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**TITLE PAGE**

# Dopamine $D_2$ Receptor Supersensitivity as a Spectrum of Neurotoxicity and Status in Psychiatric Disorders

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## RUNNING TITLE PAGE

**Running Title:** Dopamine $D_2$-R Supersensitivity & Psychiatric Disorders

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**Abbreviations:**

**5-HT**: 5-hydroxytryptamine, serotonin
**7-OHDPAT**: (+/-)-2-(dipropylamine)-7-hydroxy-1,2,3,4-tetrahydronaphthalene
**$A_{2A}$R**: adenosine $A_{2A}$ receptor
**ADHD**: attention-deficit hyperactivity disorder
**AMPH**: amphetamine
**BDNF**: brain-derived neurotrophic factor
**CGS 21680**: 2-p-(2-carboxyethyl)phenethylamino-5′-N-ethylcarboxamidoadenosine
**$D_2$R**: dopamine $D_2$ receptor
**$D_2$RSS**: dopamine $D_2$ receptor supersensitivity
**DA:** dopamine
**DARSS**: dopamine receptor supersensitivity
**DR**: dorsal raphe
**EEDQ**: 2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline
**GABA**: gamma-aminobutyric acid
**KO**: knockout
**KW 6002**: (E)-1,3-diethyl-8-(3,4-dimethoxyphenylethyl)-7-methyl-3,7-dihydro-1H-purine-2,6-dione
**MDMA**: methylenedioxymethamphetamine
**NAC**: nucleus accumbens
**NGF**: nerve growth factor
**NMDA**: N-methyl-D-aspartate
**PFC**: prefrontal cortex
**PPI**: prepulse inhibition
**RGS9**: regulator of G-protein signaling 9
**RSS**: receptor supersensitivity
**SCH 58261**: 5-amino-7-(β-phenylethyl)-2-(8-furyl)pyrazolo(4,3-e)-1,2,4-triazolo(1,5-c)pyrimidine
**TAs**: trace amines
**TAAR1**: Trace Amine-Associated Receptor 1
**VTA**: ventral tegmental area (VTA)

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**ABSTRACT PAGE**

## Abstract

Abnormality of dopamine $D_2$ receptor ($D_2R$) function, often observed as $D_2R$ supersensitivity ($D_2RSS$), is a commonality of schizophrenia and related psychiatric disorders in humans. Moreover, virtually all psychotherapeutic agents for schizophrenia target $D_2R$ in brain. Permanent $D_2RSS$ as a feature of a new animal model of schizophrenia was first reported in 1991, then behaviorally and biochemically characterized over the next 15-20 years. In this model of schizophrenia characterized by production of $D_2RSS$ in ontogeny, there are demonstrated alterations of signaling processes; as well as functional links between the biologic template of the animal model; and ability of pharmacotherapeutics to modulate or reverse biological and behavioral modalities towards normality. Another such animal model, featuring knockout of Trace Amine-Associated Receptor 1 (TAAR1), demonstrates $D_2RSS$ with an increase of the proportion of $D_2R$ in the high affinity state. Currently, TAAR1 agonists are being explored as a therapeutic option for schizophrenia. There is likewise an overlay of $D_2RSS$ with substance use disorder. The aspect of adenosine $A_{2A}$-$D_2$ heteroreceptor complexes in substance use disorder is highlighted, and the association of $A_{2A}R$ antagonists in discriminative and rewarding effects of psychostimulants is outlined. In summary, these new animal models of schizophrenia have face validity, construct validity and predictive validity, and with distinct advantages over earlier models. While the review summarizes elements of $D_2RSS$ in schizophrenia per se, and its interplay with substance use disorder, a major focus is on presumed new molecular targets attending $D_2RSS$ in schizophrenia and related clinical entities.

## Introduction

While the phenomenon of dopamine (DA) $D_2$ receptor ($D_2R$) supersensitivity (RSS; DARSS; $D_2RSS$) had long been implicated in a number of clinical states (schizophrenia and tardive dyskinesia; attention-deficit hyperactivity disorder (ADHD), Tourette; Parkinson's disease; Huntington's chorea; reactivity to substances of abuse; and other neuropsychiatric disorders), the directed experimental focus on $D_2RSS$ has been in vogue for only the past 30 years. This review highlights the production of $D_2RSS$ and its seminal role in animal modeling of schizophrenia and substance abuse. The ability of pharmacological agents to alter molecular events and thereby reverse behavioral abnormalities in relevant animal models, gives credence to the possibility of new treatments in humans directed towards ameliorating $D_2RSS$.

However, other receptor systems are also involved in schizophrenia and substance use disorder. Thus, multiple $D_2$ heteroreceptor complexes with receptor-receptor interactions exist in the ventral striatum and can modulate $D_2RSS$ (Borroto-Escuela et al., 2016a; Fuxe et al., 2014a,b). They are of relevance for schizophrenia; and the presence of the adenosine $A_{2A}R$-$D_2R$, serotonin 5-$HT_{2A}$-$D_2R$, NTS1-$D_2R$ and oxytocin R-$D_2R$ heteroreceptor complexes have been demonstrated. Thus, neurotransmitters such as serotonin (5-hydroxytryptamine, 5-HT), neurotensin, and oxytoxin, and the neuromodulator adenosine can modulate $D_2RSS$ via their receptor protomers in $D_2R$ heteroreceptor complexes.

## Production of DA $D_2RSS$: Neurochemical and Behavioral Effects Relating to a Mimic of Schizophrenia Abnormalities

Perinatal repeated treatment of rats with the DA $D_2R$ agonist quinpirole consistently results in the development of $D_2RSS$ – a priming phenomenon, with $D_2RSS$ persisting life-long (Kostrzewa 1995; Kostrzewa and Brus 1991; Kostrzewa et al., 1993a,b; 2004; 2008). These primed rats, as adults, are virtually indistinguishable behaviorally from untreated rats, except if challenged with agents that impinge on the $D_2R$. A single challenge dose of quinpirole to primed rats initially produces a short-lived enhancement of yawning (Kostrzewa and Brus, 1991; 1993a; Plech et al., 1995), an action known to be mediated by the $D_3R$ (Collins et al., 2005). In these primed rats quinpirole likewise induces oral activity (Kostrzewa et al., 1990), vertical jumping (between 3-5 weeks of age) (Kostrzewa et al., 1993b; Kostrzewa and Kostrzewa, 2012), altered antinociceptive response to a hot plate (Kostrzewa et al., 1991), and a variety of stereotypic actions (Brus et al., 2003; Kostrzewa et al., 1990). Yet, the $B_{max}$ (i.e., number of $D_2R$) and $K_d$ (affinity) for striatal $D_2R$ was unaltered in the primed rats (Kostrzewa and Brus, 1991). The general $D_2R$ template and its interaction with a ligand has been illustrated (Männel et al., 2017), while its unique deep orthosteric binding pocket was demonstrated at the molecular level (Wang et al., 2018). In rats displaying $D_2RSS$ active avoidance responding to quinpirole challenge was improved (Brus et al., 1998b), but there was a deficit in learning and memory tasks (Brown et al., 2004a; 2005; Brus et al.,

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1998a) and in the Whishaw skilled reaching task (Brown et al., 2002; 2004a). The deficits observed in learning and memory tasks were associated with a reduction in the expression of hippocampal nerve growth factor (NGF) (Brown et al., 2004b) and was reversed by prolonged treatment with olanzapine, an atypical antipsychotic agent (Thacker et al., 2006).

Important to note is that quinpirole is a DA "$D_2$-like" agonist, in that it binds to DA $D_2$R subtypes $D_2$, $D_3$, and $D_4$ with significant affinity. It is known that the $D_3$R co-localizes with the $D_1$R and forms $D_1$-$D_3$ receptor heteromers in neuronal populations in the dorsal striatum and nucleus accumbens (NAC) (Fiorentini et al., 2008; Marcellino et al., 2008). These heteromers may be involved in regulation of not only rewarding mechanisms, but emotional and cognitive processes as well as motor function (Fiorentini et al., 2010). The DA $D_1$/$D_3$ heteromer in these brain areas may play a significant role in some of the observed behavioral and neurobiological effects reported for quinpirole-primed rats.

For example, in adult rats that were quinpirole primed, an acute challenge dose of amphetamine (AMPH) is associated with a five-fold increase in evoked DA release, as assessed by *in vivo* microdialysis in awake freely-moving rats (Nowak et al., 2001). Enhanced AMPH-evoked release has been related to subsensitivity of $D_2$ autoreceptors (Seutin et al., 1991; Marinelli et al., 2003; Tammimaki et al., 2006), prompting the question of whether quinpirole priming, in part, may produce subsensitization of $D_2$ autoreceptors (Kostrzewa et al., 2016). The concept of quinpirole induction of subsensitivity of $D_2$ autoreceptors relates to the above results.

Quinpirole-primed rats displayed enhanced behavioral sensitization to AMPH in adulthood (Cope et al., 2010) and to nicotine in both adolescence (Perna and Brown, 2013) and in adulthood (Perna et al 2008). Further, quinpirole-primed rats demonstrated enhanced DA release in the NAC core in response to both AMPH and nicotine (Perna and Brown, 2013).

Using *in situ* hybridization, RGS9, the transcript regulating G-protein coupling to the $D_2$R, was shown to be decreased in the NAC and frontal cortex of these primed rats (Maple et al., 2007). Moreover, rats with ontogenetically-induced $D_2$RSS displayed deficits in prepulse inhibition (PPI), when adult, analogous to PPI-deficits found in schizophrenics (Brown et al., 2012). Notably, $D_2$RSS and diminished RGS9 expression is also reported in the brain of schizophrenics (Seeman et al., 2007). Overall, the altered neurobiological template of brain deriving from $D_2$RSS is considered to be a reasonable modeling of schizophrenia (Brown et al., 2012; Maple et al., 2015; Kostrzewa et al., 2016).

Another important research area with quinpirole has been related to the behavioral and neurobiological responses to nicotine, based on past findings that reported a high rate of cigarette smoking in the population diagnosed with schizophrenia (Winterer, 2010). In quinpirole-primed rats, alpha-7 nicotinic receptor binding was increased in striatum and hippocampus, and the enhanced behavioral effects of an acute challenge dose of quinpirole were reduced by nicotine (Perna et al., 2008).

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The latter action of nicotine was blocked by the nicotinic receptor antagonist mecamylamine (Tizabi et al., 1999). In addition, rats that were neonatally quinpirole-primed have shown increased behavioral sensitization to nicotine (Perna and Brown, 2013; Sheppard et al., 2009), conditioned place preference to nicotine (Brown et al., 2018) and enhanced DA (Perna and Brown, 2013) and BDNF (brain-derived neurotrophic factor) responses to nicotine (Peterson et al., 2017) in the NAC, a brain area that mediates drug reward. All these findings are consistent with the hypothesis that a $D_2$RSS system is enhanced in its sensitivity to the rewarding aspects of nicotine. Furthermore, prolonged nicotine treatment of quinpirole primed rats reduced the learning deficit (Morris Water Maze) and skilled reaching deficit; and reversed the reduction in hippocampal NGF and BDNF produced by neonatal quinpirole treatment (Brown et al., 2006; 2012). Interestingly, these data are consistent with the hypothesis that nicotine may be used as self-medication towards cognitive impairments known to exist in schizophrenia (Leonard et al., 2007).

In related studies on DA $D_3$R, neither quinpirole ($D_2$R, relatively selective) nor 7-OHDPAT [(+/-)-2-(dipropylamine)-7-hydroxy-1,2,3,4-tetrahydronaphthalene] ($D_3$R-selective) were able to prime $D_3$R (Oswiecimska et al., 2000), thus indicating that the priming process is mostly associated with $D_2$RSS.

The $D_1$-$D_3$ heteroreceptor complexes exist in the direct pathway, especially after degeneration of nigro-striatal dopaminergic neurons, and in the reward neurons of the NAC (Fiorentini et al., 2008; Marcellino et al., 2008); $D_3$R agonist activity of quinpirole is well-established. It should therefore be considered that $D_1$R also can participate in the above studies. $D_3$R are known to enhance the actions of $D_1$R. The enhancement of $D_1$R protomer signaling by $D_3$ protomer activation may also play a significant role.

**Production of DA $D_2$RSS: Neurochemical and Behavioral Effects Relating to a Mimic of Parkinson's Disease Abnormalities**

Quinpirole-induced striatal $D_2$RSS was demonstrated in the hemiparkinson rat model based on induction of contralateral turning behavior and enhanced inhibition of neuronal firing rates in this brain area (Strömberg et al., 2000). The mechanism related to the above finding is linked to an enhanced coupling of postjunctional $D_2$-likeR to G proteins via - inter alia – reduction in the GPCR kinase activity (Gainetdinov et al., 2003). $D_2$RSS may also develop as a result of enhanced inhibition of protein kinase tyrosine/mitogen-activated protein kinase phosphastase activity (Zhen et al., 2002). Furthermore, $D_2$-likeRSS is also associated with calcium current modulation which participates in regulation of the excitability of striatal efferent neurons (Prieto et al., 2009). In recent years it has been suggested that a reorganization of multiple $D_2$ heteroreceptor complexes in balance with each other can contribute to the development of $D_2$RSS (Fuxe et al., 2014a; Borroto-Escuela et al., 2016a). Taken together, all the above-

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mentioned mechanisms may be in operation in the dorsal and ventral striatum and have relevance for motor and reward/emotional functions, respectively, which can be disturbed both in Parkinson's disease and in schizophrenia.

**Developmental Differences in DA $D_2^{High}$ Receptors**

Dopaminergic dysfunction is a central component of many psychiatric illnesses that affects adult populations. As a natural outgrowth of this understanding, the pharmacological targeting of dopaminergic transmission is also a common strategy for treating pediatric age groups (Wall et al., 2012). This approach can be problematic as the efficacy of many of these drugs is based on adult responsiveness and may not be predictive of their effectiveness in developing organisms (Stephenson, 2005). In the preclinical literature, it is not uncommon for dopaminergic agonists and antagonists to have pronounced ontogenetic effects (Spear, 2000). Quantitative age-dependent behavioral differences are most frequently observed, as developing animals often display adult-typical responses that vary only in magnitude (McDougall et al., 2015). Occasionally, ontogenetic differences in drug responsivity can also differ in a qualitative manner, as dopaminergic drugs may either leave a particular age group unaffected or induce distinctly different behavioral patterns across ontogeny (Moody and Spear, 1992).

One striking example of this type of developmental difference is the behavioral response to the irreversible DA receptor antagonist 2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline (EEDQ). In adult rats, EEDQ reduces spontaneous behaviors and causes a dose-dependent reduction in DA agonist-induced locomotor activity. In sharp contrast, administering EEDQ to preweanling rats both increases basal locomotion and potentiates the effects of DA agonists (Der-Ghazarian et al., 2012; 2014). The cause of these age-dependent behavioral differences is uncertain; however, the potentiation effect is mediated by $D_2R$ because only drugs that directly or indirectly stimulate $D_2R$ (quinpirole and cocaine) produce an exaggerated locomotor response in EEDQ-treated preweanling rats (Der-Ghazarian et al., 2012; 2014). Receptor selectivity can also be established by pretreating rats with a reversible DA-R antagonist, such as raclopride or sulpiride, in order to protect $D_2R$ from EEDQ-induced inactivation. In this case, protecting $D_2R$ from the alkylating effects of EEDQ eliminated DA agonist-induced locomotor potentiation (Der-Ghazarian et al., 2012; 2014), thereby demonstrating that $D_2R$ is the critical receptor type underlying the potentiation phenomenon.

Ontogenetic differences in the sensitivity of the $D_2R$ may be at least partially responsible for the EEDQ-induced potentiation effect observed in young rats. Specifically, EEDQ (7.5 mg/kg) reduced $D_2R$ densities in rats of various ages; however, the surviving $D_2Rs$ of preweanling rats were preferentially in a high affinity state (McDougall et al., 2014). GTPγS binding, which is a measure of the functional relationship between G proteins and the $D_2R$, was also significantly enhanced in the dorsal striata of EEDQ-treated preweanling rats relative to adult rats. Both sets of findings suggest that EEDQ causes a

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$D_2$RSS in preweanling rats—an action that may be responsible for potentiating basal and DA agonist-induced locomotor activity.

$D_2$RSS may also be responsible for some of the quantitative differences in drug responsivity that are apparent across ontogeny. An older literature suggests that neonatal and preweanling rats, relative to adults, are more sensitive to the activational effects of DA agonists (Spear, 1979). Specifically, there is a leftward shift in the dose response curve for DA agonists, with young rats showing maximal amounts of locomotor activity at lower doses than adolescent or adult rats (McDougall et al., 2015). We hypothesized that these age-dependent behavioral effects are due to a relative excess of high-affinity $D_2$R during early ontogeny. Consistent with this explanation, non-EEDQ-treated preweanling rats have a significantly greater percentage of dorsal striatal $D_2^{High}$ receptors than adolescent or adult rats (McDougall et al., 2015).

In summary, there is accumulating evidence that behavioral responsiveness to dopaminergic drugs varies dramatically across ontogeny, and that age-dependent changes in the percentage of $D_2^{High}$ receptors may be responsible for these behavioral differences. It is well established that $D_2$RSS is correlated with a number of neuropsychiatric conditions present in adulthood. Likewise, it is possible that $D_2$RSS may contribute to some of the DA-linked disorders first expressed during childhood and early adolescence (e.g., Tourette, ADHD, etc.). More generally, the response characteristics of adults treated with dopaminergic drugs may not always be predictive of pediatric responsiveness.

**$D_2$RSS in mice lacking Trace Amine Associated Receptor 1 (TAAR1)**

The discovery of a new family of monoaminergic G protein-coupled receptors named Trace Amine-Associated Receptors (TAARs, 6 functional receptors are found in humans), has significantly added to the understanding of complexity of monoaminergic neurotransmission (Borowsky et al., 2001; Bunzow et al., 2001). The best investigated receptor TAAR1 senses trace amines (TAs) β-phenylethylamine, tyramine, tryptamine, octopamine, DA metabolite 3-methoxytyramine, thyroid derivative 3-iodothyronamine and some other biogenic amines that are found at low levels in mammalian brain (Berry et al., 2017, Grandy, 2007). TAAR1 is coupled to Gαs heterotrimeric G-protein to stimulate the production of cAMP (Borowsky et al., 2001; Bunzow et al., 2001; Barak et al., 2008). In addition to abovementioned TAs, TAAR1 also senses other compounds such as adrenergic drugs, ergolines, apomorphine, ractopamine, and psychostimulant drugs AMPH and methylenedioxymethamphetamine (MDMA) (Borowsky et al., 2001; Bunzow et al., 2001; Berry et al., 2017). In the brain, TAAR1 mRNA is found within primary monoaminergic areas such as the ventral tegmental area (VTA), substantia nigra, amygdala, frontal cortex, dorsal raphe (DR) and striatum/NAC (Lindemann et al., 2008; Di Cara et al., 2011; Berry et al., 2017). Electrophysiological experiments performed on mouse brain slices also show that TAAR1 is functionally active in the VTA and DR (Bradaia et al., 2009; Revel et al., 2011). Altogether, these studies

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clearly demonstrate that TAAR1 is expressed and functional in brain monoaminergic systems and may therefore be implicated in the modulation of DA and 5-HT neurotransmission.

Indeed, the first investigation of mice lacking TAAR1 revealed that these mutants demonstrate enhanced locomotor and neurochemical responses to psychostimulants and have an increased proportion of striatal $D_2$Rs in the high affinity state (Wolinski et al., 2007). These findings were corroborated by other groups that also reported psychostimulant supersensitivity in independently developed strains of TAAR1 knockout (KO) mice (Lindemann et al., 2008; Di Cara et al., 2011). Functional and physical interactions between $D_2$R and TAAR1 have been shown in a number of studies performed both *in vitro* and *in vivo*. Cellular mechanistic studies have revealed that $D_2$R long receptor (postsynaptic isoform) and TAAR1 form heteromers in HEK cells and that application of DA receptor antagonists results in enhanced TAAR1 signalling (Espinoza et al., 2011; Harmeier et al., 2015). Importantly, TAAR1-KO mice have reduced $D_2$R antagonist haloperidol-induced striatal c-fos expression and a reduction in haloperidol-induced catalepsy (Espinoza et al., 2011).

At the same time, both partial and full TAAR1 agonists neither cause catalepsy per se nor enhance haloperidol-induced catalepsy, but in fact somewhat reduce catalepsy (Revel et al., 2013). Further confirmation of interaction between striatal postsynaptic $D_2$R and TAAR1 is evidenced by the fact that $D_2$Rs but not DA $D_1$Rs are overexpressed, and locomotor responses to $D_2$R-, but not $D_1$R-agonists, are enhanced in TAAR1-KO mice. An allosteric antagonistic TAAR1-$D_2$R interaction in this receptor complex may contribute to these behavioral findings, implicating TAAR1 receptor antagonists as novel antiparkinsonian drugs. Further validation of interactions between striatal postsynaptic $D_2$R and TAAR1 is evidenced by the fact that $D_2$Rs but not $D_1$Rs are over-expressed, and locomotor responses to $D_2$R- but not $D_1$R-agonists are enhanced in TAAR1-KO mice. These results indicate that the TAAR1 also can exert an inhibitory transcriptional modulation of the $D_2$R.

Similarly, the profile of striatal postsynaptic signalling events in knockout animals is altered only for $D_2$Rs with selectively activated $D_2$R-mediated G protein-independent beta-arrestin 2 mediated AKT/GSK3 signaling pathway (Espinoza et al., 2015a). Co-immunoprecipitation studies directly demonstrate physical interaction of the two receptors *in vivo* in brain, resulting in altered subcellular localization of TAAR1 and an increase in $D_2$R agonist binding affinity (Liu and Li, 2018; Rutigliano et al., 2018). Also, activation of the TAAR1-$D_2$R heteroreceptor complex in cells negatively modulates beta-arrestin 2 mediated AKT/GSK3β signaling (Harmeier et al., 2015).

An altered presynaptic $D_2$R autoreceptor sensitivity is also found in TAAR1-KO mice. Electrophysiological investigations on brain slices have revealed that the lack of TAAR1 causes an increased firing rate of DA neurons in the VTA and increased $D_2$R agonist potency (Bradaia et al., 2009). Further, voltammetric and microdialysis studies in TAAR1-KO mice, and with use of selective TAAR1

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ligands, have shown that TAAR1 regulates DA release, primarily in the NAC via interaction with presynaptic $D_2$ autoreceptors (Leo et al., 2014). It may involve the existence of TAAR1-$D_2$R autoreceptor complexes in the mesolimbic DA reward neurons. The removal of the TAAR1 protomer may lead to dysfunction of the $D_2$R protomer autoreceptor due to altered allosteric receptor-receptor interactions involving recruitment of other receptor protomers and proteins to the $D_2$ autoreceptor. In view of such events knockout of TAAR1 can give different results from those obtained with TAAR1 antagonists.

Given the evidence that mice overexpressing striatal $D_2$Rs have alterations in glutamatergic transmission in the prefrontal cortex (PFC) (Kellendonk et al., 2006), it is intriguing that TAAR1 can modulate PFC processes as well (Espinoza et al., 2015b). In fact, in the PFC layer V pyramidal neurons where TAAR1 is normally expressed, TAAR1-KO mice demonstrate a functional deficit of N-methyl-D-aspartate (NMDA)-mediated current and an altered subunit composition of NMDA-R. These data indicate that TAAR1 has a major modulatory role for cortical NMDA-R-mediated glutamate transmission attending cognition (Espinoza et al., 2015b).

Finally, several recently identified selective TAAR1 agonists have been shown to effectively counteract hyperactivity induced by dopaminergic psychostimulants or NMDA-R antagonists, and improved performance in schizophrenia-related cognitive tasks in rodents, indicating that TAAR1 agonists may represent a new class of drugs for the treatment of schizophrenia (Revel et al., 2011; 2012; 2013). Additionally, TAAR1 agonists are effective in preventing addictive behaviors in various rodent models. This may involve, at least in part, modulation of presynaptic $D_2$R autoreceptor-mediated control of DA release (Leo et al., 2014; Asif-Malik et al., 2017), and it suggests the potential utility of TAAR1 agonists in addiction (Pei et al., 2016). TAAR1 agonists likely also act at postjunctional receptor complexes in the NAC and reduce the rewarding actions of substances of abuse (Liu and Li, 2018). This action may involve the formation of TAAR1-$D_2$R heteroreceptor complexes in the postjunctional plasma membrane with antagonistic allosteric receptor-receptor interactions (Espinoza et al., 2011; Harmeier et al., 2015) reducing postjunctional $D_2$R protomer signaling in the ventral striato-pallidal GABA anti-reward neurons. TAAR1 agonists acting at these postjunctional TAAR-$D_2$ heteroreceptor complexes may therefore become novel compounds for treatment of substance use disorder.

Instead, if the TAAR1 agonist used preferentially acts at TAAR1-$D_2$ autoreceptor complexes, likely having a different stoichiometry and composition, altered allosteric receptor-receptor interactions can be obtained in the mesolimbic DA neurons. As a result enhanced DA release in the mesolimbic DA reward neurons can develop. This can enhance the development of drug use disorder by impairing $D_2$ protomer autoreceptor function. It is an important area for further research to understand the true mechanisms involved. The complexity is increased through the existence of both pre and postjunctional TAAR1 homo and heteroreceptor complexes linked to mesolimbic DA neurons.

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This topic relating $D_2R$ with substance abuse is discussed in greater detail below.

**$A_{2A}$-$D_2$ Heteroreceptor Complexes Associated with Substance Use Disorder: Psychostimulant and Antagonist Interplay**

Substance Use Disorder (drug addiction) is defined as a chronic relapsing brain disorder, characterized by compulsive drug seeking and use, despite destructive consequences. Substances of abuse induce a wide range of behavioral effects including 'pleasure' and euphoria which are related to increased DA neurotransmission in the brain reward system, including ventral striatum (i.e., NAC) and PFC. Among DAR, $D_2R$ have been the most widely studied target for treatment of drug addiction. The $D_2R$ is recognized as a hub receptor that forms heteromers with multiple receptors (e.g., the adenosine $A_{2A}R$) (Fuxe et al., 2008; Borroto-Escuela et al., 2016b). The antagonistic $A_{2A}R$-$D_2R$ interaction in this heteroreceptor complex appears to be a major mechanism for the ability of $A_{2A}R$ to counteract the inhibitory role of $D_2R$ on neuronal excitability and neurotransmitter release, especially in NAC gamma-aminobutyric acid (GABA) and enkephalin expressing neurons mediating anti-reward. These are modulated by antagonistic $A_{2A}R$-$D_2R$ interactions taking place in heteroreceptor complexes located especially at the dendritic level (Fuxe et al., 2005). These actions within the $A_{2A}R$-$D_2R$ heterocomplexes were demonstrated using biochemical binding techniques (Pintsuk et al., 2016) and *in situ* proximity ligation assay (Trifilieff et al., 2011; Borroto-Escuela et al., 2013) as well as *in vivo* studies (Filip et al., 2012).

The antagonistic $A_{2A}R$-$D_2R$ interactions are also evident in cocaine reward and reinforcement as well as drug-seeking behavior. Thus, the $A_{2A}R$ agonist CGS 21680 [2-p-(2-carboxyethyl)phenethylamino-5′-N-ethylcarboxamidoadenosine] decreases, while the $A_{2A}R$ antagonists KW 6002 [(E)-1,3-diethyl-8-(3,4-dimethoxyphenylethyl)-7-methyl-3,7-dihydro-1H-purine-2,6-dione] (acting mainly postsynaptically), and SCH 58261 [5-Amino-7-(β-phenylethyl)-2-(8-furyl)pyrazolo(4,3-e)-1,2,4-triazolo(1,5-c)pyrimidine] (mixed pre- and post- synaptic receptor antagonist) do not alter cocaine self-administration in rats (Wydra et al., 2015a). There are indications that they exert their behavioral actions mainly through targeting the $A_{2A}R$ protomer of the $A_{2A}R$-$D_2R$ heterocomplexes of the ventral striato-pallidal GABA pathway mainly originating in the NAC. Thus, observations exist that disruption of these heteroreceptor complexes by $A_{2A}R$ transmembrane 5 peptide administration leads to enhancement of cocaine self-administration (Borroto-Escuela et al., 2018).

$A_{2A}R$-KO mice display a lower rate of cocaine self-administration with a reduction in the maximal effort to obtain a cocaine infusion (Soria et al., 2006). The mechanism underlying attenuated reward behavior of $A_{2A}R$-KO mice is not clear but can be related to reorganization of the balance between multiple receptor complexes in which brain $A_{2A}R$ are involved. Specifically, $A_{2A}R$ agonists inhibit, while $A_{2A}R$ antagonists potentiate the motor, discriminative, and rewarding effects of psychostimulants (Filip et

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al., 2012). While $A_{2A}R$ neuronal inactivation attenuates acute psychostimulant effects as well as psychostimulant behavioral sensitization, selective inactivation of striatal $A_{2A}R$ enhances a psychostimulant effect while $A_{2A}R$ inactivation in forebrain (including striatal, cortical and hippocampal $A_{2A}R$) attenuates psychostimulant effects (Filip et al., 2012). These findings indicate that striatal and extra-striatal $A_{2A}R$ have opposite modulatory effects on substances of abuse. The explanation may be the existence of facilitatory allosteric $A_{2A}R$-$D_2R$ interactions in the heteroreceptor complexes of the cerebral cortex related to a dominance of $D_2$ beta-arrestin2 signaling (Urs et al., 2016) which is favored by the $A_{2A}$ protomer activation (Borroto-Escuela et al., 2011; Fuxe and Borroto-Escuela, 2016). The findings indicate existence of an $A_{2A}R$-$D_2R$-beta-arrestin2 heterocomplex which is favored by the antagonistic $A_{2A}R$-$D_2R$ interaction (Borroto-Escuela et al., 2011). The $A_{2A}R$ agonist favors an enhanced recruitment of beta-arrestin2 to the $D_2R$ protomer upon $D_2R$ agonist co-treatment. This leads to co-internalization linked to a reduced time onset of AKT phosphorylation associated with a rapid dephosphorylation. In this way beta-arrestin2 resembles G protein receptor signaling by becoming fast and of short duration.

It is of high interest that the $D_2R$ structure uses a deep binding pocket to bind the atypical antipsychotic drug risperidone (Wang et al., 2018). Instead, $D_3$ and $D_4$ receptors bind substituted benzamides with $D_3$ or $D_4$ receptor selectivity in another way via distinctly extended binding sites (Chien et al., 2010; Wang et al., 2017). This opens up new possibilities to develop novel specific $D_2R$ antagonists for treatment of schizophrenia and cocaine use disorder, with expected fewer adverse-effects. It should also be considered that distinct homoreceptor and heteroreceptor complexes can be pathologically altered in the brain circuits involved in schizophrenia and cocaine use disorder versus other circuits (Borroto-Escuela et al., 2017b). Targeting specifically the vulnerable heteroreceptor complexes and restoring their balance in these circuits may also offer novel treatments with fewer adverse effects.

Cocaine self-administration was recently found to selectively increase the antagonistic $A_{2A}R$-$D_2R$ interactions in ventral striatum in [$^3$H]raclopride/quinpirole competition experiments (Pintsuk et al., 2016). An $A_{2A}R$ agonist ex vivo reduced the affinity of the $D_2R$ agonist high affinity site after cocaine self-administration vs the effects in yoked saline rats – effects not observed in dorsal striatum. Further, cocaine self-administration specifically increased the $A_{2A}R$-$D_2R$ and $D_2R$-sigma1R heterocomplexes in the NAC shell (Borroto-Escuela et al., 2017a). Thus, cocaine self-administration appears to reorganize the $A_{2A}R$-$D_2R$ heterocomplexes in this region, involving an increased presence of sigma1R in these complexes. An increased density of such complexes is observed with enhancement of their antagonistic receptor-receptor interactions. These complexes in the NAC shell may therefore be the target of the anti-cocaine actions of $A_{2A}R$ agonists.

The role of tonic activation of $A_{2A}R$ in cocaine behaviors is reported for reinstatement of cocaine-seeking behavior (Wydra et al., 2015b). In this model the $A_{2A}R$ antagonists reinstated cocaine- and cue-

induced seeking, while the $A_{2A}R$ agonist CGS 21680 evoked a dose-dependent decrease of cocaine- and cue- induced seeking behavior in rats. Also, CGS 21680 reduced quinpirole (the $D_2$-likeR agonist)-induced seeking behavior. Similar inhibitory effects on quinpirole-, cocaine-, cue- and $A_{2A}R$ antagonist-induced seeking behavior were observed after treatment with the $D_2$-like receptor antagonist raclopride, indicating that $A_{2A}R$-$D_2R$ heterocomplexes are also involved in cocaine seeking. Observed behavioral effects are in line with findings by other groups (Bachtell and Self, 2009; O'Neill et al., 2012).

Current studies support a role for $A_{2A}R$-$D_2R$ heterocomplexes in the NAC in antagonizing cocaine reward, and reinstatement and $A_{2A}R$ agonism may have therapeutic potential for preventing cue-controlled craving and relapse.

**Summary and Conclusions**

$D_2RSS$ is a phenomenon associated with an array of human clinical and/or psychiatric states, 1) being produced by repeated agonist action at $D_2R$ which 2) promotes multiple 'reorganization' of the receptor - forming heteromers with adenosine, TAAR1, or other receptor types, and which 3) alters effects of $D_2R$ activation on G protein-dependent and -independent events, and 4) causes downstream alteration of receptor transduction/signaling – any of which produces an abnormality of behavior (or thought). $D_2RSS$ has particular relevance to human disorders of thought (e.g., schizophrenia), behaviors (e.g., ADHD), and neurodegenerative disorders (e.g., Parkinson's disease, Huntington's chorea). With insight having been gained on the process of $D_2RSS$, it is now conceivable that $D_2RSS$ may represent a relevant target towards reversing abnormalities of a variety of human neural-associated disorders.

**Authorship Contribution**

The following contributed to the writing of the manuscript: Kostrzewa, Wydra, Filip, Crawford, McDougall, Brown, Borroto-Escuela, Fuxe, and Gainetdinov.

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