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REVIEW

Heteromeric Dopamine Receptor Signaling Complexes: Emerging Neurobiology and Disease Relevance

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The pharmacological modification of dopamine transmission has long been employed as a therapeutic tool in the treatment of many mental health disorders. However, as many of the pharmacotherapies today are not without significant side effects, or they alleviate only a particular subset of symptoms, the identification of novel therapeutic targets is imperative. In light of these challenges, the recognition that dopamine receptors can form heteromers has significantly expanded the range of physiologically relevant signaling complexes as well as potential drug targets. Furthermore, as the physiology and disease relevance of these receptor heteromers is further understood, their ability to exhibit pharmacological and functional properties distinct from their constituent receptors, or modulate the function of endogenous homomeric receptor complexes, may allow for the development of alternate therapeutic strategies and provide new avenues for drug design. In this review, we describe the emerging neurobiology of the known dopamine receptor heteromers, their physiological relevance in brain, and discuss the potential role of these receptor complexes in neuropsychiatric disease. We highlight their value as targets for future drug development and discuss innovative research strategies designed to selectively target these dopamine receptor heteromers in the search for novel and clinically efficacious pharmacotherapies.

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INTRODUCTION

Alterations in dopaminergic signaling have been linked to a number of mental health disorders, including schizophrenia, drug addiction, depression, and attention-deficit hyperactivity disorder (ADHD) (Beaulieu and Gainetdinov, 2011; Faraone and Biederman, 1998; Porcelli *et al*, 2011; Seeman, 2009; Volkow *et al*, 2004), and the pharmacological modification of dopamine transmission has long been employed as a therapeutic tool in the treatment of many dopamine-related disorders. The physiological effects of dopamine are mediated by five dopamine receptor subtypes, divided into two major subclasses: the D1-like (D1, D5) and the D2-like (D2, D3, D4) receptors, which are typically coupled to the stimulatory Gs/olf and inhibitory Gi/o proteins, respectively. Although traditionally G protein-coupled receptors (GPCRs), such as the dopamine receptors, have been depicted as monomeric entities, it is now widely accepted that GPCRs exist as oligomeric complexes (George *et al*, 2002; Milligan, 2004; Terrillon and Bouvier, 2004), and homomerization has been repeatedly shown to have a critical involvement in important cellular processes, such as receptor translocation to the plasma membrane (Hague *et al*, 2004; Karpa *et al*, 2000; Kong *et al*, 2006; López-Giménez *et al*, 2007; Salahpour *et al*, 2004; White *et al*, 1998). In addition, the discovery that dopamine receptors could form heteromeric complexes (Baragli *et al*, 2007; Ferrada *et al*, 2008, 2009; Ginés *et al*, 2000; Hillion *et al*, 2002; Lee *et al*, 2004; Marcellino *et al*, 2008a; Marcellino *et al*, 2008b; Scarselli *et al*, 2001; Torvinen *et al*, 2005) has opened up novel avenues of research for drug discovery, as many of these receptor heteromers may exhibit discrete distributions in brain with pharmacological and functional properties distinct from their constituent receptors. For example, the dopamine D1-D2 receptor heteromer was first identified in rat striatum (Lee *et al*, 2004) and shown to couple to the Gq/11 protein, a finding that effectively linked dopamine directly to calcium signaling in brain (Rashid *et al*, 2007a). Dopamine had been linked to calcium signalling previously in older literature, with some suggesting that the D1 receptor (D1R) itself or a 'D1-like' receptor was responsible, but the interpretation of those studies in light of what we now know suggests that much of the calcium signal in striatum would have been attributable to

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the D1-D2 receptor heteromer, with the calcium signal in cortex or other brain regions possibly to the D5 receptor (D5R) or the D2-D5 receptor heteromer.

Given the extensive involvement of dopamine receptors in the etiology and therapeutic management of mental health disorders, and the remarkable potential of dopamine receptor heteromers to access diverse signaling cascades or to modulate the nature of the transduced signal, these heteromeric complexes represent likely candidates in the search for new drug therapies. Indeed, as many of the pharmacotherapies today are not without significant side effects, or they alleviate only a particular subset of symptoms, the identification of novel therapeutic targets is imperative. This review will discuss the known dopamine receptor heteromers that have been reported to have a potential link to neuropsychiatric disorders and will review the recent advances that have contributed to the understanding of how these receptor heteromers may be important to the pathophysiology and therapeutic management of schizophrenia, addiction, depression, and ADHD.

THE RECEPTOR INTERFACE: RECEPTOR HOMOMERS VERSUS RECEPTOR HETEROMERS

Although GPCR heteromerization between class C GPCRs such as the $GABA_B$ receptor is recognized as obligatory and has been accepted for many years (Jones *et al*, 1998; Kaupmann *et al*, 1998), oligomerization between class A GPCRs is still the subject of much debate and, as such, the structural mechanism by which these receptors physically interact has been the focus of a number of research studies. What has become increasingly evident is that the interface(s) between the receptors in homomeric complexes likely involves residues located in transmembrane domains (TM), such as TM4 and TM5, as has been shown for the D2 receptor (D2R) (Guo *et al*, 2003; Lee *et al*, 2003), the alpha(1b)-adrenoceptor (López-Giménez *et al*, 2007), the 5HT1A receptor (Gorinski *et al*, 2012), and the 5HT2C receptor (Mancia *et al*, 2008). These observations have also been supported by the recent crystal structure reported for the beta1-adrenergic receptor dimer (Huang *et al*, 2013) in a lipid membrane-like environment which showed two dimer interfaces, one involving TM1, TM2, helix 8 and extracellular loop 1, and the second involving TM4, TM5, intracellular loop 2, and extracellular loop 2. The analysis of the crystal structure of the chemokine CXCR4 receptor dimer (Wu *et al*, 2010) reported receptor interfaces at TM5 and TM6. In contrast to homomeric receptor complexes, the receptor interface(s) involving class A GPCR heteromers does not appear to rely predominantly on TM interactions, resulting in concern as to whether the binding energy between the receptors in these heteromers is actually sufficient to result in stable long-lasting physical interactions (Gurevich and Gurevich, 2008). However, it has been demonstrated in several examples that certain amino-acid residues, specifically two or more adjacent arginines on one protomer and two or more adjacent glutamic acids, or aspartic acids, or a phosphorylated residue on the other protomer, is sufficient to induce the formation of stable non-covalent complexes (Jackson *et al*, 2006; Woods and Ferré, 2005). Indeed, this mechanism of interaction has been reported for both the D1-D2 receptor heteromer (O'Dowd *et al*, 2012) and the D2-D5 receptor heteromer (O'Dowd *et al*, 2013), whereby adjacent glutamic acid residues in the carboxyl tail of the D1R or D5R interacted with two different sets of adjacent arginine residues in intracellular loop 3 (IC3) of the D2R. Similarly, it has been shown for the A2-D2 receptor heteromer that there exists an arginine–phosphate electrostatic interaction between the C-terminal tail of the A2 receptor (A2R) and the IC3 of the D2R that is of high energy strength (Ciruela *et al*, 2004) and which possesses covalent-like stability (Woods and Ferré, 2005). Indeed it has been proposed that this arginine–phosphate interaction may represent a common mechanism in receptor heteromerization (Fuxe *et al*, 2010), including GPCR heteromerization with non-GPCRs such as has been suggested for the D1-NMDA receptor heteromer (Woods *et al*, 2005; Woods and Ferré, 2005).

Clearly more research is required to identify the underlying mechanisms by which class A GPCRs heteromerize, as they do not all involve electrostatic amino-acid interactions, and TM domain interactions may not have such a crucial role in the formation of at least some of these receptor complexes. Nonetheless, the mechanism of the interaction mediating receptor heteromerization does not appear to be necessary for the classification of a receptor heteromer as recommended by the International Union of Basic and Clinical Pharmacology (Pin *et al*, 2007), whereby receptor heteromers can be accepted by the scientific community provided their existence in native tissue has been firmly demonstrated. In line with this, at least two of the following criteria should be met: (1) There is evidence for physical association in native tissues or primary cells, preferably through the use of energy transfer technologies or antibodies selective for specific receptor oligomers (Wager-Miller *et al*, 2002), (2) A specific functional property for the receptor heteromer is known so receptors in native tissue can be identified, and (3) the existence of the heteromer was confirmed *in vivo* through the use of knockout animals or RNAi technology. The recommendations for the recognition and nomenclature of GPCR oligomers was adopted in a Web-based information system, the G Protein-Coupled Receptor-Oligomerization Knowledge Base (GPCR-OKB) (http://www.gpcr-okb.org), in which all available information on GPCR oligomers was included. It is important to note that although the present review will focus on dopamine receptor heteromers that have met the above criteria, it will additionally address some putative heteromer receptor species that have not yet been demonstrated to exist *in vivo*. A comprehensive characterization of the dopamine receptor heteromers discussed herein is presented in Table 1.

TABLE 1 Physical and Functional Evidence for Dopamine Receptor Heteromers

Heteromer	Physicial interaction		Functional evidence	Relevance	References
	In vitro	In vivo			
D1-D2	Co-IP, NLS FRET rat striatal neurons radioligand binding	Co-IP rat STR, PFC FRET in situ rat CP, NAc, GP	Novel Gq-coupling resulting in intracellular calcium release and BDNF expression, signaling blocked by D1R and D2R antagonists, GSK-3β inactivation	Addiction Schizophrenia	Lee *et al* (2004), Rashid *et al* (2007a), Hasbi *et al* (2009), Pei *et al* (2010), Perreault *et al* (2010, 2011, 2013), O'Dowd *et al* (2012)
D2-D4	BRET D2R and D4.4, no heteromer between D2R and D4.7 variant	Colocalization in mouse STR	Potentiation of ERK activation when D2R and D4R coexpressed but not with D4.7 variant, knock-in mice expressing D4.7 variant show no synergistic increase in striatal ERK activation	ADHD	Borroto-Escuela *et al* (2011), González *et al* (2012)
D1-D3	BRET, FRET	Co-IP rat STR	Agonist-induced D1R cytoplasmic sequestration abolished by D3R coexpression, D3R stimulation enhanced D1R agonist affinity and potentiated D1R-mediated behaviors	Addiction	Fiorentini *et al* (2008), Marcellino *et al* (2008b)
D2-D3	Co-IP	Colocalization STR	In the presence of excess D3R, the properties of partial D2R agonists transformed to antagonists	Schizophrenia	Scarselli *et al* (2001), Novi *et al* (2007), Maggio and Millan, (2010)
D2-D5	FRET, NLS	Colocalization, rat cortex, VP, CP	Gq-coupling resulting in intracellular calcium release followed by extracellular calcium influx		So *et al* (2009), Hasbi *et al* (2010), O'Dowd *et al* (2013)
A1-D1	Co-IP	Co-IP rat NAc	A1R promoted D1R G protein uncoupling and dampened receptor signaling	Addiction	Ginés *et al* (2000), Toda *et al* (2003)
A2-D2	Co-IP, FRET, BRET	Colocalization in STR	A2R promoted D2R G protein uncoupling and dampened receptor signaling	Addiction Schizophrenia Parkinson's disease	Hillion *et al* (2002), Canals *et al* (2003), Fuxe *et al* (2005), Azdad *et al* (2009), Marcellino *et al* (2010)
A2-D2-mGlu5	Biomolecular fluorescence complementation, BRET, sequential BRET-FRET	Co-IP rat STR	-	Schizophrenia	Cabello *et al* (2009), Fuxe *et al* (2010)
D1-NMDA	BRET	Co-IP rat HIP, STR PSD, PFC, pull-down assay rat HIP	Uncoupling the heteromer with a disrupting peptide upregulated NMDA-mediated LTP in rat HIP and promoted working memory	Schizophrenia	Lee *et al* (2002), Fiorentini *et al* (2003), Pei *et al* (2004), Kruse *et al* (2009), Nai *et al* (2010)
D2-NMDA		Co-IP rat STR PSD, pull-down assay STR	Heteromer formation induced by cocaine disrupted the CaMKII/NR2B interation and reduced NMDA receptor-mediated currents	Addiction	Liu *et al* (2006)
D2-5HT2A	Co-IP, FRET, BRET	Colocalization in STR	5HT2AR-mediated PLC activation was synergistically enhanced by D2R activation, D2R-mediated AC inhibition was attenuated by 5HT2AR activation	Schizophrenia	Łukasiewicz *et al* (2010), Borroto-Escuela *et al* (2011), Albizu *et al* (2011)
D1-H3	BRET	Co-IP rat STR	D1R mandatory for H3R-induced ERK activation, D1R- and H3R-induced ERK activation blocked by antagonists for either receptor	Addiction ADHD Schizophrenia	Ferrada *et al* (2009), Moreno *et al* (2011)
D2-H3	BRET	Co-IP rat STR	H3R agonists dampened D2R receptor function and D2R-induced locomotor activity	Addiction ADHD Schizophrenia	Ferrada *et al* (2008), Moreno *et al* (2007)

Abbreviations: 5HT2AR, 5HT2A receptor; A1R, adenosine A1 receptor; A2R, adenosine A2 receptor; AC, adenylyl cyclase; ADHD, attention-deficit hyperactivity disorder; BDNF, brain-derived neurotrophic factor; BRET, bioluminescent resonance energy transfer; CaMKII, calcium calmodulin kinase II; Co-IP, coimmunoprecipitation; CP, caudate putamen; D1R, dopamine D1 receptor; D2R, dopamine D2 receptor; D3R, dopamine D3 receptor; D4R, dopamine D4 receptor; ERK, extracellular signal-related kinase; FRET, fluorescent resonance energy transfer; GP, globus pallidus; GSK-3*β*, glycogen synthase kinase 3*β*; H3R, histamine H3 receptor; HIP, hippocampus; LTP, long-term potentiation; mGlu5, metabotropic glutamate receptor 5; NAc, nucleus accumbens; NR2B, NMDA receptor subunit 2B; PLC, phospholipase C; PFC, prefrontal cortex; PSD, postsynaptic density; STR, striatum; VP, ventral pallidum.

THE DOPAMINE D1-D2 RECEPTOR HETEROMER

Dopamine D1R and D2R can form heteromeric receptor complexes that occur via electrostatic interactions between specific glutamic acid residues in the carboxyl-tail of the D1R and arginine residues in the third intracellular loop of the D2R, residues present in both the long and short isoforms of the D2R (O'Dowd *et al*, 2012). The D1-D2 receptor heteromer was first identified *in vivo* by coimmunoprecipitation from rat striatum (Lee *et al*, 2004) and soon thereafter confirmed in heterologous cells (Dziedzicka-Wasylewska *et al*, 2006; So *et al*, 2005) and subsequently in primary striatal neuronal culture by confocal fluorescence resonance energy transfer (FRET) studies (Hasbi *et al*, 2009),

using the endogenously expressed dopamine receptors for the identification of receptor–receptor interactions. Indeed, a physical interaction between the endogenous GPCRs *in vivo* was first shown between the endogenously expressed D1R and D2R using quantitative confocal FRET in brain sections *in situ* (Hasbi *et al*, 2009), and expression of the D1-D2 heteromer has now been shown in regions of the rat mesolimbic and basal ganglia circuitry (Perreault *et al*, 2010; Perreault *et al*, 2011). Coexpression of D1R and D2R within a medium spiny neuron (MSN) does not necessarily indicate heteromerization *per se*, but the D1-D2 receptor–receptor distance determined to be <100 Å documented by the FRET analyses is indicative of heteromer formation. Specifically, the D1-D2 heteromer was found to be selectively expressed in MSNs with a unique phenotype, in that these neurons also expressed both dynorphin (DYN) and enkephalin (ENK) (Perreault *et al*, 2010), as well as GABA and glutamate (Perreault *et al*, 2012), and to have representation along both the direct striatonigral and indirect striatopallidal pathways (Perreault *et al*, 2010). For example, while a relatively low number of D1R-containing MSNs expressed the D2R ($\sim$6%) in caudate putamen (CP), higher coexpression levels were evident in ventral pallidum and entopeduncular nucleus, with the highest levels documented in the nucleus accumbens (NAc) shell ($\sim$17–34%) and globus pallidus ($\sim$60%) (Bertran-Gonzalez *et al*, 2008; Perreault *et al*, 2010). In addition, as D1-D2 heteromer expression has been reported to occur selectively at presynaptic but not at postsynaptic terminals of MSNs (Perreault *et al*, 2010), together these findings suggest that MSNs that express the D1-D2 receptor heteromer may have a unique physiological function at a local level as well as distal effects through their efferent projections. Together, these findings indicate that MSNs coexpressing the D1R and D2R in the basal ganglia embody a physiologically relevant subset of neurons and thus may represent a third major dopamine receptor neuronal pathway, in addition to the D1R/DYN-expressing striatonigral and D2R/ENK-expressing striatopallidal MSNs, which is involved in the regulation of thalamic output (Perreault *et al*, 2011).

The D1-D2 heteromer has been shown to exhibit pharmacological and cell signaling properties distinct from its constituent receptors (Hasbi *et al*, 2009; Lee *et al*, 2004; Rashid *et al*, 2007a; So *et al*, 2009; Verma *et al*, 2010) and the expression of dopamine D1-D2 receptor heteromers in the mesocorticolimbic system and basal ganglia nuclei suggest this receptor complex may have etiological significance in disorders characterized by abnormal dopamine signaling. More specifically, calcium signaling elicited by the D1-D2 heteromer, through activation of Gq/11 and phospholipase C (PLC), resulted in the activation of calcium calmodulin kinase IIα (CaMKII) (Ng *et al*, 2010; Perreault *et al*, 2012; Rashid *et al*, 2007a) and consequently increased expression of brain-derived neurotrophic factor (BDNF) in NAc and ventral tegmental area (VTA) (Hasbi *et al*, 2009; Perreault *et al*, 2012) (Figure 1), both of which have significant roles in the pathological processes underlying drug addiction. For instance, CaMKII in NAc shell has been shown to be critical to cocaine seeking, serving as a biochemical link between dopamine and glutamate (Anderson *et al*, 2008). In addition, whereas BDNF signaling in NAc and VTA has been shown to mediate the magnitude of the reward responses to cocaine (Bahi *et al*, 2008; Graham *et al*, 2007; Graham *et al*, 2009), BDNF in VTA suppressed the ability of morphine to increase dopamine neuron excitability and promote reward (Koo *et al*, 2012) and was a negative modulator. These findings indicate that BDNF in VTA may exert opposing effects on the reward circuitry of the brain that are specific to the psychostimulant being tested. Thus, although evidence suggests a potential role for the D1-D2 heteromer in mediating addictive processes, its exact impact on the brain reward circuitry and the mechanisms underlying addiction requires further investigation.

The dopamine hypothesis of schizophrenia postulates a hyperactivity of subcortical dopamine transmission; however, it has also been suggested that dysregulated calcium signaling may have a central role in generating the psychopathology of schizophrenia (Lidow, 2003). Although neither of the most abundant dopamine receptors (D1R or D2R) was known to directly regulate calcium signaling, it has been shown that coactivation of both receptors within the dopamine D1-D2 receptor heteromer led to a novel Gq-linked increase in intracellular calcium (Lee *et al*, 2004; Rashid *et al*, 2007a). Furthermore, D1-D2 heteromer-mediated signaling could be attenuated by D2R antagonists (as well as D1R antagonists) (Hasbi *et al*, 2009; Rashid *et al*, 2007a), indicating the D1-D2 heteromer as being a pharmacological target for antipsychotics *in vivo*. Thus, clinical administration of most antipsychotics would result in blockade of D2 receptor function, as well as D1-D2 receptor heteromer function. The antipsychotic clozapine, for instance, has been shown to uncouple the subset of D1-D2 receptor heteromers that were in an agonist-detected high-affinity state (Dziedzicka-Wasylewska *et al*, 2008; Faron-Górecka *et al*, 2008), a finding that may be of particular relevance given reports of enhanced D1-D2 receptor heteromer expression and activation in cells (Dziedzicka-Wasylewska *et al*, 2006) and striatum (Perreault *et al*, 2010) under conditions of persistent dopamine stimulation. Specifically, using FRET techniques it has been shown that the concomitant activation of D1R and D2R by subtype-specific agonists in HEK cells promoted the formation of D1-D2 heteromers (Dziedzicka-Wasylewska *et al*, 2006). Similarly *in vivo*, under conditions of hyperdopaminergia, such as occurs with repeated amphetamine administration, enhanced D1-D2 receptor interactions in rat striatum, as indicated by FRET, were apparent as was an increased proportion of the D1-D2 heteromer in the agonist-detected high-affinity state, suggestive of an increase in the functional activity of the receptor complex (Perreault *et al*, 2010). These findings indicate that there is dynamic regulation of the D1-D2 heteromer that responds to endogenous dopamine levels, such as the high levels that may occur with repeated amphetamine administration, and the increased high-affinity state of the heteromer detected under such

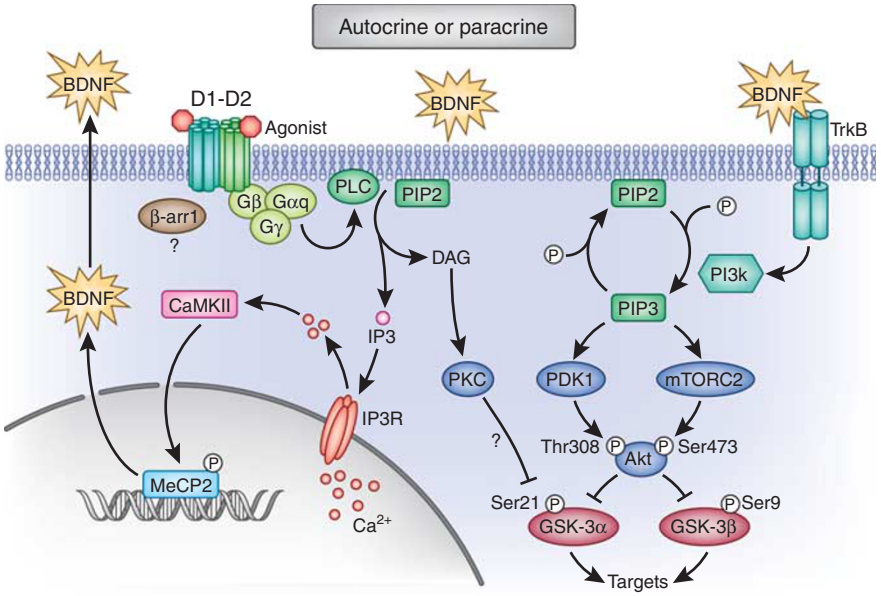

Figure 1. Signaling pathways activated by the dopamine D1-D2 receptor heteromer. Activation of the Gq-coupled D1-D2 heteromer results in PLC-dependent intracellular calcium release, the activation of CaMKII, and increased expression of BDNF potentially via phosphorylation of MeCP2. Dopamine D1-D2 heteromer activation can additionally lead to the phosphorylation, and inactivation, of GSK-3. The phosphorylation state of GSK-3 can potentially be regulated by BDNF-induced activation of TrkB and the subsequent phosphorylation and activation of Akt. Akt then phosphorylates GSK-3α, and GSK-3β resulted in their inactivation. β-Arrestin1 may also inhibit GSK-3α and GSK-3β activation. GSK-3α and GSK-3β can also be phosphorylated by PKC. BDNF, brain-derived neurotrophic factor; β-arr1/2, β-arrestin1 or β-arrestin2; CaMKII, calcium calmodulin kinase II; DAR, dopamine receptor; DAG, diacylglycerol; GSK-3, glycogen synthase kinase-3; IP3, inositol trisphosphate; IP3R, inositol trisphosphate receptor; MeCP2; methyl CpG-binding protein 2; mTORC2, mTOR complex 2; PIP2, phosphatidylinositol (4,5)-bisphosphate; PIP3, phosphatidylinositol (3,4,5)-trisphosphate; PDK1, phosphoinositide-dependent kinase-1; PLC, phospholipase C; PKC, protein kinase C; TrkB, tropomyosin receptor kinase B.

circumstances may represent a biomarker of the high dopamine-sensitized state. Postmortem analysis of globus pallidus samples from antipsychotic-treated and untreated schizophrenia patients also revealed a comparable increase in the high-affinity state of the D1-D2 heteromer (Perreault *et al*, 2010), a finding possibly reflective of increased dopamine transmission in this region. As a physiologically relevant fraction of MSNs within the NAc and globus pallidus express the D1-D2 receptor heteromer (Perreault *et al*, 2010), together these findings strongly implicate this receptor complex as being significant to dopamine transmission and should be investigated as a therapeutic target for schizophrenia. Further support for a potential role for the D1-D2 heteromer in schizophrenia comes from a recent finding demonstrating that activation of the D1-D2 receptor heteromer could inactivate glycogen synthase kinase-3β (GSK-3β) in rodent prefrontal cortex (PFC) (Perreault *et al*, 2013) (Figure 1). In schizophrenia, cortical GSK-3β activation is upregulated (Emamian *et al*, 2004) and has been implicated as contributing to cognitive dysfunction in the disorder (Freyberg *et al*, 2010; Karam *et al*, 2010). This suggests potential for D1-D2 heteromer activation as a therapeutic intervention to normalize cortical GSK-3β levels in schizophrenia patients, with potential consequential improvements in cognitive performance. However, given that D2R activation has been associated with increased GSK-3β levels via a non-canonical mechanism involving β-arrestin2 signaling (Beaulieu *et al*, 2007), and antipsychotics do not invariably improve cognitive deficits in schizophrenia, more research is clearly required to elucidate a role for dopamine-mediated changes in GSK-3β activation in cognitive dysfunction in this disorder and the potential relative importance of β-arrestin-biased signaling.

A potential involvement of the D1-D2 receptor heteromer in depression came to light when it was demonstrated in the postmortem striatum of depressed patients that there was an increased interaction between D1R and D2R (Pei *et al*, 2010). Using a disrupting peptide in rats, the authors further showed that disruption of the D1-D2 heteromer in the PFC, but not in the NAc or hippocampus, resulted in anti-depressant-like effects in the forced swim test. Similarly, in a learned helplessness paradigm, enhanced association of the D1R and D2R was reported in the PFC and striatum of rats following inescapable foot shock, an effect diminished in the presence of the antidepressant imipramine (Pei *et al*, 2010). Although the distribution of the D1-D2 heteromer in PFC has not been characterized, approximately 15–25% of the pyramidal neurons in rodent medial PFC coexpress the D1R and D2R (Zhang *et al*, 2010), implicating these neurons in the anti-depressant-like effects of D1-D2 heteromer disruption.

THE DOPAMINE D2-D4 RECEPTOR HETEROMER

Dopamine D4 receptor (D4R) expression in brain is the lowest among the types of dopamine receptors

(Missale *et al*, 1998; Rondou *et al*, 2010). Unlike the rat D4R, the gene encoding the human D4R has a number of polymorphic variants (Van Tol *et al*, 1991), due to repeats of a 16 amino-acid sequence in the third intracellular loop, numbering between 2 and 11. Three of the variants were identified to be the most abundant, D4.2R, D4.4R, and D4.7R (Borroto-Escuela *et al*, 2011).

Interactions among D4R variants, forming homomer and heteromer species, were shown to occur in transfected cell models. All three of the common variants noted above were able to form homomers. Furthermore, the D4.2R and the D4.4R variants were shown to form heteromers with each other, whereas the D4.7R variant was shown to be refractory to forming heteromer complexes with the other two variants (Borroto-Escuela *et al*, 2011; González *et al*, 2012).

Some degree of co-distribution of dopamine D2R and D4R was observed, notably in the dorsal striatum, and therefore it was investigated and confirmed that dopamine D4R variants were able to form heteromeric complexes with both the long and short forms of D2R, although with some differences (Borroto-Escuela *et al*, 2011; González *et al*, 2012). Using BRET and *in situ* Proximity Ligation Assay techniques in cotransfected cells, it was shown that the long form of human D2R (D2LR) was able to interact and form heteromers with the three human D4R isoforms (Borroto-Escuela *et al*, 2011), with the D4.7R variant being the least effective. Interestingly, allosteric modulations of receptor activity were observed using MAPK assays for the various receptor heteromers. Thus, in cells cotransfected with D2LR and each of the D4R variants, D2 agonist-induced extracellular signal-related kinase (ERK) phosphorylation was enhanced upon coactivation by a D4R agonist, PD168077, in cells coexpressing D2R with D4.2R and D4.4R but not in cells coexpressing D2LR with D4.7R. This may indicate an enhanced allosteric receptor–receptor interaction between certain protomers forming the heteromer complexes. In contrast, the D4.7R variant showed reduced ability to form a heteromer with D2LR, in keeping with the failure to observe any additive effect after combined treatment with D2R and D4R agonists on MAPK activity when these receptors were expressed together. This may suggest that the number of repeats in D4R variants may be a determinant for the formation of heteromers. The D4.7R variant showed higher propensity for homomer formation compared with the other variants tested, while the opposite was observed for heteromer formation (Borroto-Escuela *et al*, 2011).

Similar to D2LR, the short form of D2R (D2SR) was also shown to form heteromer complexes with D4.2R and D4.4R while the D4.7R failed to interact with D2SR in BRET studies (González *et al*, 2012). Biochemical crosstalk between the D2SR and cotransfected D4R variants was observed and consisted of ability to potentiate D4R-mediated ERK phosphorylation. Consistent with the failure of D4.7R to form heteromers with D2SR, this biochemical fingerprint of ERK activity potentiation was not observed in cells cotransfected with D2SR and D4.7R. Subsequently, the biochemical fingerprint (potentiation of D4R-mediated MAPK activation by D2R stimulation and not the inverse) was used in mouse striatal slices to show that D2SR was able to form heteromers with the mouse D4R (the equivalent of human D4.2R). Furthermore, when the MAPK study was performed in striatal slices taken from gene knock-in mice carrying the human D4.7R, no synergistic effect was observed, confirming that D2SR was not able to form heteromers with the D4.7R variant.

Interestingly, dopamine-induced decrease of K^+-induced glutamate release was shown to involve both D2R and D4R, as the respective antagonists, L-741626 and L-745870, were not only able to partially inhibit dopamine effect individually but were also able to completely abolish dopamine effect when co-applied (González *et al*, 2012). Moreover, striatal D4R was shown to selectively and locally modulate glutamate release, and the D2R agonist quinelorane synergistically potentiated the D4R-mediated effect, but not the inverse, suggesting a specific qualitative D2S-D4 heteromer-mediated effect in the brain similar to the biochemical fingerprint seen with MAPK activation in transfected cells. It was then postulated that the failure of D2SR to form heteromers with D4.7R may impair dopamine-induced modulation of corticostriatal glutamatergic neurotransmission, which may be linked to ADHD (González *et al*, 2012). The presence of the 7-repeat allele of D4R seems to affect neuropsychological functioning depending on age and ADHD status (Altink *et al*, 2012), and there are many genetic association studies linking this repeat allele (D4.7R) and other candidate genes (Kebir and Joober, 2011) to the development of ADHD. The role of D2R in ADHD is still not particularly clear, and the discovery of a role for the dopamine D2-D4 receptor heteromer may represent a new research target for ADHD.

ADENOSINE-DOPAMINE RECEPTOR HETEROMERS: A1-D1, A2-D2

The existence of the A1-D1 receptor heteromer was reported over a decade ago following the demonstration of coimmunoprecipitation of adenosine A1 receptor (A1R) and D1R in fibroblast cells (Ginés *et al*, 2000), and shortly thereafter, its expression was shown by the same method in rat NAc (Toda *et al*, 2003). The A2-D2 heteromer, also identified by coimmunoprecipitation, was first shown in neuroblastoma cells (Hillion *et al*, 2002) and has since been shown to exist in living cells by FRET and BRET analysis (Canals *et al*, 2003; Kamiya *et al*, 2003). It has been suggested that the A1-D1 and A2-D2 heteromers have a discrete distribution in the basal ganglia, with selective expression along the striatonigral and stratopallidal pathways, respectively (Ferré *et al*, 2007; Franco *et al*, 2007; Fuxe *et al*, 2008), and functional studies indicate that these receptor complexes may be the molecular entities responsible, at least in part, for the antagonistic interactions between adenosine and dopamine receptors, functioning to uncouple the dopamine receptors from their respective G-proteins and dampen receptor signaling (Azdad *et al*, 2009; Franco *et al*, 2007; Fuxe *et al*, 2005; Fuxe *et al*, 2008).

The ability of adenosine-dopamine receptor heteromerization to attenuate dopamine receptor function indicates that these receptor complexes are of relevance to dopamine transmission in the basal ganglia, and thus have a potential role in dopamine disorders. For example, following cocaine withdrawal the coimmunoprecipitation of A1R and D1R was reduced in rat NAc, indicating a reduction in heteromer formation (Toda *et al*, 2003). Similarly, using BRET methodology, cocaine was shown, through direct actions on D2R, to induce a conformational change in the A2-D2 complex, resulting in reduced BRETmax, potentially indicative of a reduction in heteromer expression (Marcellino *et al*, 2010). In schizophrenia animal models, evidence of antipsychotic effects of the A2 receptor (A2R) agonist CGS 21680 have been demonstrated (Andersen *et al*, 2002; Rimondini *et al*, 1997), and adenosine augmentation has been shown to ameliorate both psychotic and cognitive schizophrenia-like symptoms in mice (Shen *et al*, 2012), potentially by acting at the A2R within the A2-D2 heteromer, and thus reducing the proportion of D2R in the agonist-induced high-affinity state and D2R signaling (Fuxe *et al*, 2005; Fuxe *et al*, 2010). It has been further suggested that an imbalance in adenosine signaling, a neurotransmitter which modulates both dopamine and glutamate transmission, may be a central factor in the susceptibility to develop schizophrenia (Boison *et al*, 2012). In support of this hypothesis, it has been repeatedly shown that enhancing NMDA receptor function improves the negative and cognitive symptoms of schizophrenia (Coyle, 2012), and interestingly, heteromerization of the D2R with the A2R negatively regulated D2R-induced suppression of NMDA-mediated depolarization plateau potential (Azdad *et al*, 2009).

Thus far, studies have strongly suggested independent roles for the A1-D1 and A2-D2 receptor heteromers in regulating striatonigral and striatopallidal dopamine transmission, respectively. However, given the identification of neurons that coexpress the D1R and D2R in both the direct and indirect pathways, the expression of A1-D1-D2 or D1-D2-A2 heteromers in these neurons is a possibility, an intriguing prospect given that the sites of interaction between the D2R and D1R or A2R have been reported and are distinct (Ciruela *et al*, 2004; O'Dowd *et al*, 2012). Heterotrimeric receptor complexes involving dopamine receptors have already been identified, including the higher-order adenosine-dopamine receptor heteromer, the A2-D2-mGlu5 heteromer (Cabello *et al*, 2009), which has been suggested as a therapeutic target for schizophrenia by counteracting exaggerated D2R signaling in the ventral striatopallidal pathway (Fuxe *et al*, 2010).

THE DOPAMINE D1-D3, D2-D3 AND D2-D5 RECEPTOR HETEROMERS

The D1R and D3 receptors (D3R) show prominent colocalization in certain neurons of the direct striatonigral pathway (Ridray *et al*, 1998) and have been shown to form D1-D3 heteromers both in cells by BRET and FRET and to coimmunopecipitate from rat striatum (Fiorentini *et al*, 2008; Marcellino *et al*, 2008b). The physiological effect of this interaction was a D3R-stimulated increase in D1R-mediated responses in neurons that coexpressed both receptors (Fiorentini *et al*, 2008; Marcellino *et al*, 2008b). Although this heteromer was suggested to have potential therapeutic value as a drug target in Parkinson's disease (Ferré *et al*, 2010), these findings may also have significant future implications for disorders involving striatal D1R transmission, the most notable being drug addiction, where D1R signaling has such a critical role in drug reward.

The existence of a functional D2-D3 receptor heteromer was first shown in cells using chimeras generated from receptor fragments of the D2R and D3R in combination with coimmunoprecipitation (Scarselli *et al*, 2001). More recently, the D2-D3 heteromer was demonstrated to coexist with D2R and D3R homomers in cells at the plasma membrane using the newly developed SNAP and CLIP tag reagents (Pou *et al*, 2012). A putative role for the D2-D3 heteromer as a target for antipsychotics and, in particular, for antipsychotics with partial D2R agonism has been suggested (Maggio and Millan, 2010), an idea based on findings in cells that in the presence of excess D3R, the properties of partial D2R agonists, such as the antipsychotic aripiprazole, were transformed to antagonist actions (Novi *et al*, 2007). Thus the actions of these drugs would be postulated to have differing brain region-dependent effects, dependent on the density of expression of D3R. As a result, in the medial ventral striatum where the preponderance of D2-D3 heteromers may occur (based on D3R localization), 'partial D2R agonists' could function as D2R antagonists, whereas in the dorsal striatum, where D2-D3 heteromers would be few in number, they would exhibit partial D2R agonism (Maggio and Millan, 2010).

Expression of the dopamine D2-D5 receptor heteromer has been demonstrated in living cells (O'Dowd *et al*, 2013; So *et al*, 2009) and the two receptors have been shown to interact via electrostatic interactions between residues in the C-tail of the D5R and the third intracellular loop of the D2R (O'Dowd *et al*, 2013). Similar to the D1-D2 heteromer, the D2-D5 heteromer has been linked to increased intracellular calcium accumulation (So *et al*, 2009); however the signaling mechanisms underlying these increases in calcium mobilization are completely distinct (Hasbi *et al*, 2010). For example, in contrast to the D1-D2 receptor heteromer, which induces increased calcium release solely from intracellular stores (Lee *et al*, 2004; Rashid *et al*, 2007a), calcium mobilization induced by the D2-D5 heteromer was shown in cells to involve a small rise in intracellular calcium mediated by Gq and PLC, followed by a large influx of extracellular calcium through store-operated calcium channels (So *et al*, 2009). In addition, unlike the D1R, activation of the D5R triggered a robust calcium signal, an effect that was attenuated when the D5R heteromerized with coexpressed D2R and disinhibited when both receptors were activated (So *et al*, 2009). Therefore, together these findings suggest that in regions

where the D2R and D5R are coexpressed, such as in rat cortex and ventral pallidum, and to a much lesser degree in CP (So *et al*, 2009), the D2-D5 heteromer may have a role in regulating signaling events linked to calcium, one consequence of which may be the activation of CaMKII, a protein kinase previously discussed herein to be involved in both drug addiction and schizophrenia.

DOPAMINE-NMDA RECEPTOR HETEROMERS: D1-NR1, D2-NR2B

It was shown in rat hippocampus that the NR1 subunit of the *N*-methyl-D-aspartate (NMDA) receptor could coimmunoprecipitate with the D1R from rat hippocampal tissue (Lee *et al*, 2002). Similarly, in striatal postsynaptic density (PSD) preparations the C-terminal tail of the D1R, but not the D5R, coimmunoprecipitated with the NR1 subunit of the NMDA receptor (Fiorentini *et al*, 2003). The formation of the D1-NR1 heteromer was shown to occur through a strong and stable arginine–phosphate electrostatic interaction (Woods *et al*, 2005; Woods and Ferré, 2005) and reported to be enhanced by ligand occupancy of the NMDA/glutamate-binding site of the NMDA receptor, to slow down lateral diffusion, and stabilize D1R localization in the synapse (Scott *et al*, 2006). Presumably, increased synaptic localization would make these receptors more susceptible to activation by released dopamine, culminating in enhanced signal transduction and neuronal responsiveness. Indeed, it was shown in cells and hippocampal neurons that activation of the NDMA receptor promoted D1R translocation to the plasma membrane and enhanced D1R-mediated cyclic AMP accumulation (Pei *et al*, 2004), thus resulting in an overall increase in D1R activation and function. Conversely, direct D1R interactions with the NR1 subunit decreased NMDA currents and NMDA-mediated excitotoxicity (Lee *et al*, 2002). In addition, the D1-NMDA receptor complex may be of relevance for disorders involving cognitive dysfunction such as schizophrenia, as activation of the D1R upregulated NMDA receptor-mediated LTP in hippocampus in a CaMKII-dependent manner and promoted working memory, whereas uncoupling the D1-NMDA complex abolished the D1R-induced upregulation of NMDA-mediated LTP and impaired working memory in mice (Nai *et al*, 2010). Interestingly, an interaction between the D1R and NMDA receptor was also demonstrated in rat PFC (Kruse *et al*, 2009), a region critically involved in the cognitive impairments inherent in schizophrenia (Lewis, 2012), and in which NMDA hypofunction has been implicated (Jentsch *et al*, 1997; Mohn *et al*, 1999). It has thus been suggested that reduced D1R and NMDA function may contribute to the etiology of schizophrenia, and additionally, that the prefrontal cortical hypodopaminergia inherent in the disorder may be secondary to NMDA receptor dysfunction (Nai *et al*, 2010).

A direct interaction between the D2R and the NR2B subunit of the NMDA receptor was demonstrated in the PSD of excitatory synapses in striatum, the physiological function of which was to disrupt the interaction of CaMKII with the NR2B subunit, reduce CaMKII-mediated NR2B phosphorylation, and inhibit NMDA receptor-mediated currents (Liu *et al*, 2006). In the same study, Liu *et al* (2006) also showed that while acute cocaine administration to mice increased the physical association of the two receptors, disruption of the D2R-NR2B complex significantly reduced cocaine-induced locomotor activation and stereotypy, a finding which directly linked the D2-NR2B heteromeric complex to behavioural responses evoked by cocaine.

OTHER DOPAMINE RECEPTOR HETEROMERS

Despite the D2R and the serotonin 5HT2A receptor (5HT2AR) having distinct cell signaling properties, being linked to the Gi/o and Gq/11 proteins, respectively, they are both receptor targets for antipsychotic drugs in schizophrenia. Although the existence of the D2-5HT2A heteromer has not been definitively demonstrated *in vivo*, functional crosstalk between the two receptors has been shown at both the level of receptor pharmacology, cell signaling properties, as well as in a behavioural assay of locomotor activity (Albizu *et al*, 2011). This crosstalk was suggested by the authors to be potentially mediated by the D2-5HT2A receptor heteromer as this heteromeric complex has been reported to occur in cells using FRET, BRET, and coimmunoprecipitation techniques (Albizu *et al*, 2011; Borroto-Escuela *et al*, 2010; Łukasiewicz *et al*, 2010), and the site of interaction reported to occur between the C-tail of the 5HT2AR and the third intracellular loop of the D2R (Łukasiewicz *et al*, 2010). Interestingly, ligands for the 5HT2AR or the D2R were shown to directly influence the heterodimerization process in cells, with agonists reducing the FRET value between the 5HT2AR and the D2R in the D2-5HT2A heteromer and antagonists increasing the FRET values (Łukasiewicz *et al*, 2010). The authors therefore posited that the agonists and antagonists may have promoted the formation of homomeric or heteromeric receptor entities, respectively, although these results could represent conformational changes induced by the drugs. Given that heteromeric receptor complexes often exhibit unique functional characteristics compared with their constituent receptors, the ability of pharmacological agents to selectively target the signaling pathways being initiated in favor of homomeric or heteromeric complexes may have significant future therapeutic implications in any number of human diseases.

The histamine H3 receptor (H3R) has also been shown by BRET to form a heteromeric complex with the D1R and D2R in cells (Ferrada *et al*, 2008; Ferrada *et al*, 2009) and to coimmunoprecipitate with the D1R or D2R in the striatum (Moreno *et al*, 2011). At a functional level, it was demonstrated that H3R-induced activation of ERK phosphorylation occurred only in striatal slices of mice expressing the D1R but not in mice gene-deleted for the D1R. Conversely, both D1R and H3R antagonists attenuated D1R- or H3R-induced ERK

activation (Moreno *et al*, 2011). As the H3R has been implicated in a number of psychiatric disorders, including schizophrenia, addiction, and ADHD (Vohora and Bhowmik, 2012), these findings suggest a potential contribution of the D1-H3 heteromer in mediating some of the effects attributed solely to the H3R, and thus further investigation into the role of the D1-H3 heteromer in the etiology of these mental health disorders is warranted.

FUTURE RESEARCH DIRECTIONS

The involvement of the dopaminergic system in a wide array of mental disorders has resulted in the pharmacological targeting of dopamine receptors as the mainstay for many of the treatments currently available. Most of the ligands developed and clinically used have a single dopamine receptor type or subtype as a target. The successive demonstration of the presence of more complex physical and functional interactions among the dopamine receptors and between these receptors and other receptors, including other GPCRs and ion channels, should add a new dimension to rational drug design that may lead to the development of new approaches taking into account the presence and physiological relevance of these heteromers (George *et al*, 2002).

Bivalent Ligands

One approach based on the notion of receptor dimerization is the development of bivalent ligands. Such compounds are formed of two ligand moieties linked through a spacer capable of binding to both protomers of a dimer (Guixà-González *et al*, 2012). Some of these bivalent ligands were described in the literature for different homo- and heteromers, such as bivalent ligands for D2-D2 homodimers (Kühhorn *et al*, 2011), for D2-A2 heterodimers (Soriano *et al*, 2009), or opioid receptor heterodimers (Balboni *et al*, 2010; 2011; Zhang *et al*, 2009). Physicochemical limitations due notably to their large molecular sizes (Morphy and Rankovic, 2006; Guixà-González *et al*, 2012) may represent, however, a big challenge against their pharmaceutical development and clinical use.

In many cases, receptor heteromerization has been shown to confer novel pharmacological profiles as well as signaling properties different from those of the protomers that constitute these receptor complexes (George and O'Dowd, 2007; Maggio *et al*, 2009; Smith and Milligan, 2010). Interestingly, these receptor heteromer complexes are mostly confined to some brain regions, as is the case for the dopamine D1-D2 heteromer (Hasbi *et al*, 2009; Perreault *et al*, 2010), which makes targeting GPCR heteromers a pharmacological alternative that offers the advantage of a higher brain region specificity and a better targeting of receptor signals.

Allosterism

Receptor oligomerization through protein–protein interactions can be considered as a form of allosterism (Maggio *et al*, 2009), in that the binding of a compound to one protomer of the heteromer may positively or negatively modulate the drug occupancy of the other protomer, or in some cases, some heteromers may display a selectivity to ligands not observed in the case of individual receptors (George and O'Dowd, 2007; Maggio *et al*, 2009; Smith and Milligan, 2010). In the first type of scenario, some examples were cited for the GABAB receptor complex (Galvez *et al*, 2001), for A2-D2 receptor heterodimers (Franco *et al*, 2000), D2-D3 heteromers (Maggio *et al*, 2009), delta-kappa opioid heterodimers (Jordan and Devi, 1999), as well as for somatostatin-dopamine sSST5-D2 receptors (Rocheville *et al*, 2000) (reviewed in Maggio *et al*, 2009). For the second case, which relates to the differing specificity of certain ligands to either the heteromer or a constituent homomer, some examples were described such as for the dopamine D1-D2 heteromer (Rashid *et al*, 2007b) and dopamine D2-D3 heteromer (Maggio and Millan, 2010; Maggio *et al*, 2009). For example, two D1R-like agonists, SKF 83959 and SKF 83822, although showing high radioligand-binding affinities for the D1R in D1-D2 or D1-D1 receptor complexes, showed very specific functional effects, with SKF 83959 robustly stimulating the D1-D2 heteromer-mediated calcium signal and not activating adenylyl cyclase by the D1-D1 homomer, whereas SKF 83822 robustly stimulated adenylyl cyclase by the D1-D1 homomer with no effect on calcium release through the D1-D2 heteromer complexes (George and O'Dowd, 2007; Rashid *et al*, 2007a; Hasbi *et al*, 2009). Furthermore, examining the binding pockets within each receptor in the D1-D2 heteromer complex revealed that SKF 83959 occupied both binding pockets and acted as a full agonist at the D1R and a partial agonist at the D2R within the D1-D2 receptor heteromer (Rashid *et al*, 2007a; 2007b; George and O'Dowd, 2007). Another D1R agonist, SKF 81297, showed no specificity for the signal and robustly stimulated both Gs-mediated adenylyl cyclase activity through D1-D1 homomer complexes as well as Gq-mediated intracellular calcium release through D1-D2 heteromer complexes. Thus, these agonists show selective actions to activate either the D1-D2 heteromer (SKF 83959) or the D1-D1 homomer (SKF 83822), or both at the same time (SKF 81297), suggesting there are significant differences within the binding pockets of the receptors depending on whether they are within a homomeric/heteromeric complex (Rashid *et al*, 2007b; George and O'Dowd, 2007; Hasbi *et al*, 2009; 2010; Verma *et al*, 2010). These differences in the binding pockets of the receptors induced by heteromerization may be an aspect that can be capitalized upon to develop heteromer-specific compounds.

Selective Modulation of Signaling Pathways

In numerous cases of receptor complex formation, such as with the D1-D2 receptor heteromer, the heteromerization confers to the receptor complex a different signaling mechanism than that activated by the two individual

protomers (George and O'Dowd, 2007). The modification of the signaling properties by receptor oligomerization may constitute another manifestation of the effects of allosterism, whether these changes are minor resulting in differences in efficacy or are major with a complete signal switching (reviewed in Smith and Milligan, 2010), as is the case for the D1-D2 heteromer. The stimulation of the dopamine D1-D2 receptor heteromer, for instance, triggers a Gq-mediated intracellular mobilization of calcium that neither D1R or D2R individually are associated with, and whose specificity was shown using $D1R^{-/-}$, $D2R^{-/-}$, and $D5R^{-/-}$ gene-deleted mice (George and O'Dowd, 2007; Rashid *et al*, 2007a, Hasbi *et al*, 2009). One approach to better study the pathophysiologies linked to receptor heteromerization would be to generate ligands more specific to either heteromeric or homomeric complexes, capable of activating one signaling pathway or the other. Another approach would be to target a particular signaling pathway or even a component of a signaling pathway specific to the receptor oligomer in question. However, although targeting the signaling pathway may be an interesting path to investigate, the results may not be definitive as these signaling pathways are not specific to a particular homo- or heteromer receptor complex.

Biased Agonism

Another approach that is under scrutiny in drug development is based on a ligand's preference for one signaling pathway over another, a phenomenon also known as 'biased agonism' (Beaulieu and Gainetdinov, 2011). Of notable interest was the discovery of the ability of some GPCR ligands to activate G-protein-independent but β-arrestin-dependent signaling (Beaulieu and Gainetdinov, 2011). This biased agonism may represent another interesting path in drug development, although there are not yet many compounds with a clear demonstration of potential clinical use, notably in the case of the dopamine receptor heteromers.

Disrupting the Heteromers

Identification of the specific receptor–receptor interaction interfaces involved in the formation/stabilization of receptor heteromers may yield another approach to antagonize heteromer function, based essentially on disrupting the receptor complexes. The parts of a receptor that are involved in the interaction with another protomer may be used as a target to disrupt the interaction, using a peptide that mimics the interaction interface. For the D1-D2 receptor heteromer, it has been shown that specific amino acids in the D1R carboxyl tail (O'Dowd *et al*, 2011; 2012; Łukasiewicz *et al*, 2009) are important in D1-D2 heteromer formation. These specific amino acids in the D1R carboxyl tail interacted with a region in the third intracellular loop of D2R, common to both D2LR and D2SR (O'Dowd *et al*, 2011; 2012; Łukasiewicz *et al*, 2009). A peptide generated based on these findings from the D1R was found to disrupt the D1-D2 receptor heteromer physically in brain and to inhibit its calcium-mediated signaling pathway with behavioral consequences (Hasbi *et al*, under review). Another peptide generated from the region of D2LR that is lacking in the D2SR was also reported to disrupt the D1-D2 receptor heteromer and showed antidepressant-like effects in mice (Pei *et al*, 2010). In the absence of specific antagonists for the heteromers, this disrupting peptide strategy may be very useful in studying the biology of receptor heteromers as well as their link to disease pathophysiologies in animal models. The specificity of the peptide(s) used to target a specific heteromer should, however, be tested to avoid disrupting other receptor complexes.

In conclusion, dopamine receptors participate in homomeric and heteromeric complexes with significant implications for the deeper understanding of the complex physiological roles of these receptors in brain. The emerging significance of these receptor–receptor interactions has added an enormous degree of complexity in our attempts to understand dopamine receptor function in brain. However, these novel signaling complexes provide fascinating new possibilities and novel perspectives on physiological mechanisms and models of neuropsychiatric disease. Further, these complexes provide novel targets for drug discovery, as besides the classical tools to target a specific type or subtype of dopamine receptor, investigators now have the opportunity to open new fields of research to generate compounds that may take into account that these receptors exist as heteromeric complexes, often with distinct anatomical localization as well as signaling and functional properties. Contemporary drug discovery strategies have not incorporated the issue of receptor heteromers into the discovery process, and this presents a new challenge that must be surmounted. Different approaches are possible in the search for ligands specifically targeting receptor heteromers without affecting homomers or vice versa, such as the development of bivalent ligands, the targeting of a particular signaling pathway or one of its components, specifically probing binding pocket differences, or the use of peptides to specifically disrupt these receptor complexes. In other words, the potential for true 'designer drugs' targeting dopamine receptor heteromers may be within reach, aiming for selective activation or inactivation of these receptor complexes.

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The authors declare no conflict of interest.

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