissues show a strong expression of the D1 receptor subtype, and a medium expression of the D2 receptor subtype (Meador-Woodruff et al. 1996). In the spinal cord, all five dopamine receptors are expressed throughout the spinal gray matter (Zhu et al. 2007) but the D3 receptor subtype has its highest expression in the dorsal laminae (Levant and McCarson 2001), where the primary integration of incoming sensory signals takes place. Left panel adapted from "Brain (coronal cut, simplified)", by BioRender.com (2022). Retrieved from https://app.biorender.com/biorender-templates

and that is also the only dopamine cluster with descending projections throughout the entire length of the spinal cord, the primary site for sensory integration and the final common output of all locomotor output (Bjorklund and Skagerberg 1979; Barraud et al. 2010; Skagerberg et al. 1982; Holstege et al. 1996). With its widespread projections notably to the dorsal horn and the intermediate and ventral laminae (Holstege et al. 1996), these dopamine fibers are ideally situated to modulate sensory signals at the site of entry from the periphery to the CNS, as well as the central pattern generating (CPG) networks that coordinate locomotion and drive leg movements via motoneurons (Clemens et al. 2006; Trenkwalder et al. 2018). Intriguingly, selective deletion of the descending A11 fibers was associated with an increase in locomotor activity that could be brought back by treating animals with a D3 receptor agonist (Ondo et al. 2000). Together with data showing that D3 receptor dysfunction increased the sensitivity of spinal reflex pathways (Clemens and Hochman 2004; Keeler et al. 2012), these findings further support a link between dopamine, D3 receptors, and a potential role of the spinal dopamine system in modulating both sensory and motor symptoms as they are observed in RLS.

In situ hybridization and rt-PCR studies have reported the presence of all DA receptor mRNAs in the rodent spinal cord (Zhu et al. 2007, 2008). While in rodents all receptors are present throughout the gray matter, the ratio of labeling was Lamina-specific for each receptor subtype (Zhu et al. 2007). Additionally, across all laminae, D2 and D5 receptors had the highest abundance and were expressed in about 50% of all identified neurons, D4 receptors in ~30% of the neurons, and D1 and D3 receptors in only 15–20%. However, the spatial distribution of the receptors

was more diverse: in Laminae 1–3, D1 expression was low (each in ~10% of the neurons), D4 was expressed in ~20% of the neurons, while D2 and D5 were found in ~40% of the cells. In contrast, in Lamina IX, D1, D2, and D5 receptors were found in >80% of the neurons (including motoneurons), while D3 and D4 receptors were present in less than half of the cells. Together, these spatial characteristics suggest a preferential inhibitory role for dopamine in the dorsal (sensory) horn of the spinal cord via D2-like and, in particular, D3 receptors, compared to a preferential excitatory role of the monoamine in the ventral (motor) horn via D1-like receptors.

In addition to these mRNA studies, quantitative autoradiography assessed D1 and D3 receptor expression across the spinal gray matter in rodents and identified a two- to fourfold higher expression of the D3 receptor subtype in the superficial dorsal horn (SDH) over dorsal horn, ventral horn, and pars centralis in all areas of the cord (cervical, thoracic, lumbar, and sacral), while D1-like receptor expression was generally similar across all laminae and only slightly increased in the SDH of the lumbar cord (Levant and McCarson 2001). As dopamine directly inhibits neurons in the pain-encoding SDH (Tamae et al. 2005), it is therefore conceivable that the initial beneficial actions of D3 receptor agonists in treating RLS symptoms are mediated via this pathway. The select activation of D3 receptors in the dorsal horn would then explain the high symptomatic relief these drugs are achieving in the clinical setting (Manconi et al. 2007, 2011). At the same time however, dopamine and D3 receptor agonists will also act on receptors in other areas of the brain and the spinal cord, additionally camouflaging the mechanistic pathways that provide the symptomatic relief for RLS symptoms.

Figure 1 provides a simplified comparison of D3 receptor hotspots in the striatum and in the spinal cord, which might help explain the actions of the D3 receptor agonists in these RLS-relevant tissues.

As the nucleus accumbens (NAc) serves as the neural interface between motivation and action and the putamen regulates movement, these D3 receptor-rich brain areas are in a prime position to respond to the activation by D3 receptor agonists and modulate the locomotor components associated with RLS. In contrast, the high prevalence of D3 receptors in the dorsal spinal cord supports the concept that dopamine actions in this area are chiefly mediated through this receptor subtype, and that the high efficacy of D3 receptor agonists in treating RLS may result from their actions on these sensory and nociceptive circuits.

## 6 The Caveat of Dopamine D3 Receptor Agonists in the Treatment of RLS

Dopaminergic treatments with nonergot-derived dopaminergics, such as ropinirole, rotigotine, and pramipexole, are initially very successful in treating the symptoms of RLS. However, despite their initial success, the dopaminergics generally lose their efficacy over time and patients develop a gradual tolerance to these compounds that

can eventually lead to the emergence of adverse effects and augmentation (Allen and Earley 1996; Allen et al. 2011; Garcia-Borreguero et al. 2015; Earley et al. 2017; Clemens and Ghorayeb 2019). Further, impulse control disorders can develop with the use of the dopaminergics in a dose-dependent manner that resolves with the termination of the dopamine-based treatment (Wanner et al. 2019). In addition to side effects induced by the long-term treatment of dopamine receptor agonists, dopamine receptor antagonists and drugs that affect dopamine and other monoamines levels indirectly can also worsen treatment outcomes for RLS (Rottach et al. 2008). For example, treatment of depression with serotonin (5-HT) reuptake inhibitors (SSRIs) upregulates 5-HT levels, including in the spinal cord, where 5-HT exerts strong excitatory effects on sensorimotor and locomotor networks. It has been proposed that such SSRI-induced PLMS are likely to be the result of enhanced serotonergic availability and secondarily decreased dopaminergic effects (Yang et al. 2005).

Augmentation remains the major cause of dopaminergic treatment failure with D3 receptor agonists and has led to the use of other drug classes in the treatment of RLS (e.g., alpha-2-delta (α2δ) ligands, iron therapies, opioids, cannabinoids). For example, pregabalin, gabapentin enacarbil, and gabapentin are effective in treating RLS symptoms and their potency is similar to that of the dopaminergics (Winkelman et al. 2018) but without the side effects of augmentation (Chenini et al. 2018). In addition, new iron treatment algorithms have been developed that have shown efficacy in treating RLS (Allen et al. 2018). Opioids represent the third main group of drugs used for the treatment of RLS, and while they are approved in Europe for the treatment of RLS, they are generally only used as a second-line treatment option for patients in the refractory form of the disorder, where they have demonstrated considerable efficacy (Trenkwalder et al. 2013; Winkelman et al. 2021). Moreover, the potential benefits of opioids must be carefully balanced against the opioid-typical side effects which include fatigue, constipation, nausea, and a potential for the worsening of sleep-disordered breathing (Winkelman et al. 2018). In addition, with nearly 5% of the adult US population misusing opioids (Skolnick 2018), caution must be taken when considering this promising alternate pharmaceutical approach, to avoid the risk of tolerance and addiction. The endocannabinoid system provides another possible therapeutic target for RLS (Ghorayeb 2021), and there are some indications that cannabis is effective and well-tolerated in patients with refractory RLS (Megelin and Ghorayeb 2017). Large placebo-controlled and double-blind clinical studies on the efficacy of medical cannabis in RLS patients are urgently needed to test their short/long-term effectiveness and safety (Ghorayeb 2021).

The lack of prospective studies evaluating the treatment of RLS augmentation makes it difficult to establish evidence-based guidelines for managing augmentation under D3 receptor agonist treatment paradigms. Therefore, special monitoring for augmentation has been recommended when initiating dopaminergic treatment (Winkelman et al. 2018). Ultimately, the fast and excellent short-term efficacy of D3 receptor agonists needs to be carefully weighed against the possible long-term side effects of these drugs (Oertel et al. 2011).

L-dopa and dopamine D3 agonists produce a pulsatile stimulation of the dopaminergic system with a sudden, efficient, but often only short-term relief of symptoms. Continuous dopaminergic stimulation (e.g., rotigotine transdermal application) improves both daytime and nighttime symptoms of RLS and is associated with augmentation, albeit to a lesser degree than the other fast-acting D3 receptor treatments. It has been suggested that any new daytime symptoms (so-called breakthrough symptoms) that occur during dopaminergic therapy may in fact reflect first symptoms of augmentation (Garcia-Borreguero et al. 2016).

## 7 Dopamine Receptor Heteromers and D3-D1 Receptor Interactions

The concept that G protein-coupled receptors (GPCRs) such as dopamine receptors form independent entities in the cell membrane that act independently of each other has in the last two decades undergone a major revision. There is growing evidence that the classical view of GPCR function and signaling, i.e. of individual receptors in the membrane that float in the plasma membrane where they can couple to their ligand, is outdated and that GPCRs can form heteromers (both heterodimers and heterotetramers) that provide the basis for the interactions of the GPCRs with adenylate cyclase (Ferre 2015; Ferre et al. 2007; Fuxe et al. 2007). For example, evidence suggests that there is an anatomical basis for the existence of functional interactions between adenosine A1 and dopamine D1 and between adenosine A2a and dopamine D2 receptors in the same neurons, and that selective A1 receptor agonists affect negatively the affinity binding of D1 receptors (Franco et al. 2000). Similarly, dopamine D1 and D2 receptors can also form functional heteromers with the histamine (H3) receptor and provide a link between the MAPK pathway and the GABA-ergic neurons in the direct striatal efferent pathway (Moreno et al. 2011). Such heteromers may even alter the affinity of the neurotransmitter to either receptor subunit, thereby providing an additional layer of complexity in modulating second messenger signaling in the target neuron (Earley et al. 2017).

Further, besides functionally coupling to other receptor classes, dopamine receptors themselves can also form both homomers and heteromers with each other, thereby modifying the dopamine-evoked response at the level of the GPCRs (see also chapter “Recent Advances in Dopamine D3 Receptor Heterodimers: Focus on Dopamine D3 and D1 Receptor-Receptor Interaction and Striatal Function”; Fuxe et al. 2015; Earley et al. 2017; Verma et al. 2010; Rashid et al. 2007). Dopamine receptor heteromers, apart from their canonical action on cAMP-mediated signaling, can therefore regulate a wide range of cellular responses to fine-tune the expression of dopamine-associated behaviors and functions, and it has been proposed that such pathways may be involved in the desensitization of GPCR activity (Beaulieu et al. 2015).

With regard to RLS, D1-D3 receptor heteromers are a particularly promising target. Firstly, these receptors can display functional interactions in both heterodimer (Fiorentini et al. 2008; Marcellino et al. 2008; Cruz-Trujillo et al. 2013) and heterotetramer configurations (Guitart et al. 2014). Secondly, in striatal protein preparations the D1 receptor can co-immunoprecipitate with the D3 receptor and, as a result of this dimerization, the D3 receptor can be switched to a desensitization mechanism typical of the D1 receptor (Fiorentini et al. 2008). Thirdly, a unique characteristic of this D1-D3 receptor complex is a synergistic interaction by which D3 receptor stimulation increases D1 receptor agonist affinity, which in turn allows a stronger stimulatory coupling of the D1 receptor to the AC/cAMP system, thus potentiating D1 receptor-mediated outcomes and behaviors (Fiorentini et al. 2010). Lastly, the reciprocal regulation of D3 and D1 receptor function in the striatum points to a potential role of this mechanism in other CNS structures as well, and the development of motor dysfunctions mediated through this mechanism may be a key feature in the emergence of PLMS in RLS patients.

In addition to these potential supraspinal actions of the D1-D3 receptor system, the distinct distribution patterns of dopamine D1 and D3 receptors in the spinal cord indicate specific tasks in those neural circuits they modulate, with D3 receptors preferentially modulating sensory afferents and D1 receptors preferentially controlling locomotor output (Fig. 2). However, despite the spatial differences, there is also substantial overlap between D1 and D3 receptors (Zhu et al. 2007), which would allow for D1-D3 receptors to form and be functionally active. Externally applied drugs will induce their respective effects across spinal cord neurons in both sensory

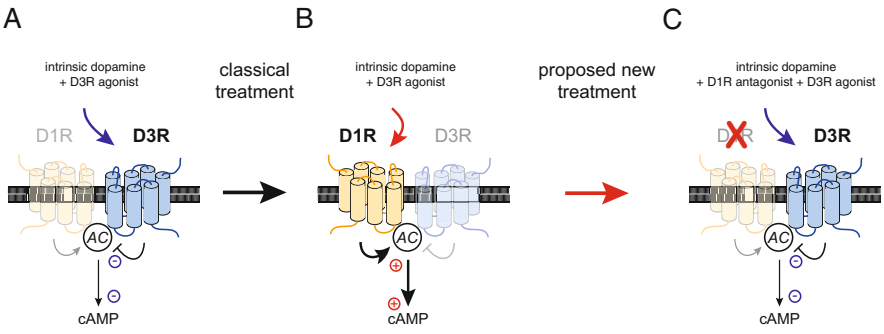


**Fig. 2** Model of changing efficacy of D3 receptor agonists in the treatment of RLS and the role of the D1 receptor. (**a**) In drug-naïve RLS patients, D3 receptor agonists (D3R) work together with intrinsic dopamine levels and activate the D3 receptor subtype, thereby reducing adenylate cyclase (AC) activities and cAMP levels in the postsynaptic neuron. (**b**) After prolonged exposure to the D3 receptor agonist, augmentation emerges and may be related to an increase in D1 receptor levels. Under these conditions, the D3 receptor is no longer fully functional and the intrinsic dopamine levels that activate the D1 receptor, leading to an upregulation of AC and increased cAMP levels. (**c**) In this state of augmentation, block of D1 receptor function, either in the presence of continued D3 receptor activation or with intrinsic dopamine levels alone, can restore the initial symptomatic relief

and motor laminae alike. Activation of the D3 pathway reduces both overall sensory (Keeler et al. 2012) and motor excitability in the isolated spinal cord (Sharples et al. 2015), while activation of the D1 receptor pathways tends to increase the excitability or the performance of neural networks that underlie or control fictive locomotion in different animal models (Han and Whelan 2009; Clemens et al. 2012).

D3 receptors can directly interact with D1 receptors, and exposure to the D3 receptor agonist PD128907 increases D1 receptor expression as a function of both time and receptor agonist concentration in rat renal proximal tubule cells (Zeng et al. 2006). In the spinal cord, the interplay between D3 and D1 receptors has recently garnered more interest, as a dysfunction of the D3 receptor system was associated with an increase of D1 receptor protein expression in the spinal cord (Brewer et al. 2014). Moreover, with age, D1R expression levels increased in both striatum and spinal cord, while D3R expression levels remained stable in the striatum or even slightly decreased in the spinal cord. The resulting D1-to-D3 receptor ratio indicated a strong upregulation of D1 receptor-mediated pathways in old animals, which was particularly pronounced in the spinal cord. These data suggest that this shift in D1 and D3 receptor-mediated signaling pathways could be an underlying factor in the emergence of RLS and its increased prevalence in the elderly (Keeler et al. 2016).

Based on the concept that the D1 receptor might be an alternate target to control the increased spinal cord excitability in RLS patients, a preliminary clinical trial was developed that used the D1 receptor antagonist as a novel treatment tool for RLS patients with augmentation. While under the level of statistical significance, the data from this study indicate an improvement of the symptoms as assessed by RLS diaries, the international RLS rating scale, and clinical global impressions (Ondo and Olubajo 2020). If these findings can be repeated in larger clinical trials, the mechanisms leading to augmentation in RLS patients after long-term use of D3 receptor drugs might then be related to a parallel stimulation of the D1R system that might be based on D1-D3 receptor heteromers (Dinkins et al. 2017). Such a transition from D3 receptor to D1 receptor-mediated actions over time would point to a synergistic role of the D1 and D3 receptor system in RLS, and it could provide a new D1 receptor-based avenue to explore as a potential pharmacological target in RLS (Fig. 2).

As outlined above, an important argument against the use of D3 receptor agonists to treat RLS symptoms is the emergence of augmentation over time, which has become the single-most important side effect (e.g., (Allen and Earley 1996; Winkelman and Johnston 2004; Williams and Garcia-Borreguero 2009; Trenkwalder et al. 2017). The presence of D1-D3 receptor heterodimers in the key nervous system tissues that regulate sensory and motor functions with regard to RLS provides a tempting background to speculate if the prolonged activation of the D3 receptor pathway during treatment may inadvertently recruit and strengthen D1 receptor-mediated excitatory pathways, as also reported from other tissues (Zeng et al. 2004; Fiorentini et al. 2010) and thus induce the opposite of the desired clinical outcome. As block of D1 receptor function has recently been reported to improve the motor outcome in Tourette's syndrome (Chipkin 2014; Gilbert et al. 2014), it is

conceivable that this receptor may also be a potential target to control increased sensory symptoms in RLS.

In support of the hypothetical model that an upregulation of the D1 receptor subtype might play a role in the augmentation phenotype of RLS, a recent preclinical study showed that the gradual decline in responsiveness to long-term treatment with D3 receptor agonists in a rodent model could be reversed or rescued by adjuvant block of D1 receptors in animals that were no longer responsive to the D3 receptor agonist alone (Dinkins et al. 2017). This rodent model was the first to explore the long-term effects of prolonged D3 receptor treatment on spinal cord-mediated reflexes, and it showed that these treatments led to a gradual decline in the efficacy of these drugs to modulate spinal reflex latencies. At the time when the modulating capacity of the D3 receptors was completely lost (4–5 weeks into the treatment), adjuvant block of the D1 receptor was sufficient to restore some of the initial modulatory effects of the D3 receptor agonists. While the behavioral test provided only limited information on the underlying mechanisms that control the change in behavioral response to continued D3 receptor exposure, these data nevertheless indicate that block of D1 receptor function is sufficient to reverse the D3 receptor agonist-induced switch after long-term treatment, and to reverse the behavioral outcome to an overall analgesic response (Dinkins et al. 2017).

## 8 Beyond Dopamine D3 and D1 Receptors as Individual Entities

Dopamine receptors can form multiple versions of heterodimers and heterotetramers (Fuxe et al. 2015; Bono et al. Chap. 3, this issue), and the D1-D3 receptor model in Fig. 2 is just one of several possible scenarios that may explain the efficacy of D3 receptor-preferring agonists in treating the initial RLS symptoms and causing the augmentation that usually occurs after long-lasting treatment. Other configurations might occur that may lead to similar functional and behavioral outcomes as in the postulated D1-D3 heteromer model. Importantly however, there is no evidence to date that all five dopamine receptors are expressed in the human spinal cord. Rodent data suggest that all five dopamine receptors are expressed in the spinal cord (Zhu et al. 2007), however a non-human primate study found no evidence of the D1 receptor subtype, but only a presence of D2–D5 receptor subtypes (Barraud et al. 2010). Thus, while the projections from the animal models indicate a likely role of dopamine that is also present in the human spinal cord, it is possible that, in man, dopamine receptor distribution may differ from those reported in rodents or the non-human primate. Immunohistochemical studies assessing receptor distributions in human spinal cord and comparing them to animal models are rare, but evidence indicates that in those studies human and animal data diverge slightly (Gillberg et al. 1988). If, as in the macaque, the human spinal cord does not express the D1 receptor, the D5 receptor subtype might functionally take over its role and emerging receptor

heterodimer could be of the D3-D5 receptor type. This putative heteromer would likely be functional similar to the D1-D3 receptor subtype, and, due to the higher affinity of the D5 over the D1 receptor subtype to dopamine, could even have a stronger impact on inhibitory and excitatory dopamine receptor homeostasis.

Alternatively, but not mutually exclusive from this scenario, the D4 receptor subtype may also play a more prominent role than previously thought. Compared to D1, D2, and D5 receptors, D3 and D4 receptors have higher affinities to dopamine, and the D4 subtype has begun to emerge as a potential new target in controlling dopamine-mediated inhibitory tone (Bonaventura et al. 2017). Thus, selective D4 receptor agonists may provide a new efficient treatment method for RLS (Yepes et al. 2017).

## 9 Is There a Future for D3 Receptor Modulators in RLS?

RLS has come a long way from an often-underdiagnosed condition to a neurological disorder in its own rights. It is now widely recognized as a disorder that heavily affects sleep and quality of life. Specific genetic risk factors increase the likelihood of developing RLS but are not required to developing the disease. Some of the risk factors appear to play a role in the early development of the nervous system, others may be epigenetic in nature.

Cabergoline is a partial D3 receptor agonist with a half-life of ~60–70 h, and studies have indicated that it has a relatively small risk of augmentation. In contrast, rotigotine, ropinirole, and pramipexole are full D3 receptor agonists with much shorter half-lives (between 6 and 12 h) and an increased risk of augmentation. As

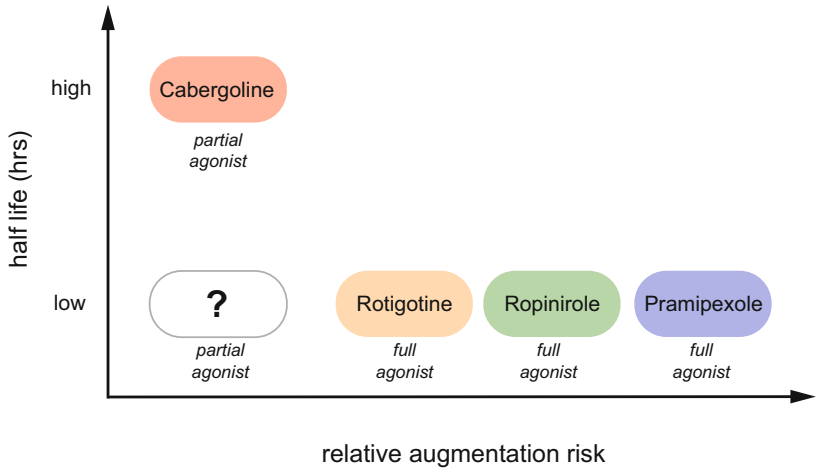


**Fig. 3** Comparison of relative augmentation risk across D3 receptor-preferring agonists currently used in the treatment of RLS, their receptor-binding profile, and their metabolic half-lives

D3 receptor agonists still provide the fastest symptomatic relief in treating RLS, it is conceivable that a novel D3 receptor partial agonist with a low metabolic half-life (Fig. 3, identified by the question mark) might combine the high efficacy of the full agonists with the low risk of augmentation observed with cabergoline. A novel compound with such pharmacological properties, continued efficacy over time, and a decreased risk of augmentation would be extremely well-received by RLS patients.

Identifying the causal links between genetic risk factors in RLS and the efficacy of D3 receptor treatments in the acute treatment setting may eventually be an alternate path for providing the tools to be more effective new D3 receptor-based treatment options that avoid the long-term side effects of the currently used therapeutics in RLS.

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# D3 Receptors and PET Imaging

**Sheida Koohsari, Yanghong Yang, and David Matuskey**

## Contents

1 Introduction ..... 252
2 Addiction ..... 254
2.1 Cocaine ..... 254
2.2 Methamphetamine ..... 256
2.3 Alcohol ..... 256
2.4 Nicotine ..... 257
2.5 Cannabis ..... 257
2.6 Food Addiction ..... 258
2.7 Gambling Disorders ..... 258
3 Neuropsychiatric Disorders ..... 258
3.1 Schizophrenia ..... 258
3.2 Parkinson's Disease ..... 261
3.3 Tourette Syndrome ..... 263
3.4 TBI ..... 265
4 Other Studies Investigating D3R in Humans ..... 265
4.1 Aging ..... 265
4.2 Social Effects ..... 265
4.3 Retinal Studies ..... 266
4.4 Next-Generation D3R Tracers ..... 266
5 Conclusion ..... 267
References ..... 267

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**Abstract** This chapter encapsulates a short introduction to positron emission tomography (PET) imaging and the information gained by using this technology to detect changes of the dopamine 3 receptor (D3R) at the molecular level in vivo. We will discuss available D3R radiotracers, emphasizing [11C]PHNO. The focus, however, will be on PET findings in conditions including substance abuse, obesity, traumatic brain injury, schizophrenia, Parkinson's disease, and aging. Finally, there is a discussion about progress in producing next-generation selective D3R radiotracers.

**Keywords** D3 · Dopamine · Humans · PET imaging

## 1 Introduction

Positron Emission Tomography (PET) is a state-of-the-art imaging modality that can investigate the living brain. The ability to investigate the brain at the molecular level with quantitative measures is a key feature of this modality that has brought critical insight to our understanding of physiologic and pathologic conditions. Mapping receptors in the brain and measuring outcomes like blood flow, metabolism, or, more recently, synaptic density is possible with PET imaging (Finnema et al. 2016). The fast-paced development of radiotracers, powerful scanners, and image analysis techniques are critical to this path of brain investigation.

A radiotracer (often shortened to tracer and also referred to as radioligand or ligand) consists of a radioactive isotope tagged to a biological molecule. After injection, when the tracer interacts with its target in the body, the scanner detects gamma rays emitted from the isotope. This information is processed into data that can be quantitively studied.

The dopamine 3 receptor (D3R) was first distinguished by Sokoloff and colleagues as a subtype of the dopamine receptor with a unique pharmacology and signaling system (Sokoloff et al. 1990). D3R is classified in the D2-like receptor family subtype, which includes D2, D3, and D4 receptors. Although this information brought attention to learning more about the new receptor, it took years to develop a suitable tracer. In particular, formulating a selective D3R radioligand has remained challenging for years due to its significant structural homogeneities with the D2 receptor (D2R). The most common dopamine tracers utilized in various studies to date are [11C]raclopride, [18F]fallypride, [11C]FLB 457, and [11C]-(+)-4-propyl-3,4,4a,5,6,10b-hexahydro-2H-naphtho[1,2-*b*][1,4]oxazine -9-ol or [11C]-(+)-PHNO (henceforth shorted to [11C]PHNO). As these tracers have a marked affinity for D2R and D3R (i.e., they are nonselective), the outcome is usually reported as D2/3R density. While [11C]raclopride, [18F]fallypride, and [11C]FLB 457 are dopamine receptor antagonists with more affinity to D2R subtypes (Halldin et al. 1995), [11C]PHNO is unique where it is a dopamine receptor agonist with a higher affinity to D3R (Gallezot et al. 2014b; Rabiner et al. 2009; Narendran et al. 2006).

Interestingly, the initial goal of developing [11C]PHNO was to measure in vivo D2R at a high-affinity state, however (Wilson et al. 2005). The D3R subtype, like other members of the D2-like family, is a G-protein-coupled receptor, and receptor agonists (hence agonist radiotracers) preferably bind to the high-affinity state of the receptor, while antagonists do not differentiate between high and low-affinity states. As the high-affinity state of the dopamine receptor is considered the functional state (George et al. 1985), an agonist radiotracer should provide different knowledge of neuroreceptor activity than antagonists because antagonist receptors are unable to differentiate between functional and nonfunctional states of the receptor.

Willeit and colleagues were one of the first groups who explored [11C]PHNO in healthy people and showed good brain uptake and kinetics for PET imaging with a high signal-to-noise ratio. Thus, the tracer could outline D2/3R rich brain regions with reasonable validity (Willeit et al. 2006). As [11C]PHNO showed different distribution compared to known D2R radioligands like [11C]raclopride, further investigations brought up the idea that this tracer has a D3R preference (Narendran et al. 2006). Occupancy studies provided evidence of this when the selective D3R antagonist GSK598809 was used to quantify D3R binding of [11C]PHNO of $BP_{ND}$ at baseline and post-drug (Searle et al. 2010; Tziortzi et al. 2011). Depending on the brain region, the binding potential values were relatively specific for D2R or D3R (Tziortzi et al. 2011; Searle et al. 2010; Graff-Guerrero et al. 2008). Thus, in vitro and in vivo studies demonstrated that, unlike other tracers, [11C]PHNO has a 30–50-fold higher affinity to D3R than D2R (Gallezot et al. 2012; Freedman et al. 1994) in some regions, with investigations attributing all of the substantia nigra (SN) and hypothalamus signals to D3R (Gallezot et al. 2012; Searle et al. 2010; Tziortzi et al. 2011). After this work, these regions are generally assumed to be D3R binding representatives, as opposed to the D2R rich areas of the putamen and caudate. Other regions are considered mixed areas, with more D3R than D2R in the ventral pallidum (VP)/substantia innominate (75% D3R) and globus pallidus (GP)(65% D3R), and the thalamus (43% D3R) and ventral striatum (VST) (26% D3R) showing D2R dominance (Tziortzi et al. 2011). Furthermore, [11C]PHNO binding values are sensitive to endogenous dopamine because D3R has a higher affinity for dopamine than other receptor subtypes, which makes D3R preferring ligands especially sensitive to endogenous dopamine levels (Sokoloff et al. 1992). For more details on the history of [11C] PHNO development and kinetic modeling, please review "Imaging the Dopamine D3 Receptor In Vivo" (Mark Slifstein et al. 2014). Given the above, [11C]PHNO is considered the best available radiotracer for D3R PET studies to date.

The primary focus of [11C]PHNO PET studies on D3R have been on addiction, schizophrenia and Parkinson's disease (PD) due to the well-established role of the dopamine circuit in the behavior and pathophysiology of these disorders (Nieoullon and Coquerel 2003; Grace and Bunney 1985). As there is evidence of an interspecies difference in brain D3R distribution (Levant 1998), this chapter reviews human in vivo D3R PET studies on these conditions.

## 2 Addiction

Abused drugs such as cocaine, amphetamine, alcohol, and nicotine have important different mechanisms of action, but share the common pathway of the mesolimbic dopamine pathway that activates dopaminergic neurons in ventral tegmental area (VTA) and elevates dopamine levels at the nucleus accumbens (NAc) (Di Chiara and Imperato 1988). As D3R is highly expressed in these limbic areas (Sokoloff et al. 1990; Diaz et al. 1995, 2000; Levesque et al. 1992; Bouthenet et al. 1991), with the introduction of [11C]PHNO imaging, these regions became a focus of multiple substance dependence studies described below. Please see Table 1 for a summary of the addiction studies.

### *2.1 Cocaine*

In preclinical models, D3R binding increased in the NAc, ventral caudate, and putamen in cocaine-experienced rats killed after 31–32 days last cocaine administration (Neisewander et al. 2004). The increased D3R binding was attenuated by a regimen that reduced cocaine-seeking behavior (Neisewander et al. 2004). In line with this work was a postmortem human brain study with [3H]-(+)-7-OH-DPAT where the D3R binding level was upregulated one- to threefold in the SN in cocaine users over controls (Staley and Mash 1996).

In cocaine use disorder (CUD), imaging studies have largely mirrored preclinical work with three studies finding higher [11C]PHNO binding potential ($BP_{ND}$) in the SN (with the resolution of PET this area also contains the ventral tegmental area) when CUD subjects are compared to controls (Matuskey et al. 2014; Worhunsky et al. 2017; Payer et al. 2014). These findings averaged an increase of 24–29% in the SN, along with years of cocaine use positively correlated to [11C]PHNO binding (Matuskey et al. 2014; Worhunsky et al. 2017). Findings in D3R-rich areas of the hypothalamus and amygdala were also reported but not confirmed with larger sample sizes (Matuskey et al. 2014; Worhunsky et al. 2017).

These D3R findings are in stark contrast to previously reported lower striatal D2/3R availability by [11C]raclopride (Martinez et al. 2004, 2007; Volkow et al. 1997, 2014a) and more recently with [11C]PHNO (Worhunsky et al. 2017). This is presumably due to the physiologic differences of cocaine desensitizing the postsynaptic D2R and a corresponding upregulation of the D3R autoreceptor. The [11C]PHNO finding did show that this tracer can also demonstrate D2R area decreases, albeit with lower sensitivity that is likely dependent on sample size (i.e., previous [11C]PHNO studies had smaller samples of ($n = 10$ CUD and $n = 15$ CUD, while the latter study had $n = 26$ CUD).

**Table 1** Dopamine receptor findings in addiction using [11C]PHNO

| Study | Addiction | Baseline/ amphetamine challenge[a] | D3R rich area (SN) | D2R rich area (striatum) | Sample size |
|---|---|---|---|---|---|
| Matuskey et al. (2014) | Cocaine | Baseline | Higher | No difference | 20 |
| Worhunsky et al. (2017) | Cocaine | Baseline | Higher | Lower | 52 |
| Payer et al. (2014) | Cocaine | Baseline | Higher | No difference | 30 |
| Boileau et al. (2012) | Meth | Baseline | Higher | No difference | 32 |
| Boileau et al. (2016) | Meth | Baseline | Higher | No difference | 29 |
| Boileau et al. (2016) | Meth | AMP challenge | Higher Δ $BP_{ND}$ than controls | No difference | 29 |
| Erritzoe et al. (2014) | Alcohol | Baseline | No difference | No difference | 29 |
| Thiruchselvam et al. (2017)[b] | Alcohol | Baseline | No difference | No difference | 8 |
| Chukwueke et al. (2021) | Alcohol | Baseline | Lower | Lower | 35 |
| Le Foll et al. (2014) | Nicotine | Baseline | No difference | Lower | 10 |
| Di Ciano et al. (2018) | Nicotine | Baseline | No difference | Lower | 28 |
| Calakos et al. (2021) | Nicotine | Baseline | No difference | Lower | 42 |
| Calakos et al. (2021) | Nicotine | AMP challenge | No difference | Lower Δ $BP_{ND}$ than baseline | 37 |
| Di Ciano et al. (2021) | Nicotine | Baseline | Higher | No difference | 10 |
| van de Giessen et al. (2017) | Cannabis | Baseline | No difference | No difference | 23 |
| van de Giessen et al. (2017) | Cannabis | AMP challenge | N/A[c] | Lower Δ $BP_{ND}$ than baseline | 23 |
| Gaiser et al. (2016) | Food | Baseline | Higher | No difference | 28 |
| Cosgrove et al. (2015) | Food | Baseline | Higher | No difference | 12 |
| Caravaggio et al. (2015) | Food | Baseline | Higher | No difference | 26 |
| Boileau et al. (2013) | Gambling | Baseline | No difference | No difference | 25 |
| Boileau et al. (2014) | Gambling | AMP challenge | No difference | Higher Δ $BP_{ND}$ than controls | 23 |

*Meth* Methamphetamine, *AMP* amphetamine
All the studies above are [11C]PHNO human studies
[a]In baseline conditions, substance abusers were compared to controls; in amphetamine challenge conditions, the change in post amphetamine scans within subjects was compared to controls
[b]Subjects from this study were healthy drinkers (binge ETOH users without alcohol dependence)
[c]This study did not investigate SN but found lower percentage changes of Δ BPND in the pallidus

## 2.2 Methamphetamine

In a preclinical study, downregulation of D3R expression was demonstrated after repeated methamphetamine administration (Jiang et al. 2018). However, in a human study, upregulation of [11C]PHNO binding levels in D3R-rich areas was in the SN (+46%), with trends in the GP and VP observed in methamphetamine users ($n = 16$) compared to controls (Boileau et al. 2012). Furthermore, after amphetamine administration, methamphetamine users had higher [11C]PHNO $\Delta BP_{ND}$ (the difference in binding potential values) levels than controls in the SN (36% vs. 20%) and GP (30% vs. 17%), which indicates greater dopamine release in D3R-rich regions in methamphetamine users (Boileau et al. 2016). Moreover, [11C]PHNO binding level changes in the SN were positively correlated to drug wanting in methamphetamine users (Boileau et al. 2016). Although there were no significant [11C]PHNO $BP_{ND}$ changes in the D2-rich area of the striatum in methamphetamine users compared to controls, years of methamphetamine use were negatively correlated (Boileau et al. 2016).

## 2.3 Alcohol

Animal and human studies have also investigated whether D3R plays an important role in alcohol addiction (Thanos et al. 2005; Vengeliene et al. 2006; Heidbreder et al. 2007; Mugnaini et al. 2013). Specifically, animal studies have elucidated a role of D3R in alcohol preference and consumption (Thanos et al. 2005), ethanol seeking behavior (Heidbreder et al. 2007), relapse-like drinking (Vengeliene et al. 2006), and binge-like consumption of ethanol behavior (Rice et al. 2015). In a preclinical study, upregulation of D3R expression was demonstrated after voluntary alcohol consumption in rodents (1 year) (Vengeliene et al. 2006) and rats (4 weeks) (Jeanblanc et al. 2006). In a human study, no difference in the SN was found between alcohol use disorder (AUD) and controls, but higher [11C]PHNO volume of distribution (+17%) was seen in the hypothalamus in an abstinent state in patients (Erritzoe et al. 2014). However, in another imaging study, lower [11C]PHNO $BP_{ND}$ in the SN and sensorimotor striatum (−16%, −12%, respectively) was observed in AUD ($n = 17$;7±4 days of abstinence) over controls (Chukwueke et al. 2021) and AUD subjects with lower D3R availability in the SN had higher desire for alcohol (Chukwueke et al. 2021). The different results in human studies might be due to different outcome measures (volume of distribution vs. $BP_{ND}$) and different regions analyzed (i.e., the hypothalamus). Chukwueke and colleagues suppose D3R upregulation may also be masked by D2R downregulation in AUD.

In binge alcohol drinkers ($n = 8$, ≥2 binge-drinking episode in last 30 days), there were no significant [11C]-PHNO binding changes in regions such as the SN or striatum after acute alcohol administration (Thiruchselvam et al. 2017). However, greater change in $BP_{ND}$ between the baseline and alcohol scans was related to the

blood alcohol concentration in the associative striatum ($r = -0.80$, $p < 0.05$) (Thiruchselvam et al. 2017), indicating dopamine level elevations may occur at low doses.

## 2.4 Nicotine

Downregulation of [11C]PHNO $BP_{ND}$ levels in the ventral striatum (10%) occurs after acute nicotine in the nonhuman primate (Gallezot et al. 2014a). In humans, smoking significantly decreased [11C]PHNO $BP_{ND}$ in the limbic striatum and VP (−12% and −15%, respectively), which are D3R-rich areas (Le Foll et al. 2014). In abstinent smokers, [11C]PHNO $BP_{ND}$ levels were also significantly decreased in the limbic striatum (around −8%) and VP (around-10%) after smoking (Di Ciano et al. 2018). D3R availability is also essential for nicotine craving behavior (Mugnaini et al. 2013). After 1 week of abstinence, there was greater [11C]PHNO binding in the SN in a smoking cue condition compared to a neutral cue with no difference in striatal area, VP, and GP (Di Ciano et al. 2021). Recently, it was shown after amphetamine administration lower [11C]PHNO $BP_{ND}$ changes (19% vs. 27%) were observed in the ventral striatum in early abstinent smokers ($n = 22$, abstinent 11±9 days) compared to nonsmokers (Calakos et al. 2021). In that work depression severity scores were also significantly negatively correlated to [11C]PHNO $BP_{ND}$ percent changes in the ventral striatum ($r = -0.627$, $p = 0.025$) (Calakos et al. 2021), which might explain mood symptoms in early abstinent smokers.

## 2.5 Cannabis

The principal psychoactive component of cannabis is Δ9-tetrahydrocannabinol (THC) which binds to the endocannabinoid receptor ($CB_1$) and regulates afferent pathways (e.g.,NAc input) to the VTA among other functions (Lupica et al. 2004). Therefore, the alterations of dopamine release in these D3R-rich regions might be differentially altered in cannabis users. In a preclinical study, [3H]PHNO binding level was significantly elevated in the D3R rich regions of the NAc (+39%) and VP (+42%) in rodents with chronic exposure to THC (1 mg/kg/day; 21 days) and was not reversed after 1 week cessation (Ginovart et al. 2012).

In contrast with this preclinical study and other in vivo addiction human studies (Trifilieff and Martinez 2014; Volkow et al. 2009), there was no difference in baseline (11C)-PHNO $BP_{ND}$ in any striatal or extrastriatal areas in cannabis users compared to controls (van de Giessen et al. 2017), which is consistent with previous studies with [11C]raclopride (Urban et al. 2012; Volkow et al. 2014b). However, under a stress condition (i.e., Montreal image stress task), there was a significantly lower percent change of displacement in chronic cannabis users in the GP compared to healthy controls (−5% vs. 6%). In a sensorimotor control task (SMCT), [11C]

PHNO $BP_{ND}$ in chronic cannabis users was significantly higher in the striatal area than healthy controls (Mizrahi et al. 2013). In a different study with an amphetamine challenge, changes in [11C]PHNO $BP_{ND}$ in the striatum and pallidus were lower in cannabis users compared to controls (−18% vs. −25%; −13% vs. −23%, respectively), interpreted as showing a blunted dopamine response (van de Giessen et al. 2017).

### 2.6 Food Addiction

Studies have shown obese and higher body mass index (BMI) individuals have higher D2/3R availability with [11C]PHNO in a brain D3R rich region (i.e., SN) (Gaiser et al. 2016; Cosgrove et al. 2015; Caravaggio et al. 2015), an area found to be increased in substance abuse as reviewed above. The similar findings in food addiction give biological evidence of similar mechanisms at work, namely extending the role of dopamine sensitization as shown by D3R increases to food consumption. A recent paper investigating BMI with CUD did not find differences between normal weight and obese CUD individuals, however, suggesting that drug addiction may obscure these natural rewarding functions (Matuskey et al. 2021).

### 2.7 Gambling Disorders

It is unclear whether behavioral addictions such as pathological gambling (PG) have similar alterations of dopamine function as substance abuse. Some human studies have shown potential D3R-related mechanisms in behavioral additions, however. Although there were no [11C]PHNO binding differences in any regions of PG ($n = 13$) compared to controls (Boileau et al. 2013), [11C]PHNO $BP_{ND}$ was found to have a greater percent change than HC after amphetamine administration in the striatum, demonstrating an elevated (or exaggerated) dopamine response. Higher baseline D3R availability in the SN was also associated with greater dopamine release in the limbic striatum, and both measures predicted severity of the addiction, which led the authors to suggest that D3R may serve as an early marker of PG vulnerability (Boileau et al. 2014).

## 3 Neuropsychiatric Disorders

### 3.1 Schizophrenia

Schizophrenia is a psychiatric disorder that can include delusions, hallucinations, disorganized speech, trouble with thinking, and lack of motivation. There are genetic

and postmortem evidence of higher levels of D3R expression in the brain and blood lymphocytes of people with schizophrenia (Ilani et al. 2001; Gurevich et al. 1997). Consequently, understanding the role of D3R changes in schizophrenia is a clear area of interest.

Graff-Guerrero and colleagues measured D2/3R availability using [11C]PHNO in 13 medication-free (for at least 2 weeks) patients with schizophrenia-spectrum disorders (schizophrenia or schizophreniform disorder). Interestingly, they did not find any significant differences between medication-free patients with schizophrenia in comparison to healthy controls in [11C]PHNO availability (Graff-Guerrero et al. 2009b).

D2/3R are the target of most antipsychotic medications. However, the underlying mechanism is not completely understood. Earlier PET studies showed the relation between clinical efficacy, adverse effects, and receptor occupancy of antipsychotics in schizophrenia (Kapur et al. 2000; Farde et al. 1988). However, because they used nonspecific radioligands like [11C]raclopride, the specific occupancy of D2R vs. D3R was unexplored. Graff-Guerrero and colleagues aimed to compare long-term effects (more than 4 weeks) of three common antipsychotics (clozapine, risperidone, and olanzapine) on D2R and D3R occupancy by measuring [11C]PHNO and [11C]raclopride availability in 20 patients with schizophrenia. Compared to healthy controls, the mean receptor occupancies in participants treated with either of the three medications on the caudate and putamen were 71% and 69%, respectively, with [11C]raclopride and 53% and 41% with [11C]PHNO. In regions with higher D3R dominance [11C]raclopride showed 59% occupancy in the GP and 72% in VST, where [11C]PHNO showed higher (but with significant variability) binding in the GP with 70% and only 17% occupancy in the VST (Graff-Guerrero et al. 2009a). This finding suggested robust in vivo D2R blockade by antipsychotics and to a lesser extent D3R as well.

Mizrahi and colleagues conducted another study on receptor occupancy during treatment that employed a longitudinal within-subject design to scan eight people with schizophrenia spectrum disorders (six schizophrenia and two schizoaffective) in a drug-naïve state and then after around 2.5 weeks of treatment with risperidone or olanzapine. After antipsychotic treatment, mean occupancies were 45% in caudate and 43% in the putamen -an expected decrease- but the values in D3R rich regions were −50% in the GP and −52% in SN. Thus, compared to their nonmedicated scan, subjects had higher binding, meaning the receptor was not occupied, but rather upregulated in [11C]PHNO scans (Mizrahi et al. 2011).

In follow-up work on D3R occupancy in antipsychotic treatments after the Graff-Guerrero and Mizrahi studies, Girgis et al. investigated [11C]PHNO availability after acute doses of risperidone in individuals with schizophrenia. Strikingly, the results showed high occupancy in all regions, including the caudate, putamen, thalamus, SN, GP, and VST. $BP_{ND}$ values decreased in all regions following treatment, and after applying regression modeling, the estimated average occupancies were 53% at D2R and 24% at D3R, with a D2:D3 selectivity ratio of 2.21. This finding was further evidence of risperidone binding to D3R in acute doses, as expected by in vitro studies (Girgis et al. 2015).

Historically, schizophrenic symptoms have been divided into positive, negative and cognitive symptoms. Negative symptoms could include social withdrawal, decreased energy, flat affect, and anhedonia. Cariprazine (RGH-188), a high-affinity D3R and D2R partial agonist with D3R preference (Kiss et al. 2010), has shown a clinical potential to treat negative symptoms in patients with schizophrenia (Debelle et al. 2014, 2015). Girgis et al. explored cariprazine's receptor occupancies in three different doses in 9 patients (3 in each dose group) with schizophrenia utilizing [11C]PHNO. Eight subjects had baseline and post-dose scans on the 1st, 4th, and 15th days of treatment. In the lowest dose group (1 mg/day), the average D3R and D2R occupancies on day 15 were 76% and 45%, respectively. In the intermediate dose group (3-mg/day), average D3R and D2R occupancies were 92% and 79%, respectively. In the highest dose group (12 mg/day), near-complete receptor occupancies (∼100%) were seen for both receptors. These in vivo occupancies show at lower doses, cariprazine is a D3-preferring medication with more equal D3/D2R partial agonist activity as the doses are increased (Girgis et al. 2016).

Blonanserin is another atypical antipsychotic with a high in vitro affinity for D3R and D2R (Baba et al. 2015). Following one clinical dose of blonanserin with [11C]PHNO, it was demonstrated that this medication occupied D3R and D2R in vivo at the same level in healthy subjects, however. Regions occupancy after 12 mg blonanserin was 64–81% in the caudate, 60–84% in the putamen, 40–88% in the VST, 65–87% in the GP, and 56–88% in the SN. (Tateno et al. 2018). Another study was conducted to investigate long-term D2R and D3R changes with blonanserin and olanzapine treatment using [11C]PHNO in 13 patients with schizophrenia. Here they used the $BP_{ND}$ values of healthy controls for the baseline to calculate receptor occupancy. Seven participants switched medications after the first scan in a cross-over design, and the second PET scan was performed after 2 weeks or more. The mean receptor occupancies following olanzapine included 32% in the caudate, 26% in putamen, −33% in GP, −112% in SN. The mean receptor occupancies following blonanserin included 61% in caudate, 55% in putamen, 48% in GP, and 34% in SN. Aligned with previous works (Baba et al. 2015; Graff-Guerrero et al. 2009a), the outcome revealed that blonanserin occupied both D2R and D3R in vivo; however, the D3R occupancy (34–48%) was slightly lower than D2R occupancy (55–61%). Additionally, olanzapine also occupied more receptors in D2R dominant areas, followed by the D3R-rich regions to a lesser extent (Sakayori et al. 2021).

A significant advantage of PET imaging is exploring developing pharmaceutical products in vivo. Previous studies brought up the idea that selective D3R medications might be efficient for a greater range of schizophrenia symptoms and have fewer side effects than current D2R preferring or D2/3R medications (Girgis et al. 2016; Sokoloff et al. 2013). F17464 is a selective D3R antagonist that showed promising efficiency on cognitive and negative symptoms of schizophrenia in preliminary investigations on animal models (Sokoloff et al. 2014). A study examined F17464 binding to D2R and D3R in 6 healthy volunteers using [11C]PHNO and found D3R occupancy at 6–9 h after administration was 89–98% following a 30 mg dose and 79–87% following 15 mg, while D2R binding was <18% for both

doses (Slifstein et al. 2020). Thus, it was confirmed that F17464 is a potent D3R selective agent following a single dose oral administration.

Outside of treatments, attempts to diagnose individuals with clinically high risk (CHR) for schizophrenia remain a high priority (Cannon et al. 2008). Mizrahi et al. investigated the effect of stress as an environmental factor for psychosis-like experience by looking at differences in [C11]PHNO signals in response to a validated psychosocial stress task (Montreal Imaging Stress Task) during scanning in 12 CHR participants, 10 antipsychotic-naive participants with schizophrenia, and 12 healthy volunteers. All participants completed two PET scans: while performing a sensorimotor control task and the stress task. They reported significant between-group differences in the associative striatum and sensorimotor striatum with higher [11C]PHNO displacement following the stress task in CHR and schizophrenia groups compared to the control task (Mizrahi et al. 2012). Moreover, they reported no differences in [11C]PHNO availability in brain regions between medication naive patients with schizophrenia, CHR, and healthy individuals while doing a sensorimotor control task or a cognitive challenge (Suridjan et al. 2013). These findings suggest a different dopaminergic reaction following acute stressors in this population, which needs further investigation.

Another study on 14 CHR individuals was conducted to compare synaptic [11C]PHNO availability with healthy controls before and after administering a single oral dose (60 mg) of methylphenidate, a dopamine reuptake inhibitor. They inferred that by blocking the dopamine transporter and preventing dopamine reuptake, they could measure intrasynaptic dopamine availability. While there were no significant between-group differences in the baseline imaging, $\Delta BP_{ND}$ was used to explore the methylphenidate effect in brain regions, and a significant binding difference was seen in the VST. Furthermore, comparing imaging values with clinical measurements showed a strong negative correlation between $\Delta BP_{ND}$ in the VST and severity of negative symptoms at baseline in the individuals with CHR and a positive correlation between $\Delta BP_{ND}$ in the midbrain and positive symptoms (Girgis et al. 2021). Please see Table 2 for a summary of all schizophrenia [11C]PHNO imaging studies.

## *3.2 Parkinson's Disease*

Parkinson's disease (PD) is a progressive neurodegenerative disorder characterized by significant motor symptoms like bradykinesia, tremor, and rigidity, as well as nonmotor symptoms like cognitive impairment, depression, and sleep problems. Hornykiewicz was the first who revealed the relationship between the depletion of dopaminergic neurons in the SN and PD symptoms (Hornykiewicz 1966), and the role of dopamine pathology in PD is now well-established. Many PET studies have focused on understanding these dopamine changes; however, despite evidence of the D3R as a noteworthy target (Yang et al. 2020), only a few studies have explored this role.

**Table 2** Dopamine receptor findings in schizophrenia using [11C]PHNO

| Study | Population | Medication (occupancy) | D3R rich area (SN) | D2R rich area[a] (striatum) | Mixed area (VST) |
|---|---|---|---|---|---|
| Graff-Guerrero et al. (2009a, b) | Medication-free SZ ($n = 13$), HC ($n = 13$) | None | No difference | No difference | No difference |
| Graff-Guerrero et al. (2009a, b) | SZ on long-term antipsychotic use ($n = 23$) and HC ($n = 23$) | Clozapine Olanzapine Risperidone | – | 47% | 17% |
| Mizrahi et al. (2011) | Drug-naive SZ at baseline and after 2.5 weeks of treatment ($n = 8$) | Risperidone olanzapine | −52% | 44% | 20% |
| Girgis et al. (2015) | SZ at baseline and after one dose of medication ($n = 5$) | Risperidone | 24% | 53% | – |
| Girgis et al. (2016) | SZ at baseline and on the 15th day of medication ($n = 8$) | Cariprazine | 1 mg/day 76% | 1 mg/day 45% | – |
| | | | 3 mg/day 92% | 3 mg/day 79% | |
| | | | 12 mg/day ~100% | 12 mg/day ~100% | |
| Tateno et al. (2018) | HC after one dose of medication ($n = 6$) | Blonanserin | 72% | 73% | 60% |
| Slifstein et al. (2020) | HC after one dose of medication ($n = 6$) | F17464 | 15 mg 83% | 15 mg <18% | – |
| | | | 30 mg 94% | 30 mg <18% | |
| Sakayori et al. (2021) | SZ ($n = 13$) with a crossover design after first scan ($n = 7$) | Blonanserin | 34% | 58% | – |
| | | Olanzapine | −112% | 29% | |
| Mizrahi et al. (2012) | CHR ($n = 12$), SZ ($n = 10$) and HC ($n = 12$) at baseline and while performing a stress task | None | – | Higher displacement following the stress task in CHR and SZ | – |
| Suridjan et al. (2013) | CHR ($n = 12$), SZ ($n = 10$), HC ($n = 12$) performing a sensorimotor control task | None | No difference | No difference | No difference |
| Girgis et al. (2021) | CHR ($n = 14$), HC ($n = 14$) before and after administering a single dose of MPH | MPH challenge | – | – | Higher in CHR |

[a]Calculated as average of caudate and putamen values. Percentages are receptor occupancies after medication. Unreported findings are marked with -. *SN* substantia nigra, *VST* ventral striatum, *SZ* schizophrenia, *HC* Healthy Controls, *CHR* clinically high risk for schizophrenia, *MPH* methylphenidate

Boileau and colleagues reported a decrease in [11C]PHNO binding in the VST (−11%) and GP (−42%) of drug-naive patients with PD, yet interestingly no significant difference in the SN of PD individuals compared to controls. They also looked into correlations between clinical symptoms and a [11C]PHNO to [11C] raclopride $BP_{ND}$ ratio, which had significant positive correlations with motor symptoms and a negative correlation with depressed mood. Although not a validated measure, it has opened the door for further investigations of dopamine receptor subtypes in PD (Boileau et al. 2009).

Nonmotor symptoms may be as devastating as motor symptoms in PD, with sleep disorders having a significant disease burden (Sauerbier et al. 2016). Pagano and colleagues looked into [11C]PHNO availability in the hypothalamus of 12 participants with PD and studied the association with severity of sleep disorders. They reported a significant correlation between reduced hypothalamic [11C]PHNO availability and excessive daytime sleepiness, but not with other measures such as sleep-onset and sleep-maintenance insomnia (Pagano et al. 2016).

The mainstay of current PD treatment is improving motor manifestations, and a common complication of treatment is levodopa-induced dyskinesias (LID). A study scanned levodopa-treated patients with PD (12 with LID and 12 without LID) and 18 healthy controls. Both PD groups had higher binding potential values in the SN, ventral pallidum, and GP than the healthy group. This finding was different than the drug-naive study discussed above (Boileau et al. 2009) and is in line with previous studies which have shown D3R upregulation following levodopa treatment (Guigoni et al. 2005; Quik et al. 2000). Also, they reported higher [11C]PHNO binding in the GP of patients with LID compared to nondyskinetic PD patients (Payer et al. 2016).

Lastly, impulse control disorders (ICDs) are another complication of PD treatment with dopaminergic treatment (Potenza et al. 2007). ICD contains different subtypes like addiction to anti-parkinsonian medication, binge eating or hypersexuality; the most reported ICD is pathological gambling in PD. The role of D3R in developing these treatment-related ICDs in PD using [11C] PHNO has been studied with 11 PD participants with ICD, 21 PD participants without ICD, and 18 healthy controls. The results showed no differences in tracer availability in D3R-rich regions of PD patients with ICD than the non-ICD PD group. There was, however, 20% lower availability in the mixed D2/3R region of the VST, an important area in reward, in patients with PD-ICD, providing evidence of dopamine dysfunction in ICDs (Payer et al. 2015). Please see Table 3 for a summary of PD [11C]PHNO imaging studies.

### *3.3 Tourette Syndrome*

Tourette syndrome (TS) is a neurodevelopmental disorder with prominent motor components, including tics. Although the general hypothesis is an imbalance of different neurotransmitters, dopamine system pathology is considered the key underlying mechanism (Buse et al. 2013). To date, only one study has investigated D2/3R

**Table 3** Dopamine receptor findings in Parkinson's disease using [11C]PHNO

| Study | Population | D3R rich area (SN) | D2R rich area (striatum) | Mixed area (VST) |
|---|---|---|---|---|
| Boileau et al. (2009) | Drug-naïve PD ($n = 10$), HC ($n = 9$) | No difference | Higher | Lower |
| Payer et al. (2015) | PD w/o ICD ($n = 21$), HC ($n = 18$) | No difference | PD w/o ICD Higher | PD w/o ICD Lower |
| Payer et al. (2015) | PD – ICD ($n = 11$), HC ($n = 18$) | No difference | No difference | PD-ICD Lower |
| Payer et al. (2015) | PD w/o ICD ($n = 21$), PD – ICD ($n = 11$), | No difference | No difference | PD-ICD Lower |
| Payer et al. (2016) | PD w/o LID ($n = 12$), HC ($n = 18$) | No difference | PD w/o LID Higher | PD w/o LID No difference |
| Payer et al. (2016) | PD – LID ($n = 12$), HC ($n = 18$) | No difference | PD-LID Higher | PD-LID Lower |
| Payer et al. (2016) | PD w/o LID ($n = 12$), PD – LID ($n = 12$), | No difference | No difference | PD-LID Lower |
| Pagano et al. (2016) | Patients with PD ($n = 12$) | Negatively correlated with daytime sleepiness (hypothalamus) | – | – |

Each population comparison in the studies is shown in a separate row. *SN* substantia nigra, *VST* ventral striatum, *PD* Parkinson's disease, *HC* Healthy Controls, *LID* levodopa-induced dyskinesias, *ICD* impulse control disorders

availability utilizing [11C]PHNO in TS. They did not find any significant differences in [11C]PHNO or [11C]raclopride binding between TS and healthy controls (Abi-Jaoude et al. 2015). This finding was consistent with most of the previous studies using [11C]raclopride (Singer et al. 2002; Turjanski et al. 1994), except for a study that found lower D2/D3R striatal receptor binding in the putamen of TS participants (Denys et al. 2013). These results might be due to many confounding factors such as the lack of gender for matched groups, higher depression and anxiety scores in the TS group, and an absence of information about possible factors such as comorbid ADHD.

### 3.4 TBI

Traumatic brain injury (TBI) patients can present with cognitive disorders immediately or after the initial injury. Attention, memory, and executive function are three general domains in cognitive deficits (Stuss et al. 1989; Leininger et al. 1990; Binder 1997; Binder et al. 1997), with memory difficulties the most reported for TBI patients (Binder 1987). As the mesocorticolimbic pathway is important in modulating memory consolidation (Coccurello et al. 2000; Setlow and McGaugh 1998), the SN and striatum are also essential in memory (Mura and Feldon 2003). Therefore, dopamine levels and/or receptors in D3R-rich regions might be changed in TBI patients. Despite this possibility, the lone study with [11C]PHNO did not find differences in D3R regions, but rather did find lower $BP_{ND}$ levels in the caudate in TBI and TBI-MDD patients compared to controls (Jolly et al. 2019), similar to other studies finding reduced striatal dopamine transporter levels in TBI patients (Wagner et al. 2005, Wagner et al. 2014).

## 4 Other Studies Investigating D3R in Humans

### 4.1 Aging

So far, two studies have focused on D3R brain changes with aging. The first group scanned 72 participants to explore an age effect on D2/D3R using [11C] PHNO. The study reported no age-related change of [11C]PHNO binding potential in D3R-specific regions. However, they reported an age-related decrease in [11C] PHNO availability in the D2R-rich caudate (Nakajima et al. 2015). Our group demonstrated a similar outcome. We scanned 42 healthy people using [11C] PHNO and reported an age-related decline in the caudate (8% per decade) and putamen (5% per decade) (D2 rich regions) but not in the SN/VTA and hypothalamus (D3 rich regions) (Matuskey et al. 2016). These studies indicate that there might be differential aging patterns in dopamine receptor subtypes in the brain.

### 4.2 Social Effects

In a very influential nonhuman primate imaging study, the reinforcing effect of cocaine was more vulnerable in subordinate monkeys over dominant monkeys and was related to their D2/3R availability in social housing, with the dominant monkeys having increased binding in the striatum (Morgan et al. 2002). In the first translational study in humans, striatal D2/3R availability level was also positively correlated to social status with (11)raclopride (Martinez et al. 2010). Work extending both studies was performed with [11C]PHNO and showed the availability of extrastriatal

D3 regions were negatively correlated to social status in both healthy people and CUD individuals (Matuskey et al. 2015). These findings were in contrast to previous social status studies and may be in part explained by the differences in D3R vs. D2R areas as measured by [11C]PHNO and (11)raclopride, similar to the differences discussed above in CUD, as well as the complexities of translating animal models into clinical populations. More recently, higher striatal D2/3R availability has been observed in areas with less education and larger local population sizes with [11C]PHNO, suggesting that living in a populous area with fewer educational resources may be accompanied by stressors detectable with PET scans (Calakosa et al. 2021).

### *4.3 Retinal Studies*

A couple of studies have investigated the possibility of D3R quantification in the retinal region using [11C]PHNO (Caravaggio et al. 2018, 2020). This work suggested that the retina could be a region of interest to evaluate with D3R, which opens a new door past the brain to investigate retinal dopamine in various disorders. Thus far, however, Caravaggio and colleagues have reported no significant between-group differences with retinal [11C]PHNO availability between first-episode drug-naïve people diagnosed with schizophrenia and healthy controls or any age or BMI-related changes in retinal D3R availability in healthy individuals.

### *4.4 Next-Generation D3R Tracers*

Although [11C]PHNO is the best present radiotracer for D3R, it is not purely selective and has an affinity to D2R that is regionally dependent as discussed. These findings demonstrate the importance of developing newer D3R selective PET tracers with fewer limitations. Different groups have been on this search, with [18F]Fluortriopride ([18F]FTP) one of the radiotracers developed for this aim. A preclinical study showed [3H]FTP (also known as [3H]LS-3-134) had favorable D3R selectivity with unmeasurable D2R affinity compared to the known D2/3R tracers [125I]IABN and [3H]raclopride (Rangel-Barajas et al. 2014). Despite promising preliminary findings in nonhuman primates, further investigation suggested [$^{3}$H/$^{18}$F]FTP binding to serotonin 5-HT1A receptors (Mach and Luedtke 2018). Very recently, the group from University of Pennsylvania presented a poster that questioned whether [18F]FTP was nonselective throughout the brain (Doot et al. 2021). Given these difficulties, groups have focused on developing new D3R piperazine analogs with promising D3R selectivity at primary stages that might lead to new radioligands like the morpholine derivatives (Micheli et al. 2016c), 1,2,4-triazolyl octahydropyrrolo[2,3-b]pyrroles (Micheli et al. 2016b) and 1,2,4-triazolyl 5-azaspiro[2.4]heptanes (Micheli et al. 2016a), along with D3R analogs with diazaspiro alkane cores (Reilly et al. 2017). These ligands are at the initial

stages of exploration, however, and have not yet been investigated in humans, leaving the need for next-generation D3R tracers ongoing.

## 5 Conclusion

From the time Sokoloff first discovered the D3 receptor three decades ago, PET imaging has added remarkable insights into D3R distribution, physiology, and involvement in various disorders, including addiction, schizophrenia, and PD. Despite these advances with [11C]PHNO, considered the most selective tracer for this receptor, the notable affinity to D2R limits its interpretation. Developing more targeted D3R tracers that allow further in vivo exploration of this important receptor remains a critical area of research.

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