DOPAMINERGIC ALTERATIONS IN SCHIZOPHRENIA

EDITED BY: Silvia Gatti McArthur, Pierre Michel Llorca, Carol A. Tamminga, Andrew J. Grottick, Mark David Tricklebank and Jean-Claude Martel

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DOPAMINERGIC ALTERATIONS IN SCHIZOPHRENIA

Topic Editors:
Silvia Gatti McArthur, McArthur and Associates GmbH, Switzerland
Pierre Michel Llorca, Centre Hospitalier Universitaire de Clermont-Ferrand, France
Carol A. Tamminga, University of Texas Southwestern Medical Center, United States
Andrew J. Grottick, Beacon Discovery Inc., United States
Mark David Tricklebank, King's College London, United Kingdom
Jean-Claude Martel, Université du Québec en Abitibi Témiscamingue, Canada

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EDITORIAL
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Editorial: Dopaminergic Alterations in Schizophrenia

Mark D. Tricklebank[1], Carol Tamminga[2], Andrew Grottick[3], Pierre M. Llorca[4], Silvia Gatti McArthur[5] and Jean-Claude Martel[6]*

[1] King's College London, London, United Kingdom, [2] UT South Western Medical Center, Department of Psychiatry, Dallas, TX, United States, [3] Beacon Discovery, San Diego, CA, United States, [4] Department of Psychiatry, CHU, Clermont-Ferrand, France, [5] McArthur and Associates GmbH, Basel, Switzerland, [6] Université du Québec en Abitibi Témiscamingue, Rouyn-Noranda, QC, Canada

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Editorial on the Research Topic

Dopaminergic Alterations in Schizophrenia

In 1952 Deniker and Delay at St. Anne hospital in Paris conducted a small clinical trial with chlorpromazine. This trial confirmed its outstanding value as a tranquiliser for agitated psychotic patients and it opened the door to understanding the biological basis of schizophrenia (Madras, 2013). Within 10 years a number of structurally distinct and efficacious antipsychotics had been discovered. In 1963, Carlsson and Lindqvist demonstrated that chlorpromazine increased the metabolism of dopamine (Carlsson and Lindqvist, 1963). The true breakthrough on the dopamine hypothesis of schizophrenia came when Snyder's lab demonstrated an association between the treatment of psychosis and the pharmacological manipulation of catecholamine receptors (Snyder et al., 1974). More importantly they went on to show that dopamine receptor binding predicts the clinical and pharmacological potencies of different antipsychotics in humans (Creese et al., 1976a,b). Seeman et al. independently demonstrated the clinical potency of these compounds to correlate with their ability to displace [^{3}H]Haloperidol from brain membranes leading to the publication of perhaps the most famous graph in schizophrenia therapeutics (Seeman et al., 1976).

While antipsychotics effectively improve the management of the psychotic symptoms of schizophrenia, cognitive deficits, and negative symptoms have remained major treatment challenges. Side effects of antipsychotics in many individuals are also a source of concern since they could lead to medication interruption (Correll and Kane, 2020). This has forced a re-imagining of the dopamine hypothesis of the disease while looking for new therapies.

In the first chapter of this issue, Conn et al. focus on the role dopamine plays in the cognitive deficits and altered decision making processes in schizophrenia. They also describe the complexities of relevant animal model design. The media attention received by this review may be an indirect indication of the critical relevance of these aspects of the disease for the daily life of patients.

We then come right up to date with a consideration of the utility of patient derived pluripotent stem cells to provide "the disease in a dish" a still, much under-utilized approach (in our view). What is also important in the work of Collo et al. is the systematic attention to the negative symptoms of schizophrenia and the relevant motivational aspects of dopamine neurocircuitries.

This is complemented by the work of Ashton and Jagannath, who review the important role that dopaminergic systems play in circadian rhythm disturbances in schizophrenia, which are considered a pathophysiological hallmark of the disease.

The proposed model of neurocircuit alterations in schizophrenia is centered on hyperactive hippocampal and prefrontal inputs and the role of the ventral tegmental area, as described by the comprehensive work of Sonnenschein et al. that convincingly includes the impact of stressors on these circuits.

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Dominique Massotte, Université de Strasbourg, France

****Correspondence:***
Mark D. Tricklebank
tricklebank.mark@gmail.com

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The work of Vidal and Pacheco address the most recent advancements in our understanding of dysregulated immune system in schizophrenia. This approach will surely receive much greater attention as more neuro-immunological tools become available. The immune response at critical developmental phases has long been recognized as a major risk factor for schizophrenia, and this concept has been also reviewed in two Frontiers Research Topics (Köhler-Forsberg et al.; Sánchez-Ramón et al.). The dopaminergic system is clearly involved with sensitization processes, which fits with a contributory role in mechanisms of neuronal network reshaping.

The main focus of the present issue is on therapeutic approaches. Understanding the multidimensional phenomenon of medication adherence attitudes in schizophrenia is therefore of paramount importance. Current dopaminergic antipsychotics are still the best therapeutic option, but rates of non-adherence, ranging from 1 to 81%, demonstrate the continuing need for improved therapeutics. El Abdellati et al. review how treatment adherence should be better monitored. They also highlight areas for improvement which include by encouraging people with psychosis to stop cannabis use and providing active support to them by family and medical professionals.

An interesting longitudinal imaging study by Andersen et al. suggests that increases in striatal volume in antipsychotic-naïve first episode people with schizophrenia correlates with how well positive symptoms are controlled with amisulpride. This study represents a link to another interesting issue of Frontiers of Psychiatry (Vita et al.) dedicated to trajectories of brain abnormalities in early schizophrenia.

Are there better ways to manipulate the dopaminergic system to improve functional outcomes in schizophrenia? The final three contributions from Kozak et al., Menniti et al., and Martel and Gatti McArthur, all focus on therapeutic approaches: D1 agonists, new monoaminergic ligands, and PDE10 inhibitors which remain among the best strategies for novel therapeutics.

One answer may come from more selective dopaminergic drugs (including trace amines) acting on dopaminergic subsystems as reviewed in Martel and Gatti-McArthur or, as suggested by the interesting work of Kozak et al. from new D1 selective agonists that show promising pro-cognitive potentials.

Perhaps the greatest challenge comes from the antipsychotic potential of phosphodiesterase PDE10 inhibitors. Why do these compounds demonstrate preclinical properties indicative of efficacious antipsychotic drugs, i.e., are functional D2 antagonists but have no affinity for D2 receptors and do not have any demonstrable clinical benefit? No simple conclusion is reached but the contribution of Menniti et al. highlights the importance of maintaining scientific analysis of these interesting new compounds. The work also questions the translational relevance of conditioned avoidance responding, a test in which all antipsychotics including PDE10 inhibitors are active. It is hoped that withdrawal of the pharmaceutical companies responsible for these compounds from psychiatric drug discovery will not impede such important investigations of translational validity.

We are indebted to all contributing authors, that have carefully crafted this update on the dopaminergic hypothesis in schizophrenia. This hypothesis, with all related questions seems still relevant 50 years on. But much still remains to be discovered. An admirable and worthy calling for those wishing to emulate Delay, Deniker, Carlsson, Seeman, and Snyder.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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REVIEW
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The Cross-Talk Between the Dopaminergic and the Immune System Involved in Schizophrenia

Pia M. Vidal[1,2] *and Rodrigo Pacheco*[2,3]*

[1] Department of Basic Science, Biomedical Science Research Lab, Faculty of Medicine, Universidad Católica de la Santísima Concepción, Concepción, Chile, [2] Laboratorio de Neuroinmunología, Fundación Ciencia & Vida, Santiago, Chile, [3] Universidad San Sebastián, Santiago, Chile

Dopamine is one of the neurotransmitters whose transmission is altered in a number of neural pathways in the brain of schizophrenic patients. Current evidence indicates that these alterations involve hyperactive dopaminergic transmission in mesolimbic areas, striatum, and hippocampus, whereas hypoactive dopaminergic transmission has been reported in the prefrontal cortex of schizophrenic patients. Consequently, schizophrenia is associated with several cognitive and behavioral alterations. Of note, the immune system has been found to collaborate with the central nervous system in a number of cognitive and behavioral functions, which are dysregulated in schizophrenia. Moreover, emerging evidence has associated schizophrenia and inflammation. Importantly, different lines of evidence have shown dopamine as a major regulator of inflammation. In this regard, dopamine might exert strong regulation in the activity, migration, differentiation, and proliferation of immune cells that have been shown to contribute to cognitive functions, including T-cells, microglial cells, and peripheral monocytes. Thereby, alterations in dopamine levels associated to schizophrenia might affect inflammatory response of immune cells and consequently some behavioral functions, including reference memory, learning, social behavior, and stress resilience. Altogether these findings support the involvement of an active cross-talk between the dopaminergic and immune systems in the physiopathology of schizophrenia. In this review we summarize, integrate, and discuss the current evidence indicating the involvement of an altered dopaminergic regulation of immunity in schizophrenia.

Keywords: schizophrenia, dopamine receptors, T cells, microglia, peripheral monocytes, neuroimmunology, behavior

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Dietmar Fuchs, Innsbruck Medical University, Austria
Belinda Lennox, University of Oxford, United Kingdom
Marie-Eve Tremblay, Laval University, Canada

***Correspondence:**
Rodrigo Pacheco
rpacheco@cienciavida.org

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DYSREGULATION OF THE DOPAMINERGIC NEURAL PATHWAYS IN THE SCHIZOPHRENIA

Schizophrenia is a mental illness that often appears during late adolescence or early adulthood. It is characterized by thought disorders, perception, cognition and volition. The prevalence of this disorder reaches almost 1% of the world population, with an annual incidence ranging between 3.89 and 4.03 per 1,000 subjects (Moreno-Kustner et al., 2018). Its etiology is still unclear, and includes genetic and environmental components promoting alterations of dopaminergic signaling. The initial dopamine

hypothesis stated that hyperactive dopaminergic transmission leads to development of schizophrenia symptoms (hallucinations, delusions, thought disorder, among others). However, several lines of evidence have shown that hypoactivity of frontal dopaminergic neurons in rodents (Pycock et al., 1980), non-human primates (Roberts et al., 1994), and humans (Ragland et al., 2007; Simpson et al., 2010) are also associated with schizophrenia. For instance, a pharmacological lesion of subcortical dopaminergic pathways in rats suggested a correlation between hyperactivation of subcortical dopaminergic neurons with hypoactivity of frontal dopaminergic neurons (Pycock et al., 1980). In addition, evidence obtained from humans has suggested that the polymorphism in the gene encoding catechol-O-methyltransferase, an enzyme involved in the degradation of dopamine, is associated with hypoactivity of prefrontal dopaminergic neurons in schizophrenia (Slifstein et al., 2008). Moreover, patients with frontal lobe damage as well as schizophrenia patients display similar alterations in the executive function (Ragland et al., 2007). Therefore, the current dopaminergic hypothesis involves hyperactive dopaminergic transmission in mesolimbic areas, striatum and hippocampus (Lodge and Grace, 2007; Patel et al., 2010; Weinstein et al., 2017), as well as hypoactive dopaminergic transmission in the prefrontal cortex of schizophrenic patients (Da Silva Alves et al., 2008). In addition, glutamatergic hypofunction has been suggested as one of the mechanisms involved in this dopaminergic dysfunction in schizophrenia (Swerdlow et al., 2009). In this regard, it has been hypothesized that DRD2-antagonism might prevent DRD1-mediated potentiation of N-Methyl-d-aspartate (NMDA) responses in the prefrontal cortex (Paz et al., 2008). Another line of evidence points to the changes in subcortical dopaminergic activity as one of the responsible circuits promoting alterations in glutamatergic neurotransmission in the substantia nigra (Mueller et al., 2004).

This, imbalance in the dopaminergic signaling has been differentially associated with the development of positive (presence of undesired cognitive/emotional functions, such as hallucinations, delusions, thought disorders, trouble concentrating, movement disorders) and negative (deficiency of desired cognitive/emotional effects, such as flattened affect, lack of pleasure, trouble with speech, apathy, concentration problems, and lack of motivation) symptoms. Positive symptoms have been related with stimulation of D2-like receptors, including DRD2, DRD3, and DRD4) (Li et al., 2016). Both primate and rodent brains express a higher density of D1-like (including DRD1 and DRD5) than D2-like receptors in healthy conditions (Weinstein et al., 2017). Meta-analysis of studies using positron emission tomography (PET) and single photon emission computed tomography (SPECT) have shown that presynaptic dopamine release is decreased in most brain regions of schizophrenic patients (Slifstein et al., 2015), except in the striatum, where the synthesis and the levels of dopamine released are increased (Mccutcheon et al., 2018; Avram et al., 2019). Furthermore, PET studies have demonstrated that prefrontal DRD1 expression is decreased in patients with schizophrenia (Kosaka et al., 2010), which has been associated with working memory deficits in the prefrontal cortex (Takahashi et al., 2008). In contrast, DRD1 expression is increased in the temporal and parietal cortex of schizophrenic patients, which might be associated with auditory hallucinations (Domyo et al., 2001). It has been described a moderate increase (10–20%) in the expression of DRD2 and DRD3 in the striatum of a subgroup of schizophrenic patients (Kestler et al., 2001). Moreover, DRD3 expression has been found to be enhanced in the basal ganglia, ventral forebrain (Gurevich et al., 1997), and blood lymphocytes of schizophrenic patients (Ilani et al., 2001). On the other hand, it has been shown that in comparison to healthy subjects, dopamine occupies a higher proportion of striatal D2-like receptors (Kegeles et al., 2010), and a bigger fraction of the dopamine transporters (DAT) in sensorimotor striatum (Weinstein et al., 2017) in schizophrenia.

Interestingly, it has been described a sub-regional heterogeneity in the dopaminergic dysregulation within the striatum. The greatest alterations in dopaminergic transmission have been observed in the associative striatum region. These alterations have been negatively correlated with verbal fluency performance in schizophrenic patients (Howes et al., 2009b). Since this brain region regulates information flow to and from the prefrontal cortex, the authors have suggested a potential link between striatal dopaminergic dysfunction and prefrontal alterations in schizophrenic patients (Howes et al., 2009b). A recent study showed that impaired connectivity between the cortico-striato-thalamo-cortical circuits is associated with cognitive difficulties in schizophrenic patients, including deficits in attention, memory, and executive function (Avram et al., 2018). Moreover, reduced striatal dopamine synthesis correlates with cognitive difficulties in patients during remission of positive symptoms, without an association with negative symptoms (Avram et al., 2019). Of note, the cohort of patients was taking antipsychotic drugs that did not seem to have a short-term clear effect on the results of the study (Avram et al., 2019). In addition, studies performed in schizophrenic patients have analyzed the expression levels of tyrosine hydroxylase (TH), the enzyme that catalyzes the first (and limiting) step in the biosynthesis of dopamine, and have found heterogeneous results, supporting either dopaminergic hyperactivity or hypoactivity (Akil et al., 2000; Mueller et al., 2004). One study has reported regional and laminar specific decrease of TH-immunoreactive axons in the entorhinal cortex of schizophrenic patients (Akil et al., 2000), whereas another study has shown increased TH mRNA levels in the dopaminergic neurons of the substantia nigra *pars compacta* of schizophrenic patients (Mueller et al., 2004).

Thus, current evidence indicates the involvement of complex alterations in the activity of neural dopaminergic pathways in the brain of schizophrenic patients, which are not completely consolidated. Therefore, further research is still needed to better understand the alterations of dopaminergic circuitry associated to the pathophysiological scenario of schizophrenia.

TARGETING THE DOPAMINERGIC SYSTEM IN SCHIZOPHRENIA

The World Health Organization estimates that costs of schizophrenia in Western countries represent 1.6–2.6% of total health care budget, whereas in the US more than $60 billion USD per year are spent in this disorder (Howes et al., 2009a; Chong et al.,

2016). The primary targets of many antipsychotic drugs for schizophrenia are striatal DRD2 and DRD3 (Howes et al., 2009a). However, the antagonism of these receptors is not always specific, and current drugs also act over other neurotransmitter receptors in the brain, including receptors for serotonin, histamine, norepinephrine, gamma-aminobutyric acid (GABA), and acetylcholine (Li et al., 2016). A DRD2 occupancy between 50% to 65%, is required in order to achieve clinical response to antipsychotic drugs and to minimize development of side-effects (e.g. extrapyramidal motor side effects) (Kapur et al., 2000). Targeting DRD2 using the antagonists chlorpromazine and haloperidol has been shown to effectively reduce positive symptoms, but ineffective at attenuating negative symptoms, cognitive deficits, and development of extrapyramidal motor side effects (Li et al., 2016). Of note, antipsychotic drugs might also increase the density of D2-like receptors in the striatum (Simpson et al., 2010). Antagonism of serotonin receptor 5-HT_{2A} in combination with DRD2-antagonism (e.g. clozapine and risperidone) have been shown to be more effective attenuating positive and negative symptoms, nevertheless, promoting the development of extrapyramidal motor side effects (Kinon and Lieberman, 1996) and others, such as gain of body weight, increase incidence of diabetes, loss of bladder control, and blurred vision (Snyder et al., 2015).

Moreover, patients who do not respond well to antipsychotic treatment have relatively normal levels of striatal dopamine compared with patients whose symptoms respond to antipsychotics (Demjaha et al., 2012). Treatment with the dopaminergic agonist apomorphine, ameliorates cognitive deficits and improves dopaminergic neurotransmission, which has been associated to the enhancement of prefrontal activity (Dolan et al., 1995), exaggerated stimulation of dopaminergic release, and potentially promoting more occupancy of D2-like receptors by dopamine in schizophrenic patients (Laruelle et al., 1996). On the other hand, the treatment with dopamine receptor blockers is more effective at ameliorating symptoms such as hallucinations or delusions. New antipsychotic drugs antagonizing preferentially DRD3 over DRD2 have shown cognitive performance improvement (Nakajima et al., 2013; Wang et al., 2017). However, further research is needed in this regard to fully understand how dopaminergic transmission, triggered through the stimulation of every single dopamine receptor subtypes, regulates the spectrum of positive, negative, and cognitive symptoms involved in schizophrenia. Of note, schizophrenia is a heterogeneous disorder and no single brain region or neurotransmitter is likely to explain all symptoms observed in all schizophrenic patients (Mccutcheon et al., 2019). Therefore, new drugs aiming to target beyond the dopaminergic system or involving modulation of multiple targets are more likely to effectively tackle positive and negative symptoms of schizophrenia. An example is the newer antipsychotic drug ITI-007, which is able to interact with the serotoninergic, dopaminergic, and glutamatergic pathways (Snyder et al., 2015). This drug has shown promising results either in safety and improving negative symptoms in a phase II randomized double-blind multicenter clinical trial (Lieberman et al., 2016).

The interaction between the dopaminergic and the immune system should also be considered for the development of new therapeutic targets in schizophrenia. In this regard, it is important to consider the role of tetrahydrobiopterin (BH4), which is an essential enzyme cofactor required for the production of tyrosine and dopamine (Felger et al., 2012). Some cytokines involved in inflammation might regulate the expression of GTP-cyclohydrolase I (GCH-1), the enzyme necessary for BH4 synthesis, thus increasing or decreasing dopamine biosynthesis rate. Nevertheless, inflammation may also increase reactive oxygen species and inducible nitric oxide synthase (NOS) activity, which lead to decreased BH4 availability and thereby reducing dopamine synthesis. For instance, the administration of Interferon alpha (IFN-α) in rats has been shown to promote a significant decrease in the levels of dopamine and BH4 in the amygdala and raphe area, an effect that was abolished upon administration of a NOS inhibitor (Kitagami et al., 2003). Similarly, IFN-α, Interleukin-6 (IL-6), and cardiotrophin-1, have also been shown to reduce the levels of BH4 in sympathetic neurons (Li et al., 2003). Conversely, IL-1β, IFN-γ, and TNF-α have been shown to increase BH4 synthesis, by inducing the expression and activity of GCH-1 in endothelial cells (Shi et al., 2004).

In addition to the effect in the biosynthesis of dopamine, some cytokines have shown to regulate dopamine storage in dopaminergic cells. In this regard, the pro-inflammatory cytokines IL-1β and TNF-α have been shown to decrease the expression of the vesicular monoamine transporter 2 (VMAT2), which is responsible for transporting cytosolic dopamine into secretory vesicles, and thereby limiting the availability of presynaptic dopamine. Conversely, TGF-α increases VMAT2 expression, favoring the storage of presynaptic dopamine (Kazumori et al., 2004). Taken together these results indicate that inflammatory cytokines exert a complex regulation in the levels of dopamine available in dopaminergic cells by modifying the biosynthesis rate and the storage of this neurotransmitter.

Adding another level of complexity in the interaction between the dopaminergic and immune system, some studies have shown that some drugs targeting dopaminergic system might regulate inflammation. According to the critical role of dopamine in the regulation of sepsis (Torres-Rosas et al., 2014), it has recently been shown that the antipsychotic drug trifluoperazine (TFP), which suppress dopamine secretion, exerted a strong regulation of pro-inflammatory cytokines and increased the survival rate in animal models of sepsis (Park et al., 2019). Another example illustrating the role of antipsychotic drugs is the study of the effect of paliperidone in neuroinflammation. The authors show that the pre-treatment of rats with paliperidone inhibited the stimulation of toll-like receptor 4 (TLR4) in a model of neuroinflammation induced by stress (Macdowell et al., 2014). In the same direction, another study has shown that haloperidol attenuates the activation of NF-κB, and consequently abrogated the production of pro-inflammatory cytokines in macrophages in response to lipopolysaccharide (LPS) (Yamamoto et al., 2016). Thus, these findings together illustrate how some antipsychotic drugs used for the treatment of schizophrenia might also induce anti-inflammatory effects by targeting the dopaminergic system in immune cells.

INVOLVEMENT OF THE IMMUNE SYSTEM IN COGNITIVE FUNCTIONS

Cognitive deficits in schizophrenia affect language, working and episodic memory, processing speed, stress resilience, social behavior, attention inhibition, and sensory processing (Kennedy and Adolphs, 2012; Brisch et al., 2014). Proper function of our cognitive and social abilities has partially been associated with the interaction between the central nervous system (CNS) and the immune system. The crosstalk between CNS and the peripheral cells is mediated by the glymphatic and meningeal lymphatic systems (Pape et al., 2019). In addition, the communication through the blood-brain barrier (BBB) and the endocrine system might also contribute to immunological alterations, affecting cognitive function. Here we analyze how some of these cognitive functions need the participation of the immune system.

Memory and Learning

Immune cell infiltration has been considered a pathological hallmark of many CNS conditions. However, cells from either the innate and adaptive immune systems might exert beneficial effects in the CNS, as long as their recruitment and activation are well controlled. Cells from the immune system have been shown to play a role in spatial memory, learning, and neurogenesis (Kipnis et al., 2004b; Ziv et al., 2006).

The hippocampus is partially responsible for spatial learning/memory. Neurogenesis occurring in the hippocampal dentate gyrus has been shown to be dependent on the infiltration of mature $CD4^+$ T-cells in the meninges (Ziv et al., 2006; Wolf et al., 2009), on the activation, phagocytic activity, and recruitment of microglia (Ziv et al., 2006), and on the increased production of brain-derived neurotrophic factor (BDNF) by glial cells (Wolf et al., 2009). Accordingly, the administration of minocycline, a non-specific anti-inflammatory drug, led to decreased neurogenesis in the dentate gyrus and abrogated the activation of meningeal of T-cells (Ziv et al., 2006; Derecki et al., 2010). Interestingly, a later study demonstrated that meningeal $CD4^+$ T-cells participating in the acquisition of spatial memory and neurogenesis in the dentate gyrus are memory T-cells with specificity for self-antigens derived from the CNS (Baruch et al., 2013). Thus, these studies provide evidence that CNS-specific T-cells are required for neurogenesis, and their activation is dependent on microglial cells (Ziv et al., 2006; Derecki et al., 2010; Baruch et al., 2013).

Surgical removal of the deep cervical lymph nodes can result in dysregulated T-cell immunity that correlates with cognitive impairment (Radjavi et al., 2014b). Moreover, a decreased relative number of circulating dendritic cells, $HLA\text{-}DR^+$ regulatory T-cells (Tregs), and $CD4^+$ memory T-cells has been associated with more severe negative and cognitive symptoms in schizophrenic patients (Fernandez-Egea et al., 2016). According to the key role described for CNS-specific $CD4^+$ T-cells in spatial memory, mice lacking peripheral mature T-cells manifest impaired spatial learning, memory capabilities (Kipnis et al., 2004b), and neurogenesis (Ziv et al., 2006) compared with the wild-type control group. This cognitive decline is reversed by transfer of T-cells (Kipnis et al., 2004b), but not when other immune cells (i.e. bone marrow-derived immune cells from T-cell depleted donors) are injected (Brynskikh et al., 2008). Similarly, it has been shown that the cognitive impairment developed by the deficiency of adaptive immune system is reversed just by the transfer of $CD4^+$ T-cells, even in the absence of B-lymphocytes and $CD8^+$ T-cells (Wolf et al., 2009; Radjavi et al., 2014b). Accordingly, a particular $CD4^+$ T-lymphocyte subset, which originates from deep cervical lymph nodes (Radjavi et al., 2014a) and resides in the choroid plexus, ventricular margins, and subarachnoid spaces (Derecki et al., 2010; Baruch et al., 2013; Radjavi et al., 2014b) has been implicated in cognitive functions (Wolf et al., 2009; Radjavi et al., 2014b). These cells have been shown to decrease drug-induced psychosis and reduce cognitive impairment (Kipnis et al., 2004b) in mice. The training in spatial memory led to the accumulation of IL-4 producing T-cells in the meninges of experimental mice. Moreover, experiments in which the entrance of T-cells into the meningeal space was attenuated by using FTY720 or an anti-VLA4 antibody showed that reduced recruitment of a subset of memory $CD4^+$ T-cells into the meninges resulted in impaired spatial memory (Derecki et al., 2010). However, these results do not rule out the indirect interaction of $CD4^+$ T-cells with local or systemic antigen presenting cells (i.e. microglia, myeloid cells) and their secreted cytokines. For example, myeloid cells acquire an inflammatory phenotype in response to cognitive tasks in absence of meningeal T-cells. This phenotype can be reversed after injection of T-cells-expressing IL-4, which act on myeloid cells, favoring the acquisition of an anti-inflammatory phenotype (Derecki et al., 2010). These results suggest that T-cell derived IL-4 is the main cytokine regulating the phenotype of myeloid cells. Furthermore, peripheral macrophages alternatively activated *in vitro* in the presence of IL-4 acquire an anti-inflammatory phenotype that might improve learning and memory in the absence of $CD4^+$ T-cells (Derecki et al., 2011).

Thus, these findings suggest that the key role of meningeal $CD4^+$ T-cells in learning and memory is associated to their participation as a source of IL-4 in the brain. Interestingly, age-related cognitive impairment has been related with a shift on the regulatory cytokines produced in the choroid plexus, which constitute the entrance gate for $CD4^+$ T-cells into the meninges. In this regard, high IL-4-to-IFN-γ ratio promotes CCL11 production, whereas low IL-4-to-IFN-γ ratio favors production of BDNF (Baruch et al., 2013). Remarkably, BDNF has been involved in neurogenesis and promoting learning and spatial memory (Derecki et al., 2010). Conversely, CCL11 is a chemokine associated with age-related cognitive impairments, whose high plasma levels correlate with reduced neurogenesis in mice and aging in humans (Villeda et al., 2011). These results highlight the importance of a cross-talk between meningeal lymphoid and myeloid cells for cognitive function. To add another piece to the puzzle, a recent study has raised new questions regarding the potential role of $CD8^+$ T-cells derived IFN-γ from neurogenic brain niches in the generation of

neurogenesis and cognition (Dulken et al., 2019). It has been shown in both rodents and humans that aging involves an increased CNS-infiltration of T-cells expressing IFN-γ, which decreases proliferation of neural stem cells (Dulken et al., 2019).

Importantly, another group of studies has provided evidence of a fundamental role of microglia in shaping neuronal circuitry by four different ways. First, by engulfing presynaptic termini in the healthy brain (Schafer et al., 2012; Meyer, 2013). Secondly, by limiting neurogenesis through the release of soluble factors, such as secretome after phagocytosis (Diaz-Aparicio et al., 2020), BDNF, insulin growth factor-1, TNF-α, pre-micro RNAs, among others (Rodriguez-Iglesias et al., 2019). Third, by inhibition of Sirt1/p65 signaling pathway in the dentate gyrus (Sellner et al., 2016). Fourth, by phagocytosis of apoptotic newborn cells in the dentate gyrus (Sierra et al., 2010). In this regard, the stimulation of the fractalkine receptor (CX3CR1) and the complement receptor 3 (CR3) signaling pathways have been shown to participate in these processes through the pruning of synaptic spines, engulfment of neurons during periods of active synaptic pruning (Schafer et al., 2012; Meyer, 2013). Indeed, the defective interaction between neurons and microglia given in *cx3cr1*-deficient animals leads to impaired synaptic maturation and reduced efficiency of synaptic transmission (Reshef et al., 2014; Basilico et al., 2019). Consequently, the deficiency in these signaling pathways results in neurological impairment. For instance, *cx3cr1*-deficient mice display impaired associative and spatial memory (Rogers et al., 2011), reduced neurogenesis in the dentate gyrus (Meyer, 2013), as well as increased levels of IL-1β in the hippocampus (Rogers et al., 2011). *Cx3cr1*-deficiency also leads to higher spine density, enhanced number of excitatory synapses (Meyer, 2013), and altered microglial morphology (Corona et al., 2010; Basilico et al., 2019) in comparison with wild-type controls. It has been suggested that one of the mechanisms involved in the learning deficits observed in *cx3cr1*-deficient mice is the inability to achieve long-term potentiation (LTP) in the hippocampus of these animals, which seems to be a consequence of the increased levels of IL-1β (Rogers et al., 2011; Liu et al., 2019). In addition, other studies have shown that CX3CL1 transiently potentiates NMDA-function, but inhibits hippocampal LTP, a process regulated through the stimulation of adenosine receptors (Maggi et al., 2009; Scianni et al., 2013). In the same direction, the pharmacological depletion of microglial cells mediated by the bilateral injection of clodronate into the dorsal hippocampus or by oral administration of PLX3397 showed alterations in spatial learning (Torres et al., 2016). Moreover, depletion of BDNF from microglial cells has been shown to reduce motor learning, recapitulating some of the behavioral alterations reported in microglia-depleted mice (Parkhurst et al., 2013). Regarding CR3, it has been shown that C3 deficiency leads to an enhanced learning and memory in mice when compared with their wild-type littermates (Shi et al., 2015; Shi et al., 2017). However, little is known regarding C3R deficiency in microglial cells. These findings together illustrate how the innate and adaptive immune response in the CNS play a relevant role in the proper development of neuronal circuitry and in cognitive tasks in healthy physiological conditions.

Finally, some studies have shown that BBB might play a relevant role modulating the immune response, thus affecting cognitive function in schizophrenia. For instance, increased expression of genes involved in immune function and inflammation have been detected in the choroid plexus of schizophrenic patients, which correlates with BBB permeability (Kim et al., 2016). On the other hand, decreased expression of the tight-junction protein claudin-5 at the BBB, correlates with impaired learning and memory, depression, anxiety, impaired social behavior, and altered locomotor activity in mice (Greene et al., 2018). Therefore, the BBB should also be considered as an important actor involved in the regulation of the cross-talk between immune system and the cognitive and behavioral impairment associated to schizophrenia.

Stress Resilience

Another behavioral response that might be significantly regulated by immune cells, including T-cells, microglia, and peripheral monocytes, is the adaptation to psychological stress. In this regard, it has been shown that T-cell deficient mice, including severe combined immunodeficient (SCID) and nude mice, develop a worst adaptation than their immunocompetent counterpart in models of post-traumatic stress disorder (Cohen et al., 2006; Scheinert et al., 2016). Adoptive transfer of T-cells into either T-cell deficient (Cohen et al., 2006) or immunocompetent mice (Lewitus et al., 2008) improves the adaptation to psychological stress (Scheinert et al., 2016). Moreover, when immunodeficient mice where reconstituted with T-cells devoid of Tregs, psychological adaptation to stress was better than in mice replenished with the total T-cell compartment, containing both Tregs and effector T-cells (Teff) (Cohen et al., 2006). Further analysis of post-traumatic stress showed an association between lymphocyte recruitment into the choroid plexus and stress resilience, as well as increased hippocampal BDNF levels (Lewitus et al., 2008). Single-cell RNAseq analysis of T-cells recruited into the CNS upon psychological stress suggests a non-encephalitogenic origin, expressing *Foxp3*, *Gata3*, and Th2 genes (Kertser et al., 2019). It has been hypothesized that increased lymphocyte recruitment into the CNS might lead to the development of memory T-cells, that are necessary in order to promote homeostasis and enhance resilience to subsequent psychological stressful experiences (Lewitus and Schwartz, 2009). Accordingly, lymphocytes from chronically stressed mice adoptively transferred into immunodeficient mice ($Rag2^{-/-}$) are able to confer anti-depressant like behavioral effects compared to the adoptive transfer of lymphocytes isolated from unstressed mice (Brachman et al., 2015; Scheinert et al., 2016). Thus, these findings support the hypothesis that psychological stress triggers the generation of memory T-cells, probably with specificity to CNS-derived self-antigens, which are recruited to the choroid plexus and confer resilience to future adverse psychological events.

Recent studies have shown that monocytes might also be involved in stress resilience. In a mouse model of severe psychological stress, leukocyte trafficking through choroid plexus was suppressed. The inhibition of glucocorticoid receptor signaling restored leukocyte trafficking through

choroid plexus, which was associated to the recruitment of Th2 and Tregs cells into the CNS, leading to attenuation of post-traumatic behavioral deficit (Kertser et al., 2019). Accordingly, corticosterone mediated an increase of inflammatory circulating monocytes in mice behaviorally susceptible to stress (Niraula et al., 2018; Gururajan et al., 2019). It has been shown that peripheral monocytes might be recruited into the brain by microglial cells and their chemokines secreted (Weber et al., 2019), where they can exacerbate neuroinflammation (Niraula et al., 2018) and anxiety-like behavior (Wohleb et al., 2014). In addition, *cx3cr1*-deficiency in mice confers resilience to chronic unpredictable stress stimuli, thus suggesting a detrimental role of microglial cells in response to psychological stress (Hellwig et al., 2016; Rimmerman et al., 2017). In this regard, it has been shown that CX3CR1-signaling induces hyper-ramification of microglial cells in response to chronic stress, which was associated with the development of depressive-like behavior (Hellwig et al., 2016). Thus, the emerging evidence indicates that the innate and adaptive immune system play a fundamental role in the behavioral response to psychological stress.

Social Behavior

Social behavior constitutes another response regulated by the immune system. In this regard, mice deficient in adaptive immunity display social deficits, as evidenced by anti-social behavior in validated behavioral tests of preference between another mouse or an inert object. This behavioral impairment has been attributed to the lack of IFN-γ production by meningeal T-cells. Accordingly, this deficit in social behavior might be reversed by administration of IFN-γ in the cerebrospinal fluid (CSF) or by the adoptive transfer of T-lymphocytes isolated from wild-type mice. The authors showed evidence indicating that IFN-γ stimulates GABAergic inhibitory neurons, triggering inhibitory neural circuits and thereby preventing hyper-excitability in the prefrontal cortex (Filiano et al., 2016). On the other hand, microglial cells have been reported to be involved in social behavior (Torres et al., 2016; Kopec et al., 2018), and to drive changes in affective behavior under exposure to psychosocial stress (Lehmann et al., 2019). In this regard, microglia abrogated the development of chronic social defeat-induced anxiety-like and antisocial behavior. Accordingly, microglia replenishment in the brain, just after psychosocial stress, leads to anxiety-like and antisocial behavior. A potential mechanism to explain the role of microglia in social behavior suggested by the authors involves the elevated reactive oxygen species produced by microglial cells during and after stress exposure (Lehmann et al., 2019). Another plausible explanation comes from a recent study involving the CSF-1/CSF-1R axis, which is required for development of most tissue macrophages including osteoclasts, brain microglial cells, and others. In this regard, it has been shown that the interference of the CSF-1/CSF-1R signaling pathway in cerebellar microglia leads to defective motor learning and impaired social interactions (Kana et al., 2019). This latter study is supported by another work that indicates a relevant role of the cerebellum as a regulator of major cognitive functions, such as expectations and reward (Wagner et al., 2017). Furthermore, it has recently been shown that the disruption of connectivity between the cerebellum and the right dorsolateral prefrontal cortex is associated with the severity of negative symptoms in schizophrenia (Brady et al., 2019). Another line of evidence shows microglial cells as key components organizing neuronal circuits involved in sex-associated social behavior during adolescence. In this regard, microglia and their complement-dependent phagocytosis promoted the elimination of D1-like receptors in the nucleus accumbens of male rats, a process that was required for natural developmental changes in male social play behavior (Kopec et al., 2018). Interestingly, a recent study provided evidence that this organized phagocytic process mediated by microglia and complement in the development of sex-associated social behavior during adolescence is promoted by the action of testosterone-induced endocannabinoids (Vanryzin et al., 2019).

Interestingly, maternal immune inflammation has also been linked to an increasing risk of developing schizophrenia in the progeny, where hippocampal microglial cells display an altered gene expression profile, including upregulation of genes involved in embryonic development, long-term neuronal plasticity, angiogenesis, and extracellular matrix organization, whereas genes involved in phagocytosis, cell migration, and inflammatory response were downregulated (Mattei et al., 2017). Thus, emerging evidence indicates that meningeal T-cells and microglial cells play relevant roles regulating social behavior.

DOPAMINERGIC REGULATION OF THE IMMUNE SYSTEM

A number of catecholamine family members, including dopamine, have been extensively involved in the regulation of the immune response (Tracey, 2009), affecting both the innate and adaptive immune system (Pacheco et al., 2014; Vidal and Pacheco, 2019). In this regard, dopamine receptors are expressed on T and B lymphocytes, dendritic and NK cells, macrophages, microglia, intermediate monocytes, neutrophils, and eosinophils (Levite, 2016; Arce-Sillas et al., 2019). In this section we focused in the analysis of the dopaminergic regulation of T cells, microglia, and monocytes, since these immune cells have been implicated in cognitive functions.

Dopaminergic Regulation of T Cells

The final outcome of dopamine effects on T-lymphocytes depends on dopamine concentrations, type of T-cells and activation status, as well as the dopamine receptors being expressed (Levite, 2016). In addition to the expression of dopamine receptors, the dopaminergic system in T-cells also involves some subsets of T-cells as sources of dopamine. For instance, human Tregs might synthesize and release dopamine, which exerts a negative feedback on their suppressive activity (Cosentino et al., 2007). More recently, another study revealed that follicular helper T (T_{FH}) cells synthesize and store dopamine, which is released upon antigen-recognition to stimulate DRD1-signaling on B-cells as a costimulatory signal to induce antibody production (Papa et al., 2017).

A group of studies has addressed the effect of dopamine in Teffs and Tregs using pharmacologic approaches in experiments *in vitro*. These studies have shown that the stimulation of D1-like dopamine receptors favors the differentiation of naive $CD4^+$ T-cells toward a Th2 phenotype (Nakano et al., 2009), and attenuates the regulatory activity of Tregs (Kipnis et al., 2004a; Cosentino et al., 2007). Pharmacologic evidence has also shown that DRD4-signaling induces T-cell quiescence (Sarkar et al., 2006). A recent study using genetic approaches revealed that DRD4-signaling in T-cells favors Th2 differentiation, promoting allergic asthma in newborn lungs (Wang et al., 2019). Addressing the role of DRD3-signaling in T-cells, pharmacologic and genetic evidence has recently shown that stimulation of this receptor potentiates T-cell activation, favoring Th1 differentiation and reciprocally dampening the acquisition of the Th2 phenotype, both *in vitro* and *in vivo* (Gonzalez et al., 2013; Franz et al., 2015; Contreras et al., 2016; Elgueta et al., 2019). In the same direction, pharmacologic evidence obtained with human T-cells has shown that DRD3-stimulation increases IFN-γ production and concomitantly decreases IL-4 and IL-10 release, along with and exacerbated expression of the activation marker CD25 (Ilani et al., 2004).

Mechanistic analyses carried out through pharmacologic and genetic approaches have shown that this DRD3-mediated potentiation of Th1-differentiation and concomitant repression of Th2-differentiation involves the upregulation of the regulatory protein SOCS5 (Contreras et al., 2016), as well as reduction of intracellular cyclic adenosine monophosphate (cAMP) levels and ERK phosphorylation (Franz et al., 2015). It is important to consider that, under chronic inflammatory conditions, DRD3-signaling in $CD4^+$ T-cells also favors the expansion of the Th17-lienage (Contreras et al., 2016). Regarding DRD5-signaling in T-cells, pharmacologic and genetic evidence has shown DRD5-stimulation on $CD4^+$ T-cells potentiates TCR-signaling (Franz et al., 2015) favoring a stronger T-cell activation *in vitro* and *in vivo* (Osorio-Barrios et al., 2018). Further analyses have shown that DRD5-signaling in Teff favors the acquisition of the Th17-lineage, whereas in Tregs increase the potency of their suppressive activity (Osorio-Barrios et al., 2018).

Despite that most studies addressing the dopaminergic regulation of T-cells have been focused in $CD4^+$ T-cells, a few studies have also analyzed $CD8^+$ T-cells. In this regard, a couple of studies using pharmacological approaches have shown that DRD3-stimulation on $CD8^+$ T-cells potentiates IFN-γ transcription (Ilani et al., 2004), increases their integrin-mediated adhesion to fibronectin and intercellular adhesion molecule 1 (ICAM-1), and synergizes lymphocytes migration toward inflammatory chemokines (Watanabe et al., 2006). In addition, a recent study has shown that pharmacologic stimulation of D1-like dopamine receptors attenuates both the generation and the suppressive activity of regulatory $CD8^+$ T-cells (Nasi et al., 2019). Taken together, these findings indicate that both $CD8^+$ and $CD4^+$ T-cells might undergo a complex dopaminergic regulation, which affects their activation, adhesion, migration, differentiation, and effector or suppressive function. Thus, alterations in physiological dopamine levels or the expression of dopamine receptors in T-lymphocytes, such as the case of schizophrenia, may exert strong changes in the behavior of T-cells.

Dopaminergic Regulation of Microglial Cells

Microglial cells constitute a key cell of the innate immune response in the CNS, which play a central role in neuroinflammation (Gonzalez et al., 2015). Since microglial cells reside in the CNS, they are exposed to dopamine released by dopaminergic neural circuits of the CNS. In this regard, dopaminergic signaling has been reported to modulate different microglial functions, including their inflammatory activity (Yan et al., 2015; Dominguez-Meijide et al., 2017; Elgueta et al., 2017), migration (Farber et al., 2005), and cell adhesion (Fan et al., 2018). Accordingly, microglial cells express both dopamine D1-like and D2-like receptors (Farber et al., 2005; Huck et al., 2015). However, under inflammatory conditions the expression of dopamine receptors might strongly change (Huck et al., 2015). This is the case of the DRD2, whose expression is induced upon inflammatory stimulation of microglial cells following stroke (Huck et al., 2015). High dopamine levels attenuate the inflammatory activation of microglia by reducing the release of nitric oxide (Farber et al., 2005) and decreasing the extent of phagocytosis (Fan et al., 2018). It has been suggested that this process may be mediated by the stimulation of low-affinity dopamine receptors in these cells, including DRD1 and DRD2 (Dominguez-Meijide et al., 2017), leading to a reduction in the phosphorylation of ERK1/2 (Fan et al., 2018), and to the inhibition of the angiotensin type-1/NADPH-oxidase/superoxide axis (Dominguez-Meijide et al., 2017). In the same direction, it has been described that DRD1-signaling in microglial cells induces the cAMP-mediated degradation of the NLRP3 inflammasome, thus exerting a potent anti-inflammatory effect *in vivo* (Yan et al., 2015). Moreover, microglial complement-dependent signaling pathway mediates elimination of D1-like receptors in the nucleus accumbens of adolescent male, leading to the development of social behavior changes in male rats (Kopec et al., 2018). In addition, the stimulation of the DRD2 in homeostatic microglial cells has been shown to attenuate the inflammatory response (Dominguez-Meijide et al., 2017) by increasing p38MAPK and reducing the number of cellular processes (Fan et al., 2018). Furthermore, indirect mechanisms involving DRD2-signaling in astrocytes and triggering anti-inflammatory effects on microglial cells have been described. Accordingly, it has been shown that high-dopamine levels might exert down-regulation of angiotensin II release by astrocytes (Dominguez-Meijide et al., 2017), and also induce the upregulation of the anti-inflammatory molecule αβ-crystallin in astrocytes (Shao et al., 2013), thus attenuating inflammatory behavior in microglial cells.

On the other hand, emerging evidence has suggested that stimulation of high-affinity dopamine receptors in microglia promotes neuroinflammation. Accordingly, the treatment of primary cultures of activated microglia with a DRD2/DRD3 agonist, has been shown to increase the release of nitrite and

IFN-γ (Huck et al., 2015). Moreover, inflammatory stimuli such as LPS, IFN-γ, or TNF-α, induced enhanced levels of intracellular BH4 in microglial cells, which promotes a higher rate of dopamine biosynthesis in mouse. A similar situation was observed in peripheral macrophages when stimulated by IFN-γ or LPS, which promoted the activity of the transcription factor NRF2 (Mcneill et al., 2015) and the consequent upregulation of GCH-1 in a rats (Sakai et al., 1995). Thereby, these findings suggest that pro-inflammatory stimuli in microglial cells promote a stronger capacity for dopamine biosynthesis in animal models. However, it is important to keep in mind that human cells are less efficient generating BH4 in comparison to other species (Schmidt et al., 2014).

Interestingly, dopaminergic regulation of microglial activity has also been studied under pathological conditions, such as those associated to amyotrophic lateral sclerosis (ALS) and Parkinson's disease. For instance, the pharmacologic stimulation of DRD4 has been shown to suppress microglia recruitment to the site of inflammation, and thereby delaying the progression of ALS in a mouse model (Tanaka et al., 2008). Moreover, the systemic DRD3-antagonism has been shown to modulate the inflammatory response of astrocytes, attenuating microglial activation in the striatum in a mouse model of Parkinson's disease (Elgueta et al., 2017; Elgueta et al., 2019). Taken together, the current evidence indicates that high-dopamine levels promote the stimulation of low-affinity dopamine receptors (including DRD1, DRD2, and DRD4), inducing an anti-inflammatory effect in microglia, while low-dopamine levels selectively stimulates high-affinity dopamine receptors (including DRD3 and DRD5), triggering inflammation, as proposed before (Pacheco, 2017).

Dopaminergic Regulation of Monocytes

Under pathological conditions, such as those associated to neurodegenerative disorders (Gonzalez and Pacheco, 2014), or CNS injury (Wattananit et al., 2016; Olingy et al., 2017; Norden et al., 2019; Vidal et al., 2019) peripheral monocytes play an important role contributing to neuroinflammation. For instance, using a rat model of peripheral inflammation it has been shown that monocytes depletion, mediated by the peripheral administration of clodronate, mitigates the production of inflammatory mediators and microglial activation without affecting dopaminergic neuronal survival (Xie et al., 2017).

Similar to microglial cells, monocytes also express both dopamine D1-like and D2-like receptors (Mckenna et al., 2002; Coley et al., 2015). A few studies have addressed the role of dopaminergic signaling in monocytes function and have found that it is associated with migration, regulation of inflammatory mediators (Gaskill et al., 2012; Coley et al., 2015), and proliferation (Bergquist et al., 2000). In this regard, *in vitro* experiments using human monocytes have shown that high dopamine levels potentiate the production of IL-10 as well as CXCL8, while low dopamine levels favor the secretion of IL-6 and CCL-2, and decrease TNF-α production in response to LPS (Gaskill et al., 2012). In addition, it has been shown that dopaminergic signaling through D1-like dopamine receptors increases monocytes migration and adhesion (Coley et al., 2015). Interestingly, a recent study has shown that DAT hypofunction in mice, a condition associated with increased dopamine levels, correlates with reduced microglial activation and a lower extent of infiltration of monocyte-derived macrophages into the brain (Castellani et al., 2019), suggesting that high-dopamine levels attenuate neuroinflammation. Furthermore, it has been shown that high dopamine concentrations (10–100 μM) decrease proliferation of peripheral blood monocytes (Bergquist et al., 2000). In summary, the current evidence suggests that high dopamine levels, probably by stimulating DRD1 or DRD2 in monocytes (Pacheco et al., 2014), reduce the production of inflammatory mediators, proliferation and CNS recruitment, thus attenuating neuroinflammation. Conversely, the stimulation of high-affinity dopamine receptors in peripheral monocytes seems to favor the production of some inflammatory cytokines in these cells.

CHANGES IN DOPAMINERGIC REGULATION OF THE IMMUNE SYSTEM ASSOCIATED TO SCHIZOPHRENIA

As described in section 3, the immune system plays an important role collaborating with the CNS to carry out some cognitive and behavioral function. Thereby, dysregulation of the immune system function might be involved in the development of neurologic diseases. In this regard, the association between psychiatric disorders, like schizophrenia, with altered immune responses has progressively gained interest over the past decade (Khandaker et al., 2015; Sellgren et al., 2019).

Several studies have reported immunological alterations associated to schizophrenia. In this regard, two meta-analysis have reported dynamic alterations in the profile of cytokine expression of schizophrenic patients depending on the stage of the disease (first episode versus relapsed patients) (Miller et al., 2011; Frydecka et al., 2018). These changes involve both inflammatory and anti-inflammatory cytokines depending on the disease duration, pharmacologic treatment, smoking status, among others (Miller et al., 2011; Muller et al., 2013). Another group of studies addressing gene expression in schizophrenic patients has suggested that dysregulation of the immune response associated to schizophrenia is a consequence of the disease progression or due to the long-term treatment with antipsychotic medication (Kumarasinghe et al., 2013; Frydecka et al., 2018).

A group of studies has analyzed samples of peripheral blood from patients and has found that dopamine receptors are differentially expressed in peripheral blood mononuclear cells (PBMCs) in schizophrenia. It has been reported that *DRD3* mRNA levels are increased in peripheral blood lymphocytes (Ilani et al., 2001). Consistently with the dopaminergic nature of drugs used in schizophrenia, it has been shown that pharmacological medication might induce changes in the expression of dopamine receptors in lymphocytes. In this regard, the transcriptional levels of DRD3 and DRD5 are increased in drug-free patients compared to medicated patients (Kwak et al., 2001). Moreover, the percentage of $CD4^+$ and $CD8^+$

T-cells expressing the DRD4, and $CD4^+$ T-cells expressing DRD2 was increased in medicated schizophrenic patients compared to controls (Brito-Melo et al., 2012). Interestingly, two studies have reported changes in the relative composition of PBMCs associated to schizophrenia. The first one showed a higher proportion of circulating Tregs without changes in $CD3^+$ or $CD4^+$ T-cells in medicated-schizophrenic patients in comparison with healthy controls (Kelly et al., 2018). The second one, showed increased frequencies of NK cells, classical monocytes, naive B-cells, and $CXCR5^+$ memory T-cells, and reduced percentages of dendritic cells, $CD4^+$ memory T-cells, and HLA-DR^+ regulatory T-cells in the blood of patients resistant to clozapine-treatment (Fernandez-Egea et al., 2016). Of note, the expression of DRD3 was shown significantly increased on peripheral $CD4^+$ T-cells in clozapine-treated schizophrenic patients, which was correlated with reduced frequency of Tregs (Fernandez-Egea et al., 2016). According to this negative correlation, DRD3-signaling in $CD4^+$ T-cells has been involved in promoting inflammatory responses, including Th1 and Th17 mediated immunity (Franz et al., 2015; Contreras et al., 2016). Following the same line, another study has shown an inverse correlation between the proportion of Tregs and the development of negative symptoms in schizophrenic patients (Kelly et al., 2018). Thus, the current evidence suggests an increased expression of high-affinity dopamine receptors, including DRD3 and DRD5, in T-lymphocytes of untreated schizophrenic patients, and enhanced levels of expression of the low-affinity dopamine receptor DRD2 in drug-treated schizophrenic patients. It is noteworthy that evidence obtained from *in vivo* approaches using animal models, or from *in vitro* approaches using human samples, has indicated that high affinity dopamine receptors DRD3 and DRD5 exert inflammatory effects while the low affinity dopamine receptor DRD2 promotes anti-inflammatory effects (Pacheco, 2017). Changes in dopaminergic regulation of the immune system associated to schizophrenia are integrated in **Figure 1**.

Interestingly, it has been shown that the treatment of schizophrenic patients with anti-inflammatory drugs in combination with anti-psychotic leads to better cognitive outcomes that the treatment with anti-psychotic drugs alone (Muller, 2010; Xiang et al., 2017). This is based on the inflammatory hypothesis of schizophrenia, which states that increased neuroinflammation contributes to the symptoms of schizophrenia. Therefore, combinatorial treatments using anti-inflammatory with anti-psychotic drugs might have synergistic effects at attenuating symptomatology, dampening the production of inflammatory cytokines, reactive oxygen species, prostaglandins and microglia function, among others (Sommer et al., 2014).

For instance, the treatment with the anti-inflammatory drug minocycline in combination with an atypical antipsychotic (risperidone, olanzapine, quetiapine, clozapine, or chlorpromazine), alleviates negative and positive symptoms in patients with schizophrenia (Levkovitz et al., 2010; Chaves et al., 2015). Of note, it has been suggested that minocycline acts not only by attenuating inflammation, but also decreasing synapse engulfment (Sellgren et al., 2019), and might potentially modify the composition of the gut-microbiota due to its antibiotic properties (Pape et al., 2019). The use of other anti-inflammatory therapies for schizophrenia are also being currently investigated in clinical trials, such as the treatment with monoclonal antibodies, such as Natalizumab, Tocilizumab, and Siltuximab (clinicaltrials.gov). It is noteworthy that a dopaminergic drug with anti-inflammatory activity is currently under study in humans as a treatment for schizophrenia. This is the case of a randomized, double-blind clinical trial ongoing in the US using a novel dopaminergic antagonist, I-tetrahydropalmatine, as an adjuvant treatment. This drug displays an anti-inflammatory activity and presents a higher affinity for D1-like receptors (clinicaltrials.gov).

IMMUNOLOGICAL ALTERATIONS DURING EMBRYONIC OR EARLY POSTNATAL DEVELOPMENT LEADING TO IMBALANCE IN DOPAMINERGIC TRANSMISSION AND NEUROPSYCHIATRIC DISORDERS IN ADULTHOOD

Diverse environmental risk factors associated with the development of schizophrenia during embryonic and early postnatal life have been reported, including perinatal hypoxia, cannabis consumption, stress, maternal infection, among others (Winter et al., 2009; Brown, 2011; Inta et al., 2011; Delpech et al., 2016). In the late 80's a novel hypothesis for the development of schizophrenia was raised, which suggested that primary cerebral insults occur during early brain development, triggering disease manifestation years later (Meyer, 2013). The revised hypothesis incorporated to the first version the brain changes occurring during the early phases of the disease at the postnatal stage (Meyer, 2013). A study carried out in Denmark showed that both infections requiring hospitalization and autoimmune disease are risk factors for developing schizophrenia in the future (Benros et al., 2011). Moreover, maternal infection or immune activation during critical periods of pregnancy enhances the risk of the offspring to develop neuropsychiatric disorders later in life (Winter et al., 2009). Little is known regarding the molecular mechanisms explaining how a broad range of infectious agents might trigger schizophrenia. Indeed, the evidence suggests that a significant immune response in the mother during pregnancy is sufficient to trigger schizophrenia in the adult offspring, irrespective of the pathogen identity (Zuckerman and Weiner, 2005). To add another level of complexity, it has been shown that exposure to Epstein-Barr virus or throat infection in early childhood have also been associated with increasing risk of experiencing psychotic symptoms and/or subsequent neuropsychiatric disorders during the adolescence (Khandaker et al., 2014; Orlovska et al., 2017). Thus, the period of time in which infections can increase the risk of developing neuropsychiatric disorders is not restricted to the prenatal stage (Khandaker et al., 2012).

Some recent studies have demonstrated how maternal immune activation (MIA) during embryonic life might lead to changes in dopaminergic transmission during adulthood. In this regard, a preliminary study in a non-human primate model of MIA reported that male offspring born to MIA-treated dams had

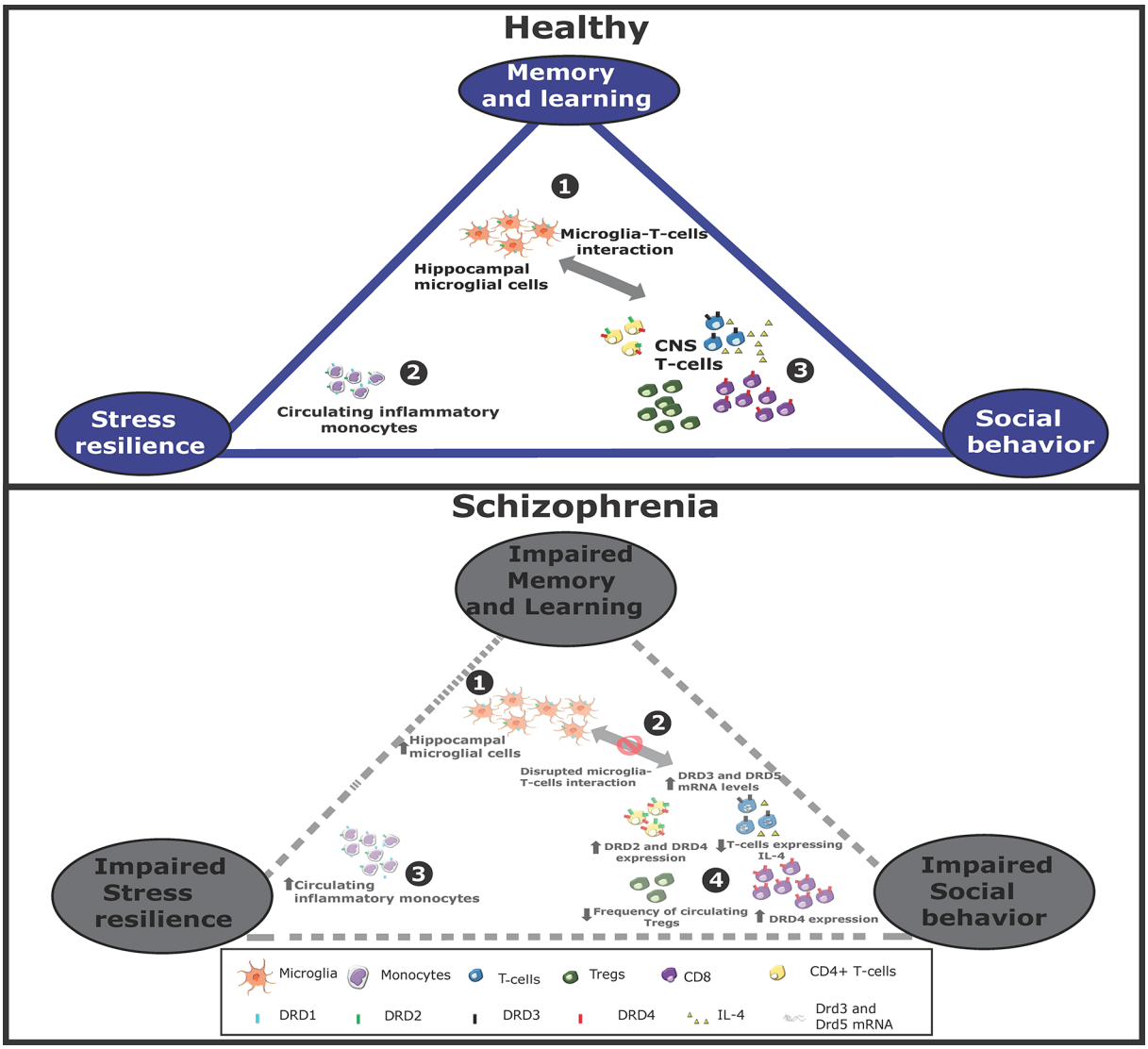

FIGURE 1 | Altered dopaminergic signaling in monocytes, T-cells, and microglia associated to schizophrenia. Schematic representation of immune cells associated to cognitive/behavioral function in healthy conditions (top panel) or in schizophrenia (bottom panel). Top panel: In healthy conditions, 1. microglial cells stimulate to T-cells infiltrated into the CNS. 2. Peripheral monocytes might also infiltrate the brain and favor T-cell activation. 3. Activated T-cells contribute to stress resilience. Some T-cells acquire Th2 phenotype and produce IL-4, which favors neurogenesis and thereby memory and learning. Some T-cells acquire Th1 features, produce IFN-γ and promote social behavior. Bottom panel: In schizophrenia, 1. the interaction between microglia and T-cells is impaired, 2. thereby attenuating T-cell activation. Moreover, the expression of different dopamine receptors is altered, changing the extent of T-cell activation and differentiation. 3. Furthermore, the extent of peripheral monocytes and hippocampal microglia are increased, 4. whereas Treg frequency is reduced. All these changes hypothetically contribute to a reduced social behavior, decreased stress resilience and in impaired memory and learning.

increased striatal dopamine in late adolescence compared with the control group (Bauman et al., 2019), changes similar to those associated with schizophrenia (see section 1). Similarly, prenatal exposure to the inflammatory agents Poly I:C or LPS in mice led to long-lasting changes in neurotransmitter levels and dopamine receptors in the brain of adult offspring. Specifically, levels of TH (Meyer et al., 2008), dopamine, and dopamine-derived metabolites were increased, whereas DRD1 and DRD2 (Meyer et al., 2008), serotonin receptors, and its metabolites were reduced in some specific brain areas (Winter et al., 2009). Moreover, organotypic cultures of ventral mesencephalon and striatum of rat fetuses exposed to LPS at embryonic day (E) E10, E14, or E18 showed a reduction in dopaminergic neurons when the cultures were kept for a long period compared with organotypic cultures of non-exposed fetuses (Snyder-Keller and Stark, 2008). In addition, prenatal exposure to inflammation also induces changes in pre- and post-synaptic GABAergic, glutamatergic, and serotoninergic neuronal circuits. Changes associated to embryonic exposure to inflammatory

stimuli are not limited to neurons, but also to glial cells affecting the number, structure, positioning, and survival of these cells, as well as morphological changes in the brain (Boksa, 2010; Chua et al., 2012). With regard to these latter analyses, controversial results have been reported about the effect of embryonic exposure to inflammation in microglial density, proliferation, phagocytic activity, and number of cells in animal models (Boksa, 2010). Some studies have reported an increase in these parameters (Juckel et al., 2011; Hadar et al., 2017) while others have reported no change (Garay et al., 2013; Mattei et al., 2014). This discrepancy can mainly be attributed to the rodent strain used and the stage of gestation where pregnant mothers receive the injection of Poly I:C (Juckel et al., 2011; Garay et al., 2013; Mattei et al., 2014). Of note, although alterations in the number and morphology of microglia cells may be a transient process, they might lead to long-lasting changes at the gene expression level increasing, for example, their phagocytic activity (Delpech et al., 2016; Mattei et al., 2017), and acquiring a "primed" pro-inflammatory phenotype prone to neuroinflammation in the adulthood (Norden et al., 2015). Microglia isolated from mice that underwent maternal immune activation in *utero* have shown altered expression of genes involved in embryonic development and phagocytosis (Mattei et al., 2017), which resemble those alterations observed upon interruption of the CSF-1/CSF-1R axis in cerebellar microglia associated with motor and social deficits (Kana et al., 2019). Intriguingly, it has been shown that cesarean section births also induce long-term changes in brain dopamine receptors, specially D1-like receptors (El-Khodor and Boksa, 2001). Thus, current evidence suggests that inflammatory events occurring during embryonic or early postnatal age lead to alterations in microglial cells which later in life result in imbalance of dopaminergic transmission and the consequent development of neuropsychiatric disorders.

Anti-inflammatory treatments have shown to partially attenuate or even fully prevent the development of schizophrenia in animal models. For instance, minocycline treatment in the offspring reduced the levels of TNF-α and IL-1β in the hippocampus of rats exposed to MIA (Mattei et al., 2014). Furthermore, the authors showed that the treatment with minocycline promoted neurogenesis, improved working memory, and social behavior as well as increased the phagocytic activity of microglia in the hippocampus (Mattei et al., 2017). In addition, it was shown that deep brain electrical stimulation treatment during the adolescence of MIA rats prevented the behavioral impairment and attenuated the increase of microglial density (Hadar et al., 2017). An important consequence of LPS-induced MIA is the induction of metallothionein, a zinc-binding protein, in the mother's liver, which promotes fetal zinc deficiency. Accordingly, maternal dietary zinc supplementation has been assessed as a treatment for offspring exposed to MIA. In this regard, it has been shown that zinc supplementation prevented the development of long-term cognitive abnormalities, reduced the number of TNF-α^+ cells, and decreased the extent of apoptotic cells in the offspring of LPS-treated mice (Coyle et al., 2009; Chua et al., 2012). Thus, prophylactic anti-inflammatory treatments intended to target maternal inflammation in experimental animal models of schizophrenia have sparked interest in the combinatorial use of anti-inflammatory drugs with atypical antipsychotic drugs during the early phases of schizophrenia. Preliminary studies in human patients with schizophrenia have shown promising beneficial effects by improving negative and cognitive symptoms compared with patients receiving antipsychotic drugs alone (Levkovitz et al., 2010; Muller et al., 2010).

CONCLUSIONS

Schizophrenia is associated with dysregulated activity of dopaminergic neural circuits, which consequently promotes several cognitive and behavioral alterations. Immune system cells, specially T-cells, microglial cells and peripheral monocytes, have been described to collaborate with the CNS to carry out some of these cognitive and behavioral functions that are altered in schizophrenia. Furthermore, dopamine might strongly affect the activity of T-cells, microglia, and peripheral monocytes, since all these immune cells express dopamine receptors. The current evidence indicates that high-dopamine levels promote the stimulation of low-affinity dopamine receptors (including DRD1, DRD2, and DRD4), inducing an anti-inflammatory effect on immune cells, while low-dopamine levels selectively stimulates high-affinity dopamine receptors (including DRD3 and DRD5), triggering inflammation. Thus, alterations in dopamine levels associated to schizophrenia might affect inflammatory response of immune cells and consequently some behavioral functions, including reference memory, learning, social behavior, and stress resilience (see a summary in **Table 1**). Interestingly, studies performed with patients have shown that drug-free schizophrenic patients display exacerbated expression of high-affinity dopamine receptors, and thereby acquiring pro-inflammatory features in response to dopamine. Conversely, medicated patients seem to switch their expression of dopamine receptors favoring the expression of low-affinity receptors in immune cells, thus acquiring anti-inflammatory profiles in response to dopamine. Accordingly, recent data obtained from clinical trials has suggested that the usage of anti-inflammatory and/or dopaminergic drugs as adjuvant therapy for schizophrenia might give a significant improvement in the symptomatology involved in this disorder. Recent evidence indicates that significant inflammatory events occurring during embryonic or early postnatal age lead to alterations in microglial cells which later in life result in imbalance of dopaminergic transmission and the consequent development of neuropsychiatric disorders. Further research is necessary in this area to decipher the molecular and cellular underlying mechanisms and, consequently, to be able to design therapeutic strategies to target specific detrimental processes without affecting the general function of the immune system. Studies about the long-term safety of combined therapies (i.e. anti-psychotics with anti-inflammatory drugs) are also still necessaries, as chronic administration of anti-inflammatory drugs could yield hepatic failure and/or an immunosuppressive state that may be involve increased susceptibility to infections and cancer. Finally, another aspect that should be further explored in the upcoming future is the validation of

TABLE 1 | Dopaminergic regulation of the immune system associated with cognitive/behavioral functions.

Immune cells	Cognitive/behavioral function	Physiological effect involved	Dopaminergic regulation	Dopaminergic alteration in schizophrenia
$CD4^+$ T-cells and microglial cells	Memory and learning (Ziv et al., 2006; Derecki et al., 2010; Baruch et al., 2013).	Neurogenesis.	Stimulation of D1-like dopamine receptors promotes the differentiation of naive $CD4^+$ T-cells toward a Th2 phenotype (Nakano et al., 2009).	Increased *DRD3 and DRD5* mRNA levels peripheral blood T-cells (Ilani et al., 2001; Kwak et al., 2001).
		Adaptation to psychological stress.	Stimulation of D1-like dopamine receptors attenuates the regulatory activity of Tregs (Kipnis et al., 2004a; Cosentino et al., 2007).	Increased percentage of $CD4^+$ T-cells expressing the DRD4 and DRD2 in medicated schizophrenic patients (Brito-Melo et al., 2012).
T-cells and Tregs	Stress resilience (Cohen et al., 2006; Lewitus and Schwartz, 2009; Scheinert et al., 2016).	Production of IFN-γ that stimulates GABAergic inhibitory neurons.	DRD3 signaling favors Th1 and inhibits Th2 differentiation (Gonzalez et al., 2013; Franz et al., 2015; Contreras et al., 2016).	Reduced percentage of Tregs in schizophrenic patients (Fernandez-Egea et al., 2016).
			DRD4 signaling induces T-cell quiescence (Sarkar et al., 2006).	
T-cells	Social behavior (Filiano et al., 2016)		DRD4 signaling promotes Th2 differentiation (Wang et al., 2019).	
$CD8^+$ T-cells	Memory and learning (Dulken et al., 2019),	Neurogenesis and cognition.	DRD3 signaling promotes IFN-γ (Ilani et al., 2004) transcription, and lymphocytes migration (Watanabe et al., 2006). D1-like dopamine receptor signaling attenuates both the generation and the suppressive activity of regulatory $CD8^+$ T-cells (Nasi et al., 2019).	Increased percentage of $CD8^+$ T-cells expressing DRD4 in medicated schizophrenic patients compared to controls (Brito-Melo et al., 2012).
Microglial cells	Memory and learning (Rogers et al., 2011; Schafer et al., 2012; Meyer, 2013).	Synaptic pruning.	Upregulation of DRD2 upon inflammation (Huck et al., 2015).	Increased hippocampal microglial cells (Busse et al., 2012).
			Attenuation of inflammatory activation of microglial cells at high dopamine levels (Farber et al., 2005; Yan et al., 2015).	
Microglial cells	Social behavior (Kana et al., 2019; Lehmann et al., 2019).	High production of reactive oxygen species under stress, interference of the CSF-1/CSF-1R signaling pathway.	DRD3-antagonism in astrocytes dampens inflammatory features of microglial (Elgueta et al., 2017; Montoya et al., 2019).	
Monocytes, Th2 and microglial cells	Stress resilience (Kertser et al., 2019; Weber et al., 2019).	Trafficking of immune cells, production of cytokines.	High dopamine levels promote an anti-inflammatory phenotype (Gaskill et al., 2012).	Increased frequency of circulating inflammatory monocytes (Mckim et al., 2018).
			D1-like dopamine receptor stimulation increases monocytes migration (Coley et al., 2015).	

the cross-talk between immune system and CNS in the development of cognitive and behavioral changes, as most research in this field has been done in animal models.

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BRIEF RESEARCH REPORT
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Striatal Volume Increase After Six Weeks of Selective Dopamine $D_{2/3}$ Receptor Blockade in First-Episode, Antipsychotic-Naïve Schizophrenia Patients

*Helle G. Andersen[1,2], Jayachandra M. Raghava[1,3], Claus Svarer[4], Sanne Wulff[1], Louise B. Johansen[1], Patrick K. Antonsen[1,2], Mette Ø. Nielsen[1,2], Egill Rostrup[1], Anthony C. Vernon[5,6], Lars T. Jensen[7], Lars H. Pinborg[4], Birte Y. Glenthøj[1,2] and Bjørn H. Ebdrup[1,2]**

[1] Center for Clinical Intervention and Neuropsychiatric Schizophrenia Research and Center for Neuropsychiatric Schizophrenia Research, Mental Health Centre Glostrup, University of Copenhagen, Glostrup, Denmark, [2] Department of Clinical Medicine, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark, [3] Functional Imaging Unit, Department of Clinical Physiology, Nuclear Medicine and PET, University of Copenhagen, Glostrup, Denmark, [4] Neurobiology Research Unit, Department of Neurology, Rigshospitalet, University of Copenhagen, Copenhagen, Denmark, [5] Department of Basic and Clinical Neuroscience, Institute of Psychiatry Psychology and Neuroscience, King's College London, London, United Kingdom, [6] Medical Research Council Centre for Neurodevelopmental Disorders, King's College London, London, United Kingdom, [7] Department of Clinical Physiology and Nuclear Medicine, Herlev Hospital, University of Copenhagen, Herlev, Denmark

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Andrew J. Grottick, Beacon Discovery Inc., United States

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***Correspondence:**
Bjørn H. Ebdrup
bebdrup@cnsr.dk

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Patients with chronic schizophrenia often display enlarged striatal volumes, and antipsychotic drugs may contribute via the dopamine $D_{2/3}$ receptor ($D_{2/3}R$) blockade. Separating the effects of disease from medication is challenging due to the lack of a proper placebo-group. To address this, we conducted a longitudinal study of antipsychotic-naïve, first-episode schizophrenia patients to test the hypothesis that selective blockade of $D_{2/3}R$ would induce a dose-dependent striatal volume increase. Twenty-one patients underwent structural magnetic resonance imaging (sMRI), single-photon emission computed tomography (SPECT), and symptom severity ratings before and after six weeks of amisulpride treatment. Twenty-three matched healthy controls underwent sMRI and baseline SPECT. Data were analyzed using repeated measures and multiple regression analyses. Correlations between symptom severity decrease, volume changes, dose and receptor occupancy were explored. Striatal volumes did not differ between patients and controls at baseline or follow-up, but a significant group-by-time interaction was found ($p = 0.01$). This interaction was explained by a significant striatal volume increase of 2.1% in patients (Cohens $d = 0.45$). Striatal increase was predicted by amisulpride dose, but not by either $D_{2/3}R$ occupancy or baseline symptom severity. A significant reduction in symptom severity was observed at a mean dose of 233.3 (SD = 109.9) mg, corresponding to $D_{2/3}R$ occupancy of 44.65%. Reduction in positive symptoms correlated significantly with striatal volume increase, driven by

reductions in hallucinations. Our data demonstrate a clear link between antipsychotic treatment and striatal volume increase in antipsychotic-naïve schizophrenia patients. Moreover, the treatment-induced striatal volume increase appears clinically relevant by correlating to reductions in core symptoms of schizophrenia.

Keywords: schizophrenia, dopamine receptor, first-episode antipsychotic-naïve, striatum, SPECT, sMRI, antipsychotic drug, longitudinal

INTRODUCTION

Schizophrenia is a mental disorder affecting approximately 1% of the population worldwide (Salavati et al., 2015). The disorder typically manifests in puberty or adolescence and is characterized by so-called positive symptoms such as delusions and/or hallucinations (Howes and Kapur, 2009), but patients also exhibit negative symptoms and cognitive deficits. Studies using structural magnetic resonance imaging (sMRI) provide evidence that patients with schizophrenia display subtle volumetric brain aberrations at the time of diagnosis as compared to healthy controls (Brugger and Howes, 2017; Dietsche et al., 2017). Moreover, the brain of chronic, medicated patients appears to undergo progressive, structural changes over the course of the illness, with ventricular volume increases, cortical thinning, and basal ganglia enlargement among the most consistent findings (Brandt and Bonelli, 2008; Puri, 2010; Haijma et al., 2013; van Erp et al., 2016; Dietsche et al., 2017). Antipsychotic drugs (APD) are the gold standard for treatment of positive symptoms (Howes and Kapur, 2009), but since illness and treatment go hand in hand, separating the effects of medication and disease on brain structure is difficult (Fusar-Poli et al., 2013).

In 1976 it was discovered that antipsychotics exert their function by antagonizing the dopamine D_2 receptors (D_2R) in striatum, and that drug efficacy is directly proportional to the affinity for the receptor (Creese et al., 1976). This led to the dopamine hypothesis of schizophrenia, which suggests that a hyperactive striatal dopamine-system leads to 'aberrant salience', meaning that wrongful interpretations of harmless stimuli can eventually lead to core psychotic symptoms such as hallucinations and delusions (Kapur, 2003). Further studies of striatum have found increased presynaptic dopamine synthesis capacity and -release compared to controls, as well as higher dopamine concentrations in the synaptic cleft (Howes et al., 2009, 2012; Brunelin et al., 2013; Salavati et al., 2015). All currently marketed antipsychotics antagonize the D_2R, thereby blocking the down-stream signaling in the post-synaptic neuron (Golan, 2012; Kusumi et al., 2015; Amato et al., 2017). However, most antipsychotics are characterized by broad receptor profiles, and bind to e.g., serotonin 2A-, histaminergic- and cholinergic receptor systems (Kusumi et al., 2015). This complex pharmacology has further limited the investigations of causal mechanisms linking antipsychotic treatment to structural brain changes. Nevertheless, longitudinal studies on antipsychotic-naïve patients as well as meta-analyses studies have reported associations between antipsychotic exposure and volumetric increase in basal ganglia (Glenthoj et al., 2007; Ebdrup et al., 2013; Jorgensen et al., 2016; Huhtaniska et al., 2017; Di Sero et al., 2019). Studies on rodents have replicated the basal ganglia volume increase in response to antipsychotic treatment (Vernon et al., 2012), and investigations in dopamine D_2 or D_3 receptor knock-out- and wild-type mice provide evidence that this increase is likely to be mediated through D_2-like receptors (Guma et al., 2018, 2019).

In humans, dopamine D_2-like receptor availability and blockade following antipsychotic treatment can be investigated with single-photon emission computed tomography (SPECT) examinations (Salavati et al., 2015). The association between antipsychotic treatment, dopamine $D_{2/3}$ receptor occupancy, and basal ganglia enlargement has, however, yet to be established in a longitudinal study of antipsychotic-naïve patients with schizophrenia.

To address this gap in our knowledge, we completed a prospective study, wherein we examined a cohort of first-episode, antipsychotic-naïve schizophrenia patients, before and after 6 weeks of treatment with amisulpride, a relatively selective dopamine $D_{2/3}$ receptor antagonist. Baseline- and follow-up examinations included sMRI, SPECT, and Positive and Negative Syndrome Scale (PANSS) examinations.

We hypothesized that selective blockade of dopamine $D_{2/3}R$ would lead to a dose-dependent striatal volume increase. Further, we explored correlations between symptom severity decrease, striatal volume increase, dose and receptor occupancy.

MATERIALS AND METHODS

Participants

We included participants between the ages of 18–45 years from 2008 to 2014. Patients with schizophrenia were first-episode, antipsychotic-naïve, and were recruited from hospitals and psychiatric out-patient clinics in the capital region of Denmark, as a part of the PECANS I (Pan European Collaboration Antipsychotic-naïve Studies, PECANS) cohort. All patients met the International Classification of Diseases (ICD-10) criteria for schizophrenia (F20) verified by the structured diagnostic interview SCAN (Schedule of Clinical Assessment in Neuropsychiatry, version 2.1). Exclusion criteria included previous exposure to antipsychotic medication, methylphenidate, or use of antidepressants less than 1 month prior to baseline examinations. Healthy controls were recruited through advertisement, and matched to patients on age, gender and parental socioeconomic status. Exclusion criteria for the healthy controls were identical to the criteria for patients, but also comprised any former or current psychiatric illnesses, psychiatric diagnoses within first-degree relatives and/or any

drug-abuse (classified by ICD-10). For all participants, previous or current medical history of serious head trauma, neurological diseases, developmental disorders or current drug dependency (by ICD-10 classification), and current pregnancy were exclusion criteria. All participants were screened for drug-use with urine samples (Rapid Response, Jepsen HealthCare) prior to SPECT scan. Included participants are a subsample of Wulff et al. (2015, 2019) from the PECANS I cohort. Wulff and colleagues also reported on binding potentials in their sample, although a different method of binding potential extraction was used. Subcortical volumes have not yet been investigated in this subgroup.

Medication

The atypical APD, amisulpride, was chosen as a tool compound because of its relative selectivity toward dopamine $D_{2/3}$ receptors (Rosenzweig et al., 2002). Amisulpride treatment was initiated after completion of baseline examinations, and dosage was slowly increased and adjusted to the individual patient, according to clinical judgment and patients' reports of adverse effects. Pharmaceutical treatment against adverse effects was not allowed. Follow-up examinations were conducted after six weeks, and treatment dose in mg was recorded. To ensure a steady concentration at examinations, dosage was kept stable in the week prior to follow-up. Compliance was continuously ensured through dialogue with the patient, and measurement of serum-amisulpride (S-amisulpride) levels at follow-up. Benzodiazepines were allowed on an "as-needed basis" to secure sleep and reduce anxiety but were not allowed 12 h prior to SPECT examinations. Healthy control subjects were not treated.

Symptom Severity

Symptom severity was assessed with PANSS (Kay et al., 1987) within the same week as MRI and SPECT scan examinations. PANSS total score as well as sub-scores (positive-, negative-, and general sub-scores) was assessed at baseline and at follow-up. To ensure consistency in PANSS ratings between clinicians, ratings were regularly evaluated using systematic video recordings of the interviews. Duration of untreated illness was assessed from the patient history of worsening in functions due to symptoms. Healthy controls did not undergo PANSS examinations.

Magnetic Resonance Imaging

T1-weighted scans of the whole head (sagittal 3D sequence, TR = 10 ms, TE = 4.6 ms, FA = 8°, voxel size = 0.79 mm × 0.79 mm × 0.80 mm) were acquired with an 8-channel SENSE head coil on a 3T Philips Achieva scanner (Philips Healthcare, Best, Netherlands) at baseline and after 6 weeks. MRI scans were acquired within the same week as SPECT and PANSS. Subcortical segmentation and volume extraction were performed with tools from the FSL, FMRIB software library v5.0.10 (Patenaude et al., 2011). In this study we focused on striatum as our region of interest, estimated as a sum of volumes from the bilateral subregions of caudate nucleus, putamen and nucleus accumbens (**Figure 1**). Anatomically, striatum is also referred to as a part of basal ganglia (Waschke and Paulsen, 2011).

Single Photon Emission Computed Tomography

Single-photon emission computed tomography acquisition has previously been described (Wulff et al., 2015). In short, SPECT images were acquired using a Siemens Symbia T2 series SPECT-CT scanner, with the [^{123}I]-Iodobenzamide ([^{123}I]-IBZM) as the radioactive ligand, because of its dopamine $D_{2/3}$R selectivity (Kung et al., 1990; Barnas et al., 2001). After 180 min of rest, a CT scout and 2 × 30 min tomography were performed. CT-scout and tomography were performed to optimize positioning in the scanner and for attenuation correction. Patients underwent both baseline and follow-up SPECT scans, whereas controls only underwent baseline SPECT to minimize their exposure to radiation. At follow-up, the individual dose of amisulpride was administered 3 h prior to the scan, and s-amisulpride was measured prior to and at 60, 120, 150, 180, 210, and 240 min after administration. The mean s-amisulpride during SPECT-scan was calculated.

Image Processing

Because SPECT images contain limited anatomical information, it was not possible to automatically extract SPECT counts (counts/s) directly from our regions of interest. First, we co-registered CT- and MR anatomical images using a statistical parametric mapping method (SPM8) to calculate the transformation matrix. This step was visually inspected with an image overlay method, and manually adjusted if needed (Willendrup et al., 2004). Next, the CT-MR transformation matrix was used to co-register the SPECT images to the MRIs, and FSL subcortical region segmentations were resliced to fit the individual SPECT images. Subsequently, SPECT counts were extracted from FSL-MRI defined regions.

Lastly, extracted SPECT counts were scatter- and decay corrected. The specific binding potentials were calculated by subtracting non-specific binding from a reference region from total binding in the regions of interest divided by the metabolite corrected plasma counts. Cerebellum as defined in Svarer et al. (2005) was used as reference region for non-specific binding, as in our previous study on binding potentials (Wulff et al., 2015). Dopamine receptor occupancy was calculated using the following equation:

$$Occupancy\,(\%) = \left(1 - \frac{Specific\ binding\ potential\,(follow\text{-}up)}{Specific\ binding\ potential\,(baseline)}\right) \times 100\%$$

Statistical Analyses

Statistical analyses were conducted using IBM SPSS version 25. Normal distributions were assessed by Shapiro–Wilk. Equality of variance was assessed by Box's- or Levene's test. For between-groups comparisons, unpaired students t-test was used for normally distributed data and Mann–Whitney for non-normally distributed data (demographics, volumes, and binding

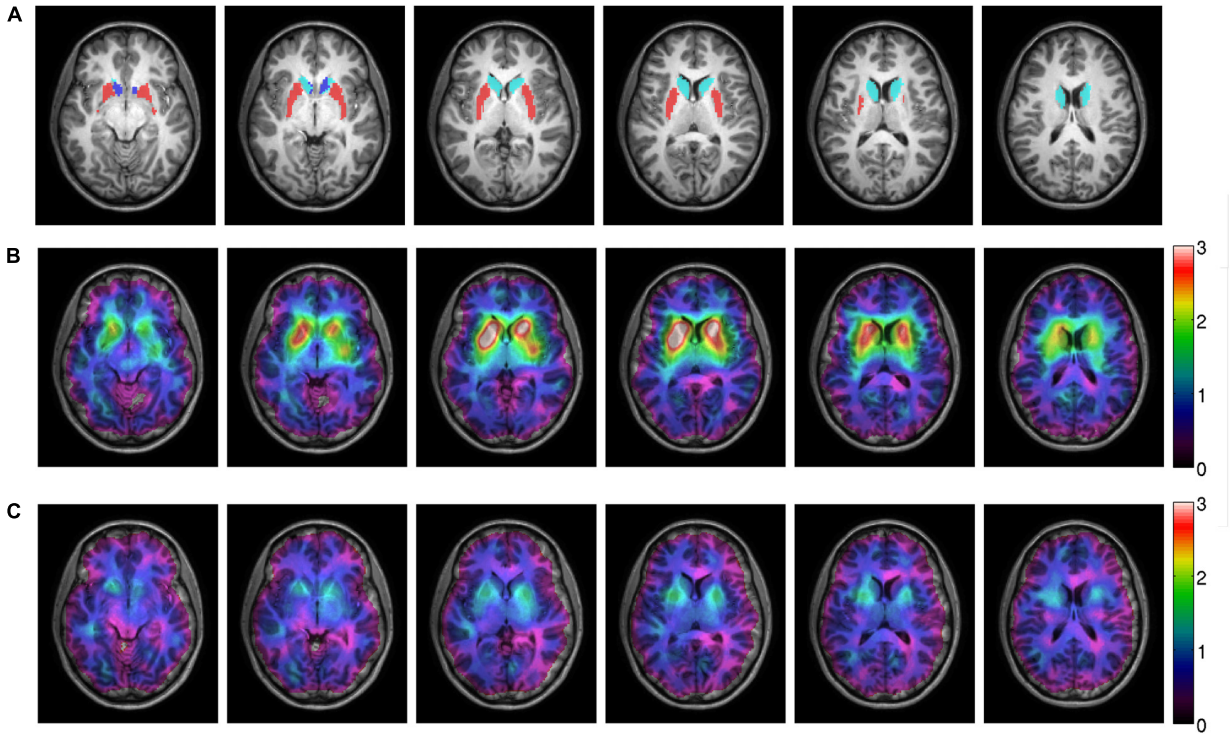

FIGURE 1 | MRI and SPECT images of one patient, treated with 300 mg amisulpride displaying a mean dopamine D_2 receptor occupancy of 56%. Panel **(A)** shows the sMRI image with the caudate nucleus (light blue), putamen (red) and accumbens (dark blue) from the subcortical Harvard-Oxford atlas depicted. Panels **(B,C)** show the co-registered SPECT image on top of the MRI image. The color scale corresponds to the specific binding potential before treatment **(B)** and after six weeks of treatment **(C)**.

potentials). Within-group comparisons were analyzed with paired students t-test and Wilcoxon for non-normally distributed data. Cohens *d* was used to calculate effect sizes, with effect size 0.2 considered low, 0.5 considered medium and 0.8 considered high. Pearson's Chi^2 was used for nominal data. When correlating data, Pearson's correlation coefficient was used for parametric data, otherwise Spearman's rho was used.

Our primary hypothesis was tested in two steps. First, striatal volume changes over time were tested with a repeated measure analysis. Significant group-by-time interactions were further investigated with *post-hoc* t-tests. The repeated measures analysis was initially performed for striatum, and afterward we separately analyzed the striatal subregions, i.e., caudate nucleus, putamen and nucleus accumbens. Second, we applied a multiple regression analysis to investigate the individual predictive effect of a set of variables on striatal volume increase, whilst controlling for the following included variables: amisulpride dose, striatal receptor occupancy, and baseline PANSS positive score. PANSS baseline positive scores were included in the model to control for the disease severity. Assumptions of normal distribution and no multicollinearity (Variance Inflation Factor <10) were met. If variables were initially non-normally distributed, they were transformed to normal distributions using log10- or square root functions.

Finally, we explored Spearman correlations between changes in symptom severity, striatal volumes, amisulpride dose, and $D_{2/3}R$ occupancy. Explored correlations were Bonferroni corrected for number of hypotheses tested on the same data, with a threshold of α/m, where α-level was set at 0.05, and m was number of hypotheses tested. For all other analyses, a two-sided *p*-value less than 0.05 was accepted as significant.

RESULTS

Patients Compared to Healthy Controls

We included 21 patients and 23 controls with full datasets in our analyses (**Supplementary Figure S1**). Patients had higher use of tobacco and fewer years of education compared to controls (**Table 1**) but did not differ in other demographic factors. No difference in mean striatal volumes between patients and controls was found at baseline ($p = 0.82$) or at follow-up ($p = 0.28$). No difference in mean specific binding potentials to dopamine $D_{2/3}R$ was found between patients (2.49 ± 0.82) and controls (2.68 ± 0.71) ($p = 0.25$).

Symptom Severity and Receptor Occupancy in Patients After Treatment

After six weeks of treatment, patients' PANSS total-, positive- and general symptom scores were significantly decreased,

TABLE 1 | Demographic and clinical data.

Between-groups	**Group; mean ± SD [mean]**		
	Patients (*n* = 21)	**Controls (*n* = 23)**	***p*-value**
Demographics			
Age, years	23.5 ± 4.8	24.1 ± 5.01	0.92[b]
Sex, male:female	10:11	12:11	0.76[c]
Handedness, right:ambidextrous:left	16:3:2	20:2:0	0.31[c,f]
Handedness score,−100:100	59.2 ± 60.7	54.6 ± 68.7	0.78[b]
Parental socioeconomic status, high:moderate:low	4:11:6	5:14:4	0.68[c,f]
Educational level, higher education/self employed, medium education, uneducated, student	0:3:4:9	0:2:0:15	0.06[c,f]
Years of education	11.9 ± 2.0	14.3 ± 2.5	**0.001**[a]
Weight, kg	78.5 ± 20.6	68.5 ± 11.0	0.058[a]
Height, cm	172.8 ± 9.5	175.1 ± 10.3	0.54[a]
Substance use, alcohol, tobacco, cannabis, benzo, opioids, stimulants	16:13:4:0:1:3	20:3:1:0:0:0	**<0.001**[c,e,f]
Volumes (cm^3)			
Baseline	18.31 ± 2.3	18.04 ± 2.5	0.82[b]
Follow-up	18.67 ± 2.3	17.92 ± 2.3	0.28[a]
Specific binding potentials (counts/s)			
Baseline	2.49 ± 0.82	2.68 ± 0.71	0.25[b]
Follow-up	1.38 ± 0.68	–	–
Within patients	**Baseline**	**Follow-up**	
PANSS scores[d]			
Positive	19.8 ± 4.0	13.4 ± 3.4	**<0.001**
Negative	18.7 ± 7.2	20.3 ± 5.8	0.081
General	40.1 ± 8.5	30.2 ± 7.5	**<0.001**
Total	78.5 ± 16.4	64.0 ± 13.8	**<0.001**
Medication			
Dose amisulpride (mg/day)	–	233.3 ± 109.9	
S-amisulpride (ng/ml)	–	399.7 ± 283.8	
Duration of untreated illness (weeks)	80.8 ± 96.2	–	
Receptor occupancy			
Striatum	–	44.65% ± 18.7%	

SD, standard deviation. [a]t-test, [b]Mann-Whitney U, [c]Pearsons Chi, [d]Wilcoxon, [e]p < 0.05 only for tobacco use, [f]Groups have expected counts less than 5. Significant p-values are in bold.

but negative symptoms were not (**Table 1**). Patients were treated with a mean dose of 233.3 (SD = 109.9) mg amisulpride. Oral dose and s-amisulpride correlated positively (r^2 = 0.76, p < 0.001). Mean receptor occupancy was 44.65% (SD = 18.7%) and correlated positively with oral dose (r^2 = 0.60, p = 0.004) and s-amisulpride (r^2 = 0.68, p = 0.001). Receptor occupancy is illustrated in **Figure 1**. Amisulpride dose did not correlate with symptom severity (PANSS total) at baseline (r^2 = 0.292, p = 0.199).

Striatal Volume Increase Is Predicted by Amisulpride Dose, But Not $D_{2/3}R$ Occupancy

The repeated measures analysis revealed no volume difference between groups at either time-point, but instead a significant group-by-time interaction was observed (p = 0.01). The *post hoc* analysis revealed that the interaction was driven by a significant volume increase in striatum of 2.1% (95% CI = 0.52–3.68%, p = 0.01, Cohens d = 0.45) in patients. Sub-regional increases were observed in left and right caudate nucleus (2.6%) and right putamen (2.4%) (**Table 2**). The multiple regression model significantly predicted striatal volume increase (r^2 = 0.411, p = 0.026) (**Figure 2**), with amisulpride oral dose as the only unique, predictive factor (beta = 0.553, p = 0.028) (**Supplementary Table S1**).

Symptom Severity Exploratory Correlations

Reduction in positive symptoms correlated significantly with striatal volume increase (r^2 = −0.472, p = 0.031) and this correlation was driven by a reduction in hallucinations (r^2 = −0.515, p = 0.017). The correlations did not survive Bonferroni correction. Changes in PANSS total- or subscores did not correlate to either amisulpride dose, s-amisulpride or receptor occupancy.

DISCUSSION

Primary Findings

In line with our hypothesis, we found a significant volume increase in striatum in patients (2.1%) with a medium effect size (Cohens d = 0.45) after six weeks of amisulpride treatment. Our predictive model showed that dose was a predictor of volume increase, but positive symptom severity at baseline and $D_{2/3}R$ occupancy were not. Our exploratory correlation analyses indicated that striatal volume increase was associated with an improvement in positive symptoms, particularly hallucinations.

Results Compared to Previous Findings

Structural brain differences between patients and healthy controls at the time of diagnosis have previously been reported, but are not consistently replicated, and often no differences are found, indicating that changes are subtle (Glenthoj et al., 2007; Puri, 2010; Dietsche et al., 2017). Consistent with this, we did not find any significant differences in striatal volumes between patients and healthy controls. Specific $D_{2/3}R$ binding potentials did not differ between patients and controls prior to treatment, which is a replication of previous findings (Howes et al., 2009; Salavati et al., 2015). In this study, mean amisulpride dose was relatively low (233.3 mg), and approximately half of the dose used in phase one of the OPTiMiSE study (488.0 mg for completers) (Kahn et al., 2018). However, included patients in the OPTiMiSE study

TABLE 2 | Volumes of regions of interest.

Volume (cm^3)	Patients			Controls		
	Baseline mean ± SD [mean]	**Follow-up mean ± SD [mean]**	***p*-value**	**Baseline mean ± SD [mean]**	**Follow-up mean ± SD [mean]**	***p*-value**
Striatum	18.31 ± 2.3	18.67 ± 2.3	**0.01**	18.04 ± 2.5	17.92 ± 2.3	0.121[a]
Caudate	7.68 ± 1.1	7.88 ± 0.1	**<0.001**	7.45 ± 0.9	7.37 ± 0.8	0.187
Left	3.74 ± 0.5	3.88 ± 0.5	**0.003**	3.68 ± 0.4	3.61 ± 0.4	0.067
Right	3.94 ± 0.6	4.00 ± 0.6	**0.004**	3.77 ± 0.5	3.76 ± 0.4	0.770
Putamen	9.66 ± 1.3	9.82 ± 1.3	**<0.001**	9.63 ± 1.6	9.66 ± 1.5	0.224[a]
Left	4.87 ± 0.7	4.91 ± 0.6	0.347	4.81 ± 0.9	4.88 ± 0.9	0.670[a]
Right	4.79 ± 0.6	4.91 ± 0.7	**0.007**	4.82 ± 0.8	4.80 ± 0.7	0.212[a]
Accumbens	0.97 ± 0.2	0.98 ± 0.2	0.732	0.96 ± 0.2	0.97 ± 0.2	0.484[a]
Left	0.53 ± 0.1	0.55 ± 0.1	0.627	0.55 ± 0.1	0.55 ± 0.1	0.879[a]
Right	0.43 ± 0.09	0.43 ± 0.08	0.614[a]	0.41 ± 0.1	0.42 ± 0.1	0.346[a]

Equal variances were tested. p-values marked with [a] was tested with Wilcoxon. All other volumes were tested with paired students t-test. Significant p-values are in bold.

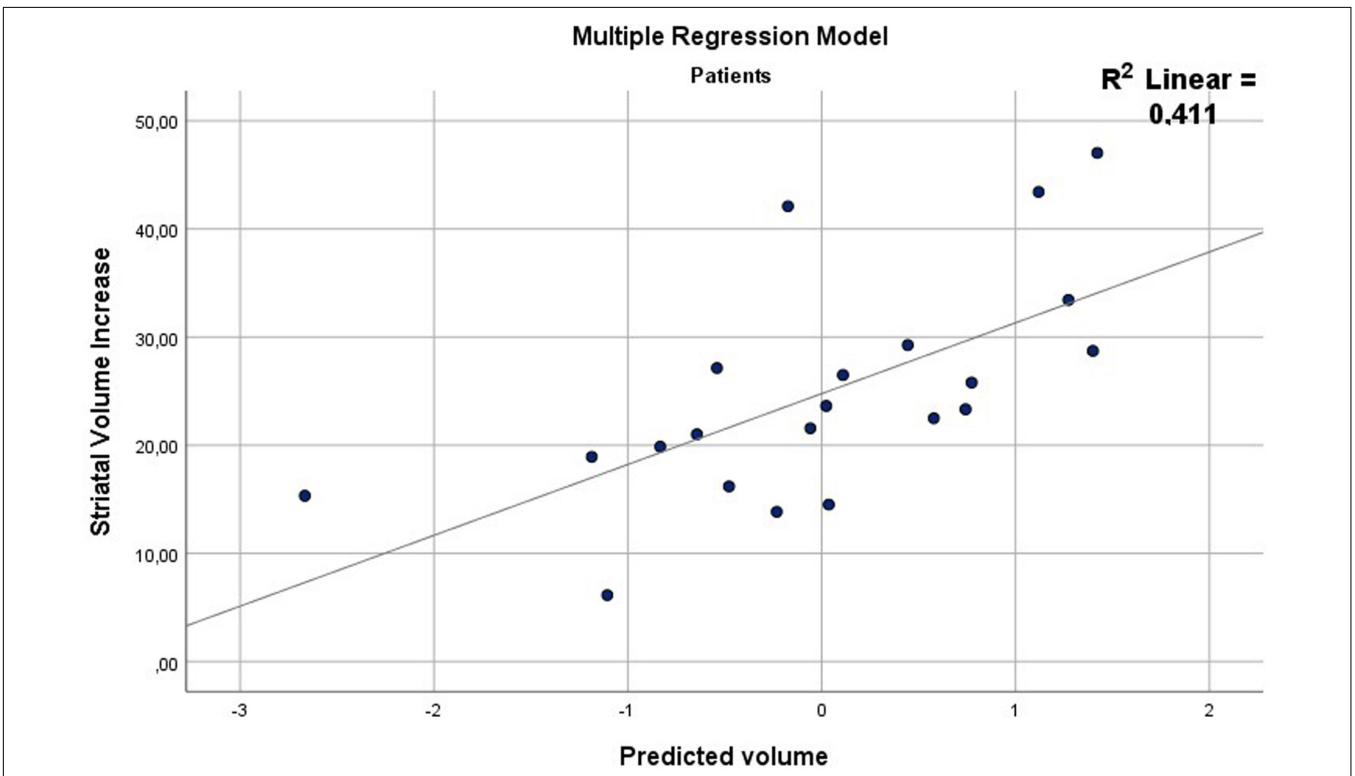

FIGURE 2 | Scatter plot of the multiple regression model including dose, striatal receptor occupancy and PANSS positive score as predictive/independent variables. The dependent variable, striatal volume increase, is depicted on the y-axis, the independent variables on the x-axis. The model significantly predicted striatal volume increase (r^2 = 0.411, p = 0.026). Only dose was a unique predictor of volume increase when controlling for the other variables (r^2 = 0.553, p = 0.028). All model coefficients can be seen in **Supplementary Table S1**.

were not all antipsychotic-naïve, and due to a potential compensatory upregulation of D_2R in response to antipsychotic treatment (Oda et al., 2015; Yin et al., 2017), this could explain the need for higher treatment doses compared to our antipsychotic-naïve patients. Furthermore, medication against adverse symptoms was not allowed in this study, which made clinicians upregulate dose slowly.

Treatment dose and blood levels are inherently linked to occupancy, and it is generally accepted that a striatal dopamine receptor occupancy of 65–80% is necessary for clinical response (Uchida et al., 2011; Yilmaz et al., 2012). However, we found a significant decrease in symptom severity at mean receptor occupancy-levels of 44.65%. Most recent studies investigating occupancy levels in patients

use estimations, but a cautious comparison can be made to the CATIE data (Moriguchi et al., 2013). Authors found that for patients in stable remission, approximately half did not have continuous dopamine D_2 blockade of ≥65%. A prospective PET study further found an optimal therapeutic window between 50 and 60% receptor occupancy on clinically stable patients with late-life schizophrenia (Graff-Guerrero et al., 2015). Altogether, this indicates that lower doses/D_2 receptor occupancies in selected patient populations are sufficient, and also reduce risk of adverse effects such as extrapyramidal symptoms.

We found no correlation between reduction in positive symptoms and dose or occupancy as previously found for amisulpride (Sparshatt et al., 2009) and other antipsychotics (Yilmaz et al., 2012), but a negative finding has also been reported (Batail et al., 2014). Different patient groups, different antipsychotics and different methodology makes results difficult to compare. Considering that amisulpride in low doses has a higher affinity toward the presynaptic dopamine D_2 auto receptors rather than post synaptic receptors (Rosenzweig et al., 2002), the discrepancy may be explained by the relatively low dose used in our study. We did, however, find a correlation between symptom reduction and volume increase. When specific positive symptoms were examined, this correlation was linked to a decrease in hallucinations. Similar results were found in a study by Li et al. (2012), in which PANSS decrease correlated to volume increase in putamen. Our results did not survive a Bonferroni correction and should be interpreted with caution. However, this plays well into hypotheses related to altered striatal structure and connectivity linked to symptom severity (Sarpal et al., 2015).

Basal Ganglia Volume Increases

Correlation between antipsychotic dose and volume changes in striatum is a subject of much debate (Roiz-Santianez et al., 2015; Huhtaniska et al., 2017) in part because separating the effect of disease and medication is inherently difficult. Vernon and colleagues found proof of concept in healthy rodent models, in which chronic (8 weeks) exposure to antipsychotics, but not other psychotropics (e.g., lithium) using clinically comparable dosing, leads to structural brain changes in naïve rats, including striatal enlargement (Vernon et al., 2012). In line with these data, we found a dose-dependent volume increase in the striatum after six weeks of treatment with an atypical APD with predominant $D_{2/3}$R blockade. It is still, however, unknown what causes this volume increase. Investigation has been made into the cellular components of the volume increase, but linking structural MR changes to their cellular correlates is challenging, and although antipsychotic exposure has been found to moderate microglial activation, neuronal dendritic spine density and astrocytes (Vernon et al., 2014; Cotel et al., 2015; Amato et al., 2017), no studies to date have linked any of these changes to striatal volume increase.

Another explanation for the volume increase could be augmented blood flow to striatum. This was found in a functional MRI study in healthy males after one dose of APD (Hawkins et al., 2018), as well as in patients treated with a mean of 27 days (Corson et al., 2002). Increased blood flow could possibly lead to an "apparent" volume change, but the difference in flow did not seem to have an impact on volume changes or brain structure investigated by Hawkins et al. (2018). Notably, Vernon et al. (2012) reported that striatal volume increases in rodents chronically exposed to haloperidol (8 weeks) was normalized after an equivalent period of drug washout (Vernon et al., 2012). The same tendencies of volume decrease in putamen after withdrawal of antipsychotics was reported from a small schizophrenia patient cohort (Boonstra et al., 2011). Finally, our previous functional MRI study on a subset of the current cohort showed changes in the task-related blood oxygen level-dependent activation in striatal regions after amisulpride treatment (Nielsen et al., 2012). Collectively, these data suggest that the effects of antipsychotics on brain structure, including the basal ganglia, are dynamic and potentially reversible.

It has long been discussed whether striatal volume changes are specific to so-called typical antipsychotics (Ebdrup et al., 2013), but our findings together with (Jorgensen et al., 2016) and (Glenthoj et al., 2007) show that this is not the case. The assumption may have been due to striatal volume decreases seen in patients treated with atypical clozapine (Garcia et al., 2015; Jorgensen et al., 2016) or quetiapine (Ebdrup et al., 2011). Both drugs, however, have low affinity toward dopamine D_2-like receptors, whereas typical antipsychotics have high affinities (Creese et al., 1976; Kusumi et al., 2015; Jorgensen et al., 2016). Dopamine D_2-like receptor knock-out mice also show striatal volume increases, mirroring the effects of chronic exposure (9 weeks) to different APDs (Guma et al., 2018, 2019). Notably, when chronically exposed to the same antipsychotics as wild-type mice, no additional basal ganglia volume increases were found. Taken together, these data strongly support that volume increases following antipsychotic exposure are mediated via the dopamine D_2-like receptor (Guma et al., 2018, 2019), rather than depending on the drug-class ('typical' vs. 'atypical' antipsychotic).

Dopamine Receptor Occupancy

We expected the volume increase to be predicted by striatal dopamine $D_{2/3}$R occupancy, because occupancy may be considered a more direct measure of effect than oral dose. This was not the case. We speculate that it may be due to several issues regarding SPECT imaging. First, receptor occupancy is calculated as the difference in available dopamine receptors between baseline and follow-up, and is therefore subjective to interfering factors such as changes in endogenous dopamine levels, which in turn affect dopamine receptor availability and occupancy. Second, as previously mentioned, studies also suggest a possible compensatory upregulation of D_2R in response to treatment (Oda et al., 2015; Yin et al., 2017), which again may affect occupancy, and a potentially decreased effect of drug dose in the long-term. Third, SPECT measurements are subjective to noise, which may have obscured a potential true correlation. Another issue to consider is that the multiple regression analysis assumes linearity, which might not be the case between volume increase and receptor occupancy. Lastly, a measure of

cumulative dose (although likely correlated to the mean dose) might have been a more accurate measure, but unfortunately not possible within our study. To our knowledge, only one study has done a similar investigation, also reporting no association between occupancy and volume increase (Di Sero et al., 2019). However, since amisulpride primarily acts by blocking $D_{2/3}R$, we argue that the observed striatal volumetric increases still can be mediated through occupancy, and we assign the negative association with occupancy to the aforementioned issues.

Strengths and Limitations

We conducted a clinically challenging prospective study on a cohort of antipsychotic-naïve first-episode schizophrenia patients and matched, healthy controls. Men and women were equally represented and confounding effects of previous exposure to antipsychotics could be ruled out. Amisulpride was chosen for treatment because of its selectivity toward dopamine $D_{2/3}R$, thereby excluding potential involvement of other neuroreceptors.

Because of the extensive examination program, the study included a limited sample of patients, and therefore selection bias cannot be ruled out. On the other hand, with a mean baseline PANSS total score of 78.5, the patients in our study may be considered moderately ill (Leucht et al., 2005). The limited number of patients restricted the degrees of freedom in the multivariate linear regression model, and therefore it was not possible to include- and control for further variables in our analyses. The effect of nicotine on basal ganglia volumes is unresolved (Van Haren et al., 2010; Das et al., 2012).

CONCLUSION

We found a dose-dependent striatal volume increase in antipsychotic-naïve schizophrenia patients in response to six weeks dopamine $D_{2/3}$ receptor blockade with an atypical antipsychotic compound. Thus, our findings contrast the notion that striatal volume increase is restricted to "typical" antipsychotics. However, the underlying mechanisms warrant further investigation. We found the striatal volume increase to be clinically relevant, since it appears correlated to a reduction in positive symptoms.

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

ETHICS STATEMENT

The study was conducted in accordance with the Helsinki declaration II, and approved by the Danish research ethics committee (H-D-2008-088), as well as the Danish Data Committee (RHP-2016-025, I-suite no. 05181). Clinical Trials.gov Identifier: NCT01154829. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

BG and BE conceived and designed the study. SW and MN collected the data. JR, CS, SW, ER, LJ, PA, and LP contributed with data processing and analysis tools. HA, JR, LBJ, ER and BE conducted the statistical analyses. HA and BE drafted the manuscript. HA, JR, CS, and SW particularly contributed with method section. AV contributed to the interpretation and discussion. All authors fulfill authorship criteria of the ICMJE by substantial contribution to the conception and design, to acquisition of data, or to the analysis and interpretation of the data contributed to manuscript revision, read and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnins.2020.00484/full#supplementary-material

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REVIEW
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Subcortical Dopamine and Cognition in Schizophrenia: Looking Beyond Psychosis in Preclinical Models

Kyna-Anne Conn[1], Thomas H. J. Burne[1,2] and James P. Kesby[1,3]†*

[1] Queensland Brain Institute, The University of Queensland, St Lucia, QLD, Australia, [2] Queensland Centre for Mental Health Research, Wacol, QLD, Australia, [3] QIMR Berghofer Medical Research Institute, Herston, QLD, Australia

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***Correspondence:**
James P. Kesby
j.kesby@uq.edu.au

†ORCID:
James P. Kesby
orcid.org/0000-0002-5814-8062

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Schizophrenia is characterized by positive, negative and cognitive symptoms. All current antipsychotic treatments feature dopamine-receptor antagonism that is relatively effective at addressing the psychotic (positive) symptoms of schizophrenia. However, there is no clear evidence that these medications improve the negative or cognitive symptoms, which are the greatest predictors of functional outcomes. One of the most robust pathophysiological observations in patients with schizophrenia is increased subcortical dopamine neurotransmission, primarily in the associative striatum. This brain area has an important role in a range of cognitive processes. Dopamine is also known to play a major part in regulating a number of cognitive functions impaired in schizophrenia but much of this research has been focused on cortical dopamine. Emerging research highlights the strong influence subcortical dopamine has on a range of cognitive domains, including attention, reward learning, goal-directed action and decision-making. Nonetheless, the precise role of the associative striatum in the cognitive impairments observed in schizophrenia remains poorly understood, presenting an opportunity to revisit its contribution to schizophrenia. Without a better understanding of the mechanisms underlying cognitive dysfunction, treatment development remains at a standstill. For this reason, improved preclinical animal models are needed if we are to understand the complex relationship between subcortical dopamine and cognition. A range of new techniques are facillitating the discrete manipulation of dopaminergic neurotransmission and measurements of cognitive performance, which can be investigated using a variety of sensitive translatable tasks. This has the potential to aid the successful incorporation of recent clinical research to address the lack of treatment strategies for cognitive symptoms in schizophrenia. This review will give an overview on the current state of research focused on subcortical dopamine and cognition in the context of schizophrenia research. We also discuss future strategies and approaches aimed at improving the translational outcomes for the treatment of cognitive deficits in schizophrenia.

Keywords: operant tasks, goal-directed behavior, reversal learning, rodent, translation

INTRODUCTION

The dopaminergic system is thought to be involved in both the etiology of schizophrenia and the regulation of a number of cognitive domains. Examination of the relationship between dopamine and cognition has largely focused on the role of cortical dopamine because the prefrontal cortex (PFC) in particular, is known to regulate a number of executive functions (Braver and Cohen, 1999; Orellana and Slachevsky, 2013). The role of subcortical dopamine systems and cognition in schizophrenia has received less attention. This is a consequence of the fact that the therapeutic action of all antipsychotic medication features the blockade of dopamine transmission, based on a number of molecular imaging studies (Seeman and Lee, 1975; Creese et al., 1976; Richtand et al., 2007; Howes et al., 2009a; Miller, 2009), but seemingly fails to improve cognitive impairments (Swartz et al., 2008). In some cases, antipsychotics may even exacerbate these deficits (Stip, 2006). While research into the pharmacodynamics of antipsychotic medication has advanced significantly, the relationship between dopamine and cognition is an important avenue to explore considering its potential influence on functional outcomes.

Currently, the overall consensus is that antipsychotic treatments seemingly have little to no effect on improving the cognitive symptoms, observed with both first- and second-generation antipsychotic medications (Hill et al., 2010; Frazier et al., 2012). Previously, a number of studies attempted to delineate the effects of both types of antipsychotics, with most suggesting second-generation antipsychotic administration had a more marked improvement in cognitive functioning (Lee et al., 1994; Meltzer and McGurk, 1999; Meltzer and Sumiyoshi, 2003; Sumiyoshi et al., 2013). While these studies reported significant improvements in cognition, the results were domain-specific and were confounded by issues such as duration of treatment and practice effects (Keefe et al., 2007). Other major inadequencies highlighted in these studies included poor experimental design, lack of appropriate control groups, insufficient washout periods, use of several medications and failure to account for dosage or duration of administration. It is also important to note that second-generation antipsychotics can induce serious metabolic side effects such as obesity and type II diabetes, illnesses that are strongly linked with cognitive impairments on their own (MacKenzie et al., 2018).

While most studies focus on cortical dopamine and cognition, subcortical regions such as the basal ganglia (a group of nuclei responsible for the coordination of a variety of motor functions) also have a primary role in complex cognitive processing (Middleton and Strick, 2000). Recent clinical evidence indicates that alterations in dopaminergic function in schizophrenia are primarily driven by changes in the associative striatum (Laruelle et al., 2005; Howes et al., 2009b; Kegeles et al., 2010). The associative striatum is heavily involved in a range of cognitive and decision-making processes and is anatomically defined as being part of the medial caudate and ventral putamen (Kesby et al., 2018). This suggests that understanding the role of subcortical dopamine in the cognitive deficits observed in schizophrenia may provide a better understanding of cognition in general, and identify novel approaches to treating these complex symptoms.

Cognitive dysfunction is thought to be one of the greatest predictors of functional outcomes in patients (Green et al., 2004). Impairments are observed in those at ultra-high-risk and with first-episode psychosis, as well as first-order relatives (Keshavan et al., 2010; Morales-Munoz et al., 2017; Lam et al., 2018). As cognitive symptoms present before the prodromal period and persist throughout the development of schizophrenia, cognitive impairment could be a biomarker for at-risk patients and a target for early prevention (Heinrichs and Zakzanis, 1998). Given the role of the associative striatum in decision-making processes, understanding the effects of altered dopamine function in this region on cognitive function is essential. For example, the associative striatum is engaged during two different components of decision-making, goal-directed action and reversal learning, both of which are impaired in schizophrenia (Redgrave et al., 2010; Morris et al., 2015). In this review, we will address the role of subcortical dopamine in the decision-making deficits observed in schizophrenia and discuss the evidence from preclinical studies which have sought to identify the underlying neural circuitry. We believe that a new approach is necessary to develop novel therapeutic targets to treat the cognitive symptoms of the disorder. To reduce the current translational gap between basic and clinical research, we suggest a shift in focus from categorical clinical measures to experimental psychopathology, i.e., elucidating the mechanisms that contribute to the etiology, exacerbation or maintenance of abnormal behavior (Forsyth and Zvolensky, 2001). With advances in genetic tools for use in animal models, manipulations of the neural circuitry and measurement of the consequent effects on cognition will also provide an avenue to improve translational outcomes.

SUBCORTICAL DOPAMINE ABNORMALITIES IN SCHIZOPHRENIA

Dopamine regulates a range of motor, limbic and cognitive functions. Based on evidence from a number of disorders (e.g., Parkinson's disease, attention deficit hyperactivity disorder, obsessive-compulsive disorder and schizophrenia), dysfunction of the dopamine system is thought to contribute to a range of neuropsychiatric symptoms. Dopamine neurons are located primarily in the midbrain, specifically in the substantia nigra and ventral tegmental area. Dopaminergic projections from the midbrain are divided into the mesocortical and mesolimbic systems (dopamine cells that arise in the ventral tegmental area and project to the PFC and limbic striatum, respectively), and the nigrostriatal system (dopamine cells that arise in the substantia nigra and project to the associative striatum). The associative striatum also receives rich connections from cortical areas including the dorsolateral PFC, anterior cingulate cortex (ACC), and orbitofrontal cortex (OFC), and has reciprocal thalamic connectivity (Haber, 2016). It is the associative striatum's role in gating incoming cortical input that makes it fundamental in maintaining the ability to adapt our choices to environmental changes (i.e., decision-making; Sharpe et al., 2018).

Alterations in dopamine neurotransmission have long been associated with the pathophysiology of schizophrenia. Early perturbations in the dopaminergic system were hypothesized to be a causative factor in the development of the disorder (Weinberger, 1987), driving both psychotic and cognitive symptoms (Laruelle et al., 2003). Recent evidence suggests that cortical dopamine function is decreased in schizophrenia (Slifstein et al., 2015), which may contribute to cognitive dysfunction. However, this does not preclude a role for subcortical dopamine systems. As such, this review will focus on subcortical dopamine systems and discuss cortical dopamine only when relevant to these cognitive processes (and to confirm when functional outcomes are insensitive to cortical dopamine changes).

In contrast with earlier hypotheses centered on mesolimbic dopamine (Laruelle et al., 2003), the current evidence supports a role for associative striatal dopamine dysfunction in schizophrenia. For example, a landmark study by Laruelle et al. (2005) demonstrated that the striatal localization of dopaminergic hyperfunction was primarily restricted to the associative, and not the limbic striatum. The results of this positron emission tomography (PET) imaging study challenged the widely accepted view that the therapeutic effects of antipsychotic drugs are derived from actions in the limbic striatum whereas actions in the associative striatum are responsible for the motoric side effects (Laruelle et al., 2005). It has subsequently been shown that dopaminergic hyperactivity is present before the onset of the disorder, is predominately found in the associative striatum, and increases in those who transition to schizophrenia (Howes et al., 2009b). Dopamine hyperactivity also correlates with the severity of symptoms, as well as cognitive dysfunction (Howes et al., 2009b). In addition, elevated dopamine synthesis capacity was seen in the midbrain origins of dopamine neurons as well as their striatal terminals, with this finding also being linked to symptom severity in the disorder (Howes et al., 2013). Together, these studies support the notion that subcortical dopamine dysfunction and, in particular, dopaminergic alterations in the associative striatum, may be the main impetus for multiple symptoms of schizophrenia.

THE ROLE OF THE ASSOCIATIVE STRIATUM IN COGNITIVE DYSFUNCTION

Cognitive dysfunction in schizophrenia spans a range of domains, including working memory, verbal speed, attention and executive function, and greatly impacts on patients' lives (Green et al., 2000; Fujii et al., 2004; Green et al., 2004). Widespread functional and structural changes are observed in most cortical areas in schizophrenia (Brugger and Howes, 2017; Li et al., 2017) and undoubtedly contribute to cognitive dysfunction. However, subcortical dopamine systems also play specific roles in regulating multiple aspects of cognitive performance. Therefore, cognitive deficits driven by alterations in subcortical dopamine systems are likely located in substructures that feature dense cortical connectivity (Nieoullon, 2002), such as the associative striatum.

A number of clinical research findings support the involvement of the associative striatum in the cognitive deficits observed in schizophrenia patients. For example, structural changes in the size of the associative striatum in those with schizophrenia correlate with performance in cognitive tasks assessing executive functions (Levitt et al., 2013). Decreased striatal dopamine synthesis capacity, in patients with symptomatic remission of positive symptoms, mediates a range of cognitive symptoms (Avram et al., 2019). Changes in associative striatal activation during goal-directed behavior have also been shown to underlie performance deficits in schizophrenia (Morris et al., 2015). These examples support the established understanding that the associative striatum contributes directly to decision-making, specifically in action selection and initiation, integrating sensorimotor, cognitive and motivational information (Balleine et al., 2007). These processes are critical for instrumental learning and the ability to adapt behavior in the face of changing information. When understanding the role of the associative striatum in cognition, we must also consider the complexity of subcortical dopamine signaling more generally. The mesolimbic dopamine system encodes signals that allow the prediction of reward outcomes and are thought to mediate reward-related adaptation and learning (Gradin et al., 2011; Hauser et al., 2017). Limbic dopamine therefore impacts autoshaping behavior as well as reward learning processes, such as probabilistic learning (Markou et al., 2013), and is thought to contribute to motivational and reward deficits in schizophrenia (Der-Avakian et al., 2016).

Multiple studies have observed the absence of a relationship between antipsychotic use and cognitive improvement in those with schizophrenia, suggesting that dopamine D_2 receptor signaling does not account for these findings *per se*. However, it is known that blockade of D_2 receptors in the striatum is a major factor in causing acute drug-induced extrapyramidal side effects (EPS). EPS can further complicate the relationship between antipsychotic medication and cognitive function (Meltzer et al., 1999). The extrapyramidal system, as used in anatomy, defines part of the motor system network (other parts of the motor cortex reach their targets via the pyramidal tract). Thus, symptoms of EPS include dystonia, akathisia, parkinsonism, bradykinesia, tremor, and tardive dyskinesia, and antipsychotic treatment is often discontinued due to these intolerable side effects. The main distinguishing features between first- and second-generation antipsychotics is that second-generation antipsychotics tend to have a more potent blockade of serotonin receptors (5HT-2A) and weak blockade of D_2 receptors, which results in lower rates of EPS (Meltzer et al., 1999). So even though all efficacious antipsychotic medications target the aforementioned dopaminergic abnormality in the striatum, there is little evidence to support improvements in cognition (Miller, 2009).

Both the Clinical Antipsychotic Trials of Intervention Effectiveness (CATIE) and the European First Episode Schizophrenia Trial (EUFEST) failed to show any effectiveness of second-generation antipsychotics in the treatment of cognitive symptoms in schizophrenia (Keefe et al., 2007;

Davidson et al., 2009). These trials encompassed a large sample size with features reflective of the general schizophrenia population, showing that antipsychotic drugs are very similar in their action across chemical classes with these similarities extending to their effects on cognition. Higher lifetime dose-years were significantly associated with poorer cognitive performance and the effects of first- and second-generation antipsychotics did not differ (Husa et al., 2017). So, the superiority of second-generation antipsychotics was also called into question during these trials, with mixed results (Desamericq et al., 2014; Nielsen et al., 2015). Most importantly, the effect size for any cognitive improvement observed in these trials was small with spurious clinical significance (Heinrichs, 2007; Keefe et al., 2007).

Furthermore, to add to the complexity of understanding this relationship, there is some evidence suggesting that antipsychotic medication may worsen cognitive dysfunction. The therapeutic effects of these medications are known to treat the psychotic symptoms via a blockade of the D_2 receptors and a study that stemmed from the CATIE trials attempted to elucidate the effects that this blockade had on neurocognitive performance (Creese et al., 1976). By evaluating the impact of estimated D_2 receptor occupancy with antipsychotic drugs on cognitive performance, they were able to show that depending on the level of occupancy, these medications may increase the risk of EPS and also increase the chance of worsening cognitive impairment (Sakurai et al., 2013). This has been shown to impact on specific cognitive domains as well, for example, excessive D_2 receptor occupancy correlates with attention deficit in late-life schizophrenia and a decrease in working memory performance (Uchida et al., 2009; Kim et al., 2013). Furthermore, in first episode psychosis patients, neuropsychological impairments are seemingly related to the pharmacodynamics and antipsychotic medication dosing regimens, specifically for verbal memory and motor function (Baitz et al., 2012).

Other effects of current antipsychotic treatments include alterations in functional connectivity in patients with long-term use (Bolding et al., 2012). This can be problematic when dysconnectivity in schizophrenia is considered to be a phenotype that may be due to either degenerative, developmental or genetic mechanisms (Meyer-Lindenberg and Weinberger, 2006). Another possible reason for the inefficacy of antipsychotic medication not alleviating cognitive symptoms is the potential role of the D_1 receptor system, and not the D_2 receptor system, contributing to cognitive dysfunction. It has been shown in a PET imaging study that binding of radioligand to D_1 receptors was reduced in the PFC of drug-free patients with schizophrenia in comparison to healthy controls, and this correlated with severity of cognitive symptoms and performance on a set shifting task measuring cognitive flexibility (Okubo et al., 1997).

Seemingly, most research on cognition in schizophrenia has focused on executive functions. This may be problematic considering that executive functions include any process that relies on the PFC. The importance of cortico-striatal circuits, and the associative striatum in particular, suggests that the prevailing presumption that the PFC is the sole contributor to deficits in executive function, may have overlooked an important avenue for better understanding these deficits. Since it is clear that dopamine plays a role in both cognition and the therapeutic action of current drugs, it is important to understand how dopamine alterations in the brain may lead to cognitive dysfunction. The recent evidence supporting subcortical dopamine's definitive role in the pathogenesis of the disorder may be key to predicting outcomes and responses to antipsychotic treatment (Kaar et al., 2019).

The Functional Neuroanatomy of the Striatum

The striatum is involved in the coordination of multiple aspects of cognition, including motor- and action-planning, decision-making, motivation, reinforcement and reward perception (Balleine et al., 2007). However, the striatum can be parcellated into functional subregions which include the aforementioned associative and limbic, as well as the sensorimotor striatum (Heilbronner et al., 2016; Kesby et al., 2018). In rodents, these approximately correlate anatomically with the dorsomedial, ventral and dorsolateral striatum, respectively (see **Table 1** for more detailed anatomical descriptions). In this current review, we will primarily use the functional names (i.e., associative, sensorimotor and limbic), and in the case of experimental manipulations, classified only by their neuroanatomical description (dorsomedial etc.), we will include the equivalent functional nomenclature in parenthesis. Each functional division of the striatum has a differing role in features of cognitive and reward processing. The associative learning of stimuli (i.e., formation of action-outcome associations) and action selection between competing alternatives is dependent on associative striatal function. The process of habit formation is thought to be dependent on activity in the sensorimotor striatum, whereas the motivational modulation of motor behavior is dependent on the limbic striatum (Liljeholm and O'Doherty, 2012). Generalized hypotheses of information flow during decision-making processes suggest that the limbic striatum encodes motivational variables, which are used by cortical subregions and the associative striatum for action selection and implementation. After sufficient training/repetition, this information is encoded by the sensorimotor striatum into a habit-based response (Pessiglione et al., 2007; Schmidt et al., 2012).

The associative striatum plays an important role in instrumental learning, whereby reinforcement or punishment is used to increase or decrease the probability that a behavior will occur again in the future (Hall, 2002; Day et al., 2007). Instrumental learning can be goal-directed, which is a highly

TABLE 1 | Comparative striatal functional and neuroanatomical nomenclature.

FUNCTIONAL REGION	HUMAN	RODENT
ASSOCIATIVE	Medial caudate Ventral putamen	Dorsomedial striatum/caudate putamen
SENSORIMOTOR	Dorsolateral caudate Dorsolateral putamen	Dorsolateral striatum/caudate putamen
LIMBIC	Ventral striatum Nucleus accumbens	Ventral striatum Nucleus accumbens

adaptive form of learning that requires the recruitment and integration of information from higher cortical regions such as the PFC, ACC and OFC. Essentially, the associative striatum accumulates this information to direct action-selection and decision-making (Yartsev et al., 2018). This is of relevance to schizophrenia, as it has been shown that corticostriatal control of goal-directed action is impaired. Specifically, those with schizophrenia are unable to integrate action-outcome learning to guide choice, a finding which has been shown to correlate with a reduction in associative striatal activity (Morris et al., 2015). The role of the limbic striatum is centered on motivational behavior, as evidenced by its involvement in the ability to predict the outcome of rewards (Schultz, 2000; Knutson et al., 2001; Tanaka et al., 2004). Not surprisingly, reduced activation in the ventral striatum has been correlated with the severity of negative symptoms in medication-free patients and in the response to cues predicting the outcome of rewards (Juckel et al., 2006; Nielsen et al., 2012). While research has predominantly focused on the role of the limbic striatum in the pathogenesis schizophrenia, little is known about the role of the associative striatum in the aberrant encoding of cortical decision-making processes observed in patients (Brunelin et al., 2013; Strauss et al., 2014).

REDUCING THE TRANSLATIONAL GAP WITH IMPROVED PRECLINICAL TESTS

Although our knowledge of brain circuitry and schizophrenia neurobiology has advanced considerably in the past decade, drug development is at a standstill. Better translation between preclinical and clinical studies is necessary in order to identify novel treatment approaches (Pratt et al., 2012; Kesby et al., 2018). The lack of cognitive improvement in response to antipsychotic medication has led to a shift in research, focusing more on the development of drugs to improve cognition in those with schizophrenia (Floresco et al., 2005; Young and Geyer, 2015). Unfortunately, drugs that appear to improve performance in animal models often do not show the same positive effects in the clinical population (Castner et al., 2000; George et al., 2007). Consequently, a number of initiatives have been established to examine dimensions of human behavior (e.g., attention, reward learning, memory) in order to facilitate novel research approaches to understand how structure and function of the brain impact neuropsychiatric impairments (Marder and Fenton, 2004; Carter and Barch, 2007; Insel, 2014). Importantly, these approaches have led to the development of comparative preclinical cognitive protocols and recommendations to improve the translational capacity in schizophrenia research (Young et al., 2009; Moore et al., 2013; Nikiforuk, 2018).

The combined use of sensitive and highly translatable cognitive tasks in combination with manipulations of the brain, relevant to schizophrenia, will help to reduce the current translational gap (Carandini and Churchland, 2013; Kesby et al., 2015). A range of pharmacological and genetic tools are now available in preclinical research that will allow us to elucidate the brain regions and molecular mechanisms behind some of the cognitive deficits in schizophrenia. As the associative striatum is involved in goal-directed behavior and reversal learning, both of which are impaired in schizophrenia, understanding the ability to select actions that guide choices is integral to understanding the link between striatal dopamine, cognition and schizophrenia (Kesby et al., 2018; McCutcheon et al., 2019).

EXAMINING THE ROLE OF THE ASSOCIATIVE STRIATUM IN GOAL-DIRECTED AND FLEXIBLE DECISION-MAKING

We have recently advocated a move in research focus to behavioral phenotypes that are consistent with the underlying neuroanatomical and biological features of schizophrenia (Kesby et al., 2018). Based on emerging evidence supporting the role of the associative striatum in this disorder, it is clear that the cognitive domains of associative learning, goal-directed action and reversal learning are key targets for further investigation, and will be the focus for the rest of this review. The rationale is that the striatum is heavily involved with the selection of a motor plan (goal-directed action) by integrating the relationship between outcomes and their relative values (associative learning), and is how an animal can make a choice or adapt its behavior (Cox and Witten, 2019). These processes are encompassed under the umbrella of "decision-making," a core but complex part of daily functioning that requires the use of higher-order cortical areas and subcortical brain structures such as the striatum (Goulet-Kennedy et al., 2016).

In terms of circuitry, the striatum is situated within multiple cortico-subcortical loops, receiving input from the cortex and thalamus, with reciprocal outputs to the cortex via the thalamus, making striatal function an integral part of decision-making (Redgrave et al., 2010). A number of cognitive processes are required to make a decision, including perception, attention, working memory, associative learning, long-term memory, adaptation and planning, before a choice or action selection is made (Young and Geyer, 2015). There are also a variety of tasks that are dependent on subcortical regions, with these mainly relating to decision-making based on action-outcome learning and reward feedback (Carandini and Churchland, 2013). It should be noted that associative learning is an integral component of both goal-directed action and reversal learning. By focusing on the aforementioned cognitive processes, we may be able to reveal behavioral responses that are consistent with the altered pathophysiological features of schizophrenia.

Goal-Directed Behavior in Schizophrenia

Goal-directed behavior is wide ranging and allows us to understand the complex process of decision-making. The main associative account of goal-directed action is a response-outcome account that begins with the consideration of possible response alternatives and is followed by the evaluation of their consequences. This is underpinned by the formation of action-outcome contingencies via associative learning processes and has been extensively examined in rodents and humans alike (Friedel

et al., 2014). A number of studies have proposed models for how goal-directed behavior is impacted in schizophrenia (Frith, 2000). One model in particular suggests that negative symptoms are associated with a deficit in action initiation and positive symptoms are associated with deficits in cognitive control, with disorganized symptoms associated with deficits in contextual information integration (Rinaldi and Lefebvre, 2016). In a study investigating goal-directed planning and action in a virtual environment, impairments in these processes were observed in those with schizophrenia (Siddiqui et al., 2019). In the context of a simulated everyday errands task, people with schizophrenia exhibited both a reduced capacity and efficiency to complete the task, indicating that goal-directed behavioral impairments can manifest as diminished real-world motivational and functional behavior. Understanding the interaction between schizophrenia pathophysiology and goal-directed behavior may therefore be essential for improving functional outcomes in patients.

Imaging studies in human participants have helped to establish the brain areas and circuits that mediate goal-directed behavior. For example, enhanced medial PFC and posterior cingulate cortex activity has been observed during action selection in the training phase of a goal-directed behavioral task (Eryilmaz et al., 2017). In the same study, early phases of associative learning, i.e., goal-directed learning, were associated with increased activation in the frontoparietal control network (which serves to instantiate new task states by flexibly interacting with other control networks) and the caudate (which encompasses most of the associative striatum). In contrast, late phase learning, i.e., habit formation, showed activation of default mode regions that are more active during times of rest as opposed to times of cognitive activity.

When examining the neural substrates of action-outcome contingency learning, a number of studies have pointed to the role of the medial PFC and caudate, as activity in these regions varies based on the probability of an action being followed by an outcome (Tanaka et al., 2008; Liljeholm et al., 2011). Furthermore, subregions of the PFC appear to have specific roles in encoding the value of outcomes. For example, the dorsolateral PFC has been shown to mediate action-value comparisons and modulate action control (Morris et al., 2014), whereas, the ventromedial PFC is important for tracking post-choice values in order to update action values accordingly (Valentin et al., 2007; Tanaka et al., 2008; Morris et al., 2014). It has been suggested that connections between the dorsolateral PFC, OFC and caudate work as a circuit to compare action values for selection and, once a choice is made, update the action values (Morris et al., 2014). Another frontal cortical region implicated in goal-directed action is the ACC, with activity in this region reflecting the use of reward-type information to guide action selection (Noonan et al., 2011). This conclusion is supported by computational modeling, as the ACC has also been identified as being responsible for tracking the progression of goal-directed action sequences (Holroyd and Yeung, 2012; Shahnazian and Holroyd, 2018). This has direct implications for schizophrenia where there is abnormal functional connectivity with multiple brain regions, in particular the caudate and putamen (Yan et al., 2012), as seen in **Figure 1**. The role of the thalamus in subcortical integration has also been argued to be a key mechanism for maintaining and updating internal representations (Wolff and Vann, 2019).

In schizophrenia, caudate function appears to be central to deficits in goal-directed action. The outcome-specific devaluation task allows for the separate assessment of limbic and associative striatal involvement in decision-making, and is specific to goal-directed action because habitual behavior is resistant to outcome devaluation (Rossi and Yin, 2012). Using this task, it has been found that people with schizophrenia are capable of understanding changes in the value of outcomes after devaluation, but are unable to update their action selections accordingly (Morris et al., 2015). These behavioral deficits are driven by a decrease in caudate activity during valued actions, but not with changes in medial PFC activity, compared with healthy subjects. In a follow-up study, a contingency degradation task was used to further elucidate whether this impairment exists alongside habit formation or an impairment in instrumental learning (Morris et al., 2018). In this modified task, one of the action-outcome contingencies was degraded by delivering the outcome in the absence of an action. Those with schizophrenia were able to learn the best action to obtain rewards, but after contingency degradation, patients were unable to determine the more causal action. This suggests a core impairment in the learning of action-outcome associations, whereby people with schizophrenia are unable to encode the causal consequence of an action. Therefore, this impairment in goal-directed action is not driven by habit formation or an inability for instrumental learning but rather by an associative learning impairment.

Preclinical Evidence of a Role for Dopamine and the Associative Striatum in Goal-Directed Behavior

A range of tools have been applied to manipulate the circuitry involved in goal-directed behavior in animal models (Rescorla, 1992; Johnson et al., 2005; Matamales et al., 2016). It is important for established operant tasks of relevance to schizophrenia to be used when assessing decision-making in rodents (Markou et al., 2013; Morris et al., 2015; Young and Markou, 2015; Der-Avakian et al., 2016). The neural basis of goal-directed action in rodents has been extensively examined, and suggests a complex convergence of multiple circuits that constitute the cortico-striatal thalamo-cortical feedback loop (Balleine et al., 2009), as illustrated in **Figure 2**. As described in schizophrenia patients, deficits in goal-directed action are seemingly driven by pathology in either the converging inputs to the associative striatum or their encoding within this region. Given that the associative striatum is the entry point for the basal ganglia, it is clear that this region has a highly regulatory role in action selection, planning and decision-making (Balleine and O'Doherty, 2010).

In rats, two components of cortico-striatal circuitry have been identified as being critical for goal-directed learning, the prelimbic cortex and the dorsomedial striatum (associative) which receives its input from the former region (Groenewegen et al., 1990). Using either outcome devaluation or contingency degradation, it has been shown that lesions of either of the aforementioned regions in rats impair the acquisition of

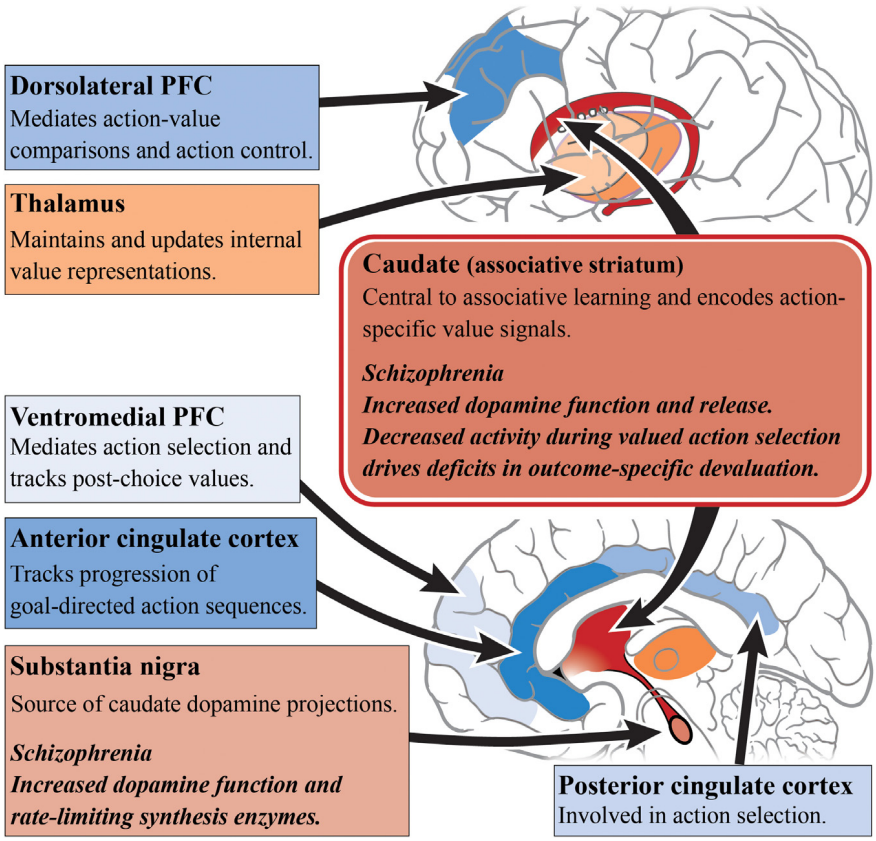

FIGURE 1 | Goal-directed action and schizophrenia. A simplified diagram of the circuitry, subcortical (reds) and cortical regions (blues), and their roles in goal-directed action. Impaired dopamine function and release in the caudate/associative striatum (dark red) of patients living with schizophrenia may be the cause of impairments in goal-directed behavior. Increased dopamine function in the associative striatum may directly alter associative learning and the understanding of action-specific values. Alternatively, increased dopamine function may impair the integration of incoming cortical inputs. In particular, subregions of the prefrontal cortex have differing roles in the encoding of outcome values. Other cortical regions such as the anterior cingulate cortex and posterior cingulate cortex have also shown to have differing roles in action selection. *PFC,* prefrontal cortex.

associative learning, causing deficits in goal-directed action (Balleine and Dickinson, 1998; Corbit and Balleine, 2003; Yin et al., 2005b). Bilaterally disconnecting the prelimbic to associative striatal pathway in rats was shown to disrupt the acquisition of goal-directed actions, further supporting the functional roles of these regions in a corticostriatal circuit to mediate goal-directed behavior (Hart et al., 2018). Single-unit recordings in primates also have also demonstrated action-specific value signals in the dorsal striatum (associative), confirming the role of this region in the expression of goal-directed action as well as its aforementioned role in learning (Samejima et al., 2005; Lau and Glimcher, 2008). N-methyl-D-aspartate receptors in the posterior dorsomedial striatum (associative) are also important for encoding action-outcome associations during instrumental conditioning (Yin et al., 2005a).

The thalamostriatal pathway, linking the parafascicular thalamus with cholinergic interneurons in the posterior dorsomedial striatum (associative), is responsible for reducing interference between new and existing goal-directed learning (Bradfield et al., 2013). Moreover, the thalamocortical pathway is responsible for integrating current causal relationships (Alcaraz et al., 2018). Therefore, the preclinical evidence implicating the dorsomedial striatum (associative), and in particular the posterior portion, in goal-directed action supports the findings in humans suggesting a role for the caudate (associative striatum) in encoding action-outcome associations and establishing causal relationships (Balleine and O'Doherty, 2010). The infralimbic cortex has also been implicated in goal-directed action. Infralimbic inactivation in rats exhibiting habitual behavior (i.e., overtrained rats) saw reinstatement of sensitivity to outcome devaluation, suggesting heightened activity may impair goal-directed behavior (Coutureau and Killcross, 2003). In addition, neurons in the ACC have been shown to map anticipated effort and reward to their associated action sequences, further supporting the aforementioned studies in humans (Cowen et al., 2012).

In the context of dopamine systems, subcortical dopamine appears more relevant than cortical dopamine in the devaluation task. For example, dopamine function in the PFC is not necessary for the acquisition of instrumental learning, and although animals with dopaminergic lesions of the prelimbic cortex fail to adapt their actions to changes in contingency, their

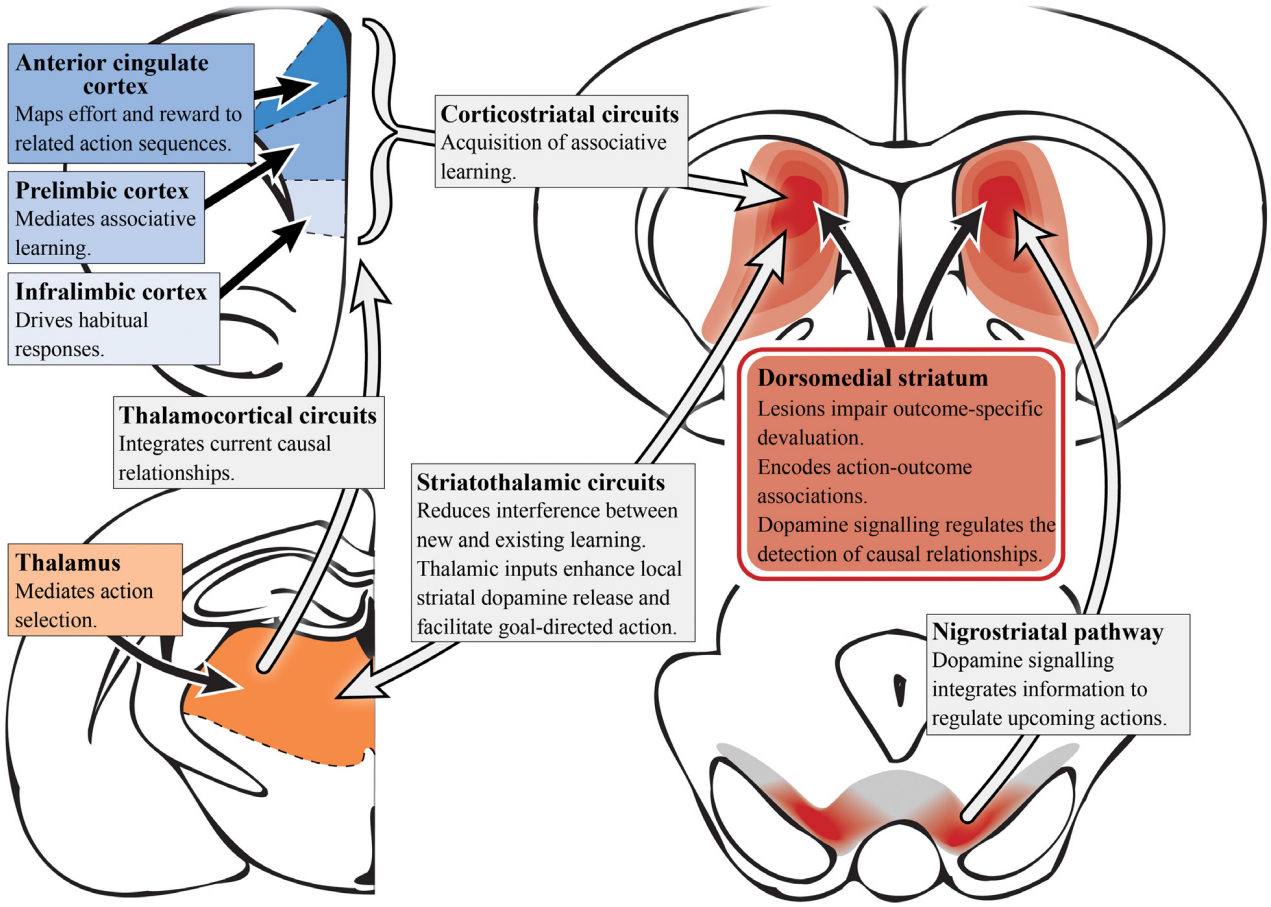

FIGURE 2 | Goal-directed action and dopamine (preclinical studies). A simplified summary of the preclinical research on subcortical (reds) and cortical (blues) regions involved in goal-directed behavior with potential relevance to schizophrenia. Dopamine signaling driven by the nigrostriatal pathway projecting into the dorsomedial/associative striatum (dark red) is essential for associative learning. Aberrant functioning in the associative striatum could impact goal-directed behavior via multiple circuits. In particular, integrating and encoding inputs from cortical regions such as the anterior cingulate cortex, prelimbic and infralimbic cortices, which have distinct roles in terms of action selection, associative learning and habit formation. However, it is the corticostriatal circuit as a whole that is responsible for the acquisition of action-outcome associations. The thalamus also has an important role in mediating action selection with the thalamocortical circuit integrating causal relationships. Striatothalamic circuitry is important to managing learning in goal-directed behavior and also has a role in regulating striatal dopamine release.

responses remain sensitive to outcome devaluation (Naneix et al., 2009). Moreover, dopamine depletion of the prelimbic cortex modulates the instrumental lever pressing rate but does not have a role in instrumental conditioning *per se* (Lex and Hauber, 2010). In contrast, studies on dorsomedial striatum (associative) dopamine signaling have shown no role in instrumental lever pressing but instead, the detection of causal relationships between an action and its outcome, i.e., associative learning (Lex and Hauber, 2010). It has also been demonstrated that the glutamatergic projections from the thalamus to the dorsal striatum (associative), activate striatal cholinergic interneurons to enhance local striatal dopamine release and improve goal-directed behavior (Cover et al., 2019). Stimulation of the substantia nigra induces striatal long-term potentiation and may positively reinforce the learning of behavior via dopamine D1 receptor-dependent potentiation of cortical inputs to the striatum (Reynolds et al., 2001; Wickens et al., 2007). Nigrostriatal dopamine signaling seemingly integrates diverse information required for the regulation of upcoming actions, as changes in the firing rate of nigrostriatal dopamine neurons, as well as dopamine signaling in the dorsal striatum (associative), have been found to accompany action selection (Howard et al., 2017). This dopaminergic signaling profile was found to be specific to behavioral choice and didn't reflect reward prediction error, timing or value as single factors alone (Howard et al., 2017).

The role of dopamine in the dorsomedial striatum (associative) elucidated in these preclinical studies converges with the outcomes observed in schizophrenia, i.e., impaired associative learning and an inability to encode the causal consequences of their actions (Morris et al., 2018). This highlights the associative striatum as a prime target underlying impaired cognitive function in schizophrenia (Griffiths et al., 2014). This could in turn facilitate, or act in addition to, the corticostriatal dysconnectivity observed in schizophrenia, including reduced connectivity between the putamen and the medial PFC (Karcher et al., 2019), and large-scale disturbances in thalamo-cortical

connectivity (Anticevic et al., 2014). Importantly, the available translational devaluation task provides a direct avenue to dissect the role of specific circuitry in preclinical models and explore targets that may rescue cognitive performance.

Cognitive Flexibility in Schizophrenia

Decision-making behavior can also be controlled dynamically; a response or action can be selected when the outcome is desired, and equally, it can be withheld when the outcome is unwanted (Furlong and Corbit, 2018). This process is known as cognitive flexibility, an executive function that is underpinned by characteristics such as the formation of/shifting between attentional sets, response inhibition, perseveration and reversal of stimulus-response or action-outcome associations (i.e., reversal learning). Since cognitive flexibility is made up of several component processes, it has been shown that these differing forms of cognitive flexibility are governed by divergent forms of underlying neurocircuitry (Eslinger and Grattan, 1993). In humans and animal models, attentional set-shifting depends largely on the role of the medial PFC and ACC, as these regions are critical for flexibly shifting from one strategy to another (Birrell and Brown, 2000; Bissonette et al., 2013; Heisler et al., 2015). Response inhibition requires the recruitment of the dorsolateral PFC, ventrolateral PFC, ACC and the parietal cortex (Blasi et al., 2006; Hardung et al., 2017). It has also been shown that dorsal striatal D_2-like receptor function mediates response inhibition in corticostriatal neural circuitry in humans (Ghahremani et al., 2012). Poor performance on an attentional set-shifting task has been observed in patients with schizophrenia due to a failure of inhibitory control and/or perseverative errors (Morice, 1990). Attentional set-shifting is also dependent on working memory, another cognitive process that relies on cortical function and is impaired in schizophrenia (Pantelis et al., 2009).

In contrast, reversal learning appears to be particularly sensitive to associative striatal function (Ragozzino, 2007; Braun and Hauber, 2011). However, as seen in studies in human and non-human primates, rules or strategies adopted during reversal learning may eventually dominate a response, advance too quickly and stifle learning assessments (Murray and Gaffan, 2006). As a result, reversal learning is primarily assessed using a probabilistic reversal learning task, which is used to reduce the ability to operate a basic strategy and to force the participant to apply accumulated evidence of previous actions and outcomes to guide choice (Hampton et al., 2007; Walton et al., 2010). This task examines flexible decision-making in the face of misleading feedback and the ability to rapidly shift responses based on positive or negative feedback (the increase or decrease in the likelihood of receiving a reward) when reward contingencies are reversed (Cools et al., 2002).

The striatum has been implicated in reversal learning based on a number of functional imaging studies of reversal learning, with recruitment of both the ventral (limbic) and dorsal (associative) striatum being observed, as shown in **Figure 3** (Rogers et al., 2000; Cools et al., 2002; Clarke et al., 2008; Tanaka et al., 2008). In the caudate (associative) specifically, dopamine receptor availability after methylphenidate administration accompanied drug-induced changes in reversal learning performance, i.e., larger increases in dopamine release corresponded with more reversal learning errors (Clatworthy et al., 2009). This is vital to our understanding of reversal learning impairments in schizophrenia as increased dopamine neurotransmission from the substantia nigra to the associative striatum is now considered a hallmark of the disorder. The nigrostriatal dopaminergic system has also been implicated in reversal learning, given that patients with Parkinson's disease (where the neuropathology of the disease involves the degeneration of dopamine cells in the substantia nigra) exhibit a compromised ability to adapt to the reward contingency reversal (Peterson et al., 2009).

A host of cortical subregions, including the lateral OFC, inferior frontal gyrus, the dorsomedial PFC, the dorsolateral PFC and the posterior parietal cortex, have also been implicated in aspects of reversal learning performance (O'Doherty et al., 2001; Cools et al., 2002; Glascher et al., 2009; Mitchell et al., 2009). The OFC is particularly important in reversal learning as increased activity has been observed while participants perform reversals (as opposed to during the initial discrimination) which indicates the OFC's role in the reformation of established associations (Ghahremani et al., 2010). People with OFC lesions also exhibit reversal learning deficits, suggesting an inability to learn from reward feedback and thereby indicating that the OFC is important for monitoring changes in reward value to guide behavior (Hornak et al., 2004).

A number of studies focusing on reversal learning have reported that limbic striatal dysfunction is tightly linked with specific reinforcement-driven reversal learning deficits observed in schizophrenia, most likely due to the interference with reward prediction error processing (Schlagenhauf et al., 2014). Some studies suggests that there are preliminary results in schizophrenia patients showing abnormal prediction error signaling, however, these findings remain inconsistent (Ermakova et al., 2018). Those with schizophrenia are able to acquire the initial probabilistic contingencies but achieve significantly fewer reversals than healthy matched controls, suggesting that OFC dysfunction is a prevalent aspect of the pathophysiology (Waltz and Gold, 2007). Therefore, there is a deficit in the ability to use this feedback and the prediction of reward outcome, in order to update internal reward value representations and guide choice (Waltz and Gold, 2007; Reddy et al., 2016). Interestingly, in a study examining probabilistic learning alone, no differences in limbic striatal reward-prediction-error activation were demonstrated between medicated patients and healthy controls, indicating that deficits in probabilistic learning in the disorder, may instead stem from processes outside of the limbic striatum (Culbreth et al., 2016b).

In a version of a probabilistic reversal learning task, schizophrenia patients achieved significantly fewer reversals than healthy controls and also showed a decrease in Win-Stay/Lose-Shift decision-making behavior (i.e., a decrease in the use of "winning" strategies) (Culbreth et al., 2016a). Furthermore, this behavioral deficit was linked with reduced activation (in comparison to controls) in striatal regions, and brain regions associated with cognitive control (Culbreth et al., 2016a). Studies in people experiencing first-episode psychosis have shown that there are both reinforcement and reversal learning deficits

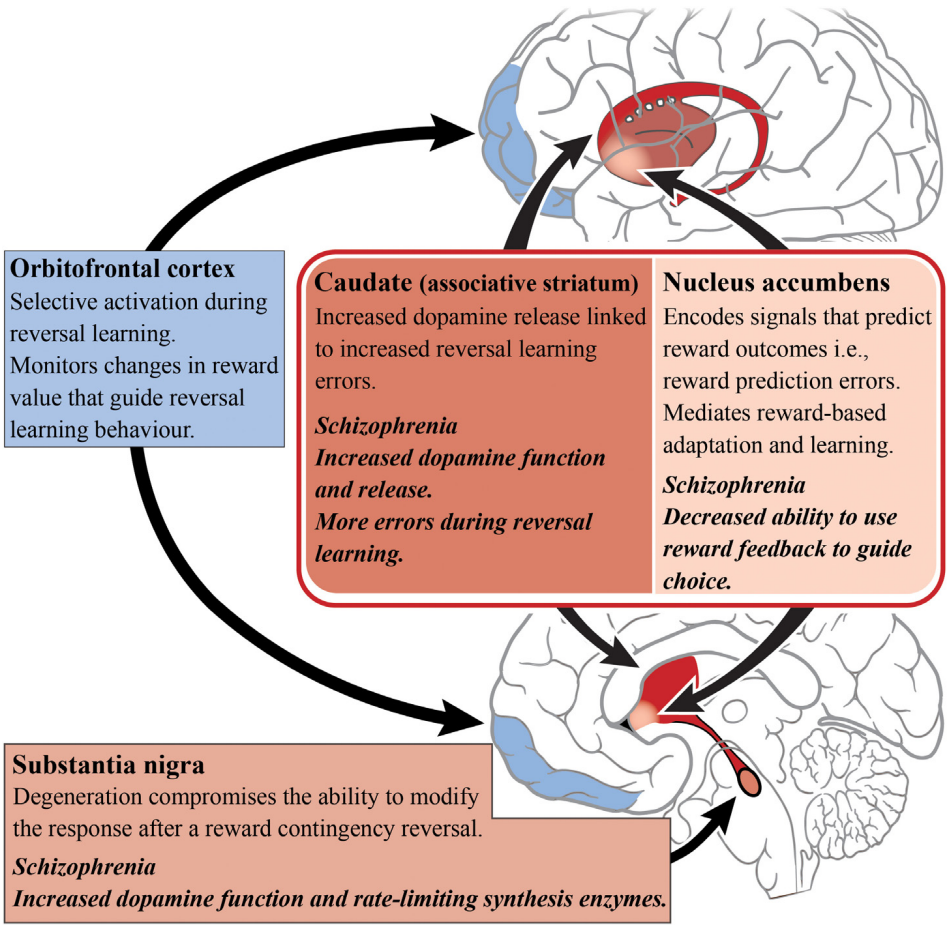

FIGURE 3 | Reversal learning and schizophrenia. A simplified diagram of the circuitry, subcortical (reds) and cortical regions (blues), and their roles in reversal learning. Impaired dopamine function and release in the caudate/associative striatum (dark red) of patients living with schizophrenia may be the cause of reversal learning impairments. The dopamine enriched substantia nigra is involved in modifying responding to changes in reward contingencies and dopamine release in the caudate is related to reversal learning errors. In contrast, the nucleus accumbens has a role in predicting reward outcome. The orbitofrontal cortex is responsible for monitoring changes in reward value that guide reversal learning behavior.

(Murray et al., 2008). These deficits in reversal learning are observed even when discrimination learning and attentional set-shifting remained intact, suggesting reversal learning may be a promising target for translational studies in early-stage schizophrenia (Leeson et al., 2009; McKirdy et al., 2009).

Preclinical Evidence Dissecting the Circuitry Involved in Reversal Learning

Development of a translational task to examine probabilistic reversal learning in rodents has emerged in recent years, allowing researchers to probe the underlying neural circuitry involved (Bari et al., 2010; Ineichen et al., 2012; Dalton et al., 2016), as seen in **Figure 4**. Preclinical evidence supports a role for the associative striatum in action selection and for the OFC as an important cortical area for transforming affective feedback to behavioral adjustment (Xue et al., 2013; Izquierdo et al., 2017). Lesions of the dorsomedial striatum (associative) have been shown to impair a range of reversal learning paradigms in animals highlighting its complex role in managing cortical inputs to select and maintain particular computational strategies. For example, dorsomedial striatum (associative) lesions in monkeys produce a reversal learning phenotype similar to that observed after OFC lesions (Clarke et al., 2008; Castane et al., 2010), suggesting that the integration of OFC inputs can be selectively perturbed in the associative striatum. Lesions of the dorsomedial striatum (associative) in rats do not effect initial discrimination learning (Featherstone and McDonald, 2004; Ragozzino, 2007) but appear to affect the maintenance and execution of a selected strategy after a reversal (Ragozzino, 2007). Moreover, these lesions do not impact effort-related reward processes (Braun and Hauber, 2011), suggesting a specific role of the associative striatum in the computation of the reversal learning strategy rather than in the motivation toward a goal.

In contrast to the associative striatum, the role of the limbic striatum is more contentious. Lesions of the nucleus accumbens (limbic) in non-human primates disrupt spatial reversal learning but has no effect with visual cues, while in rats similar lesions

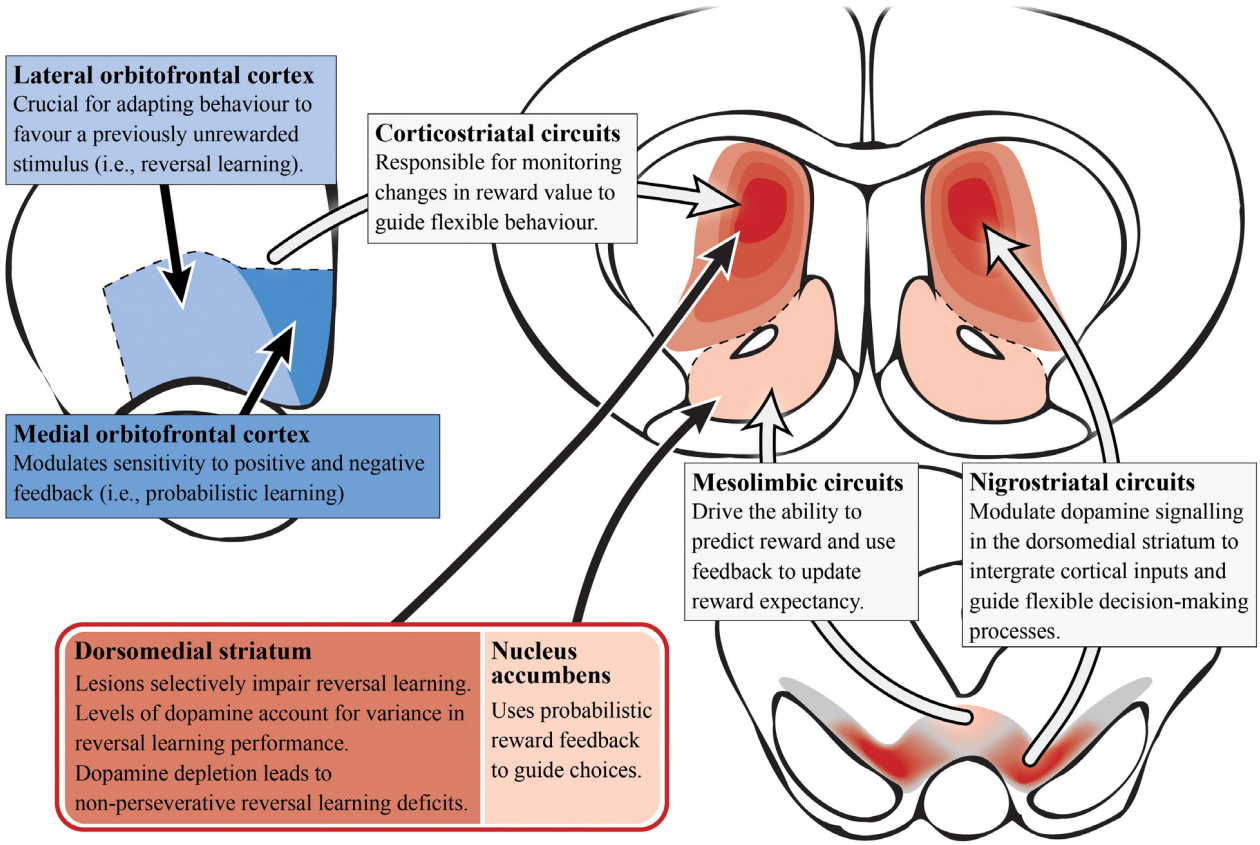

FIGURE 4 | Reversal learning and dopamine (preclinical studies). A simplified summary of the preclinical research on subcortical (reds) and cortical (blues) regions involved in reversal learning with potential relevance to schizophrenia. Alterations in dopamine signaling in the dorsomedial/associative striatum (dark red; which is essential for reversal learning) could impair integration and encoding of inputs from other regions/circuits involved in probabilistic reversal learning behavior. This is most likely driven by the nigrostriatal circuit that modulates dopamine signaling in the striatum. In contrast, the nucleus accumbens is important for using probabilisitic reward feedback to guide choices (i.e., probabilistic learning). Corticostriatal circuitry monitors changes in reward value to guide choices. Specifically, the lateral orbitofrontal cortex allows the adaptation of behavior for reversal learning while the medial orbitofrontal cortex modulates reward feedback sensitivity for probabilistic learning.

have been shown to impair probabilistic reversal learning as they impact on the ability to use probabilistic reward feedback to guide action selection (Stern and Passingham, 1995; Dalton et al., 2014). However, based on a number of animal studies, there is also evidence of unaffected reversal learning following lesions to the nucleus accumbens (limbic), where dopamine dynamics are responsible for reward prediction errors (Burk and Mair, 2001; Schoenbaum and Setlow, 2003; Castane et al., 2010).

In rodent preclinical experiments, lesions of the OFC have also induced reversal learning deficits, while infralimbic and prelimbic cortical lesions (subregions of the rodent medial PFC) did not affect this process (Boulougouris et al., 2007; Ragozzino, 2007). Furthermore, the medial OFC modulates sensitivity to positive and negative feedback (indicating its importance for probabilistic learning), while the lateral OFC is crucial for adapting behavior to favor a previously unrewarded stimulus (important for reversal learning; Dalton et al., 2016). Interestingly, inactivation of the rat prelimbic and infralimbic cortices showed impairments in extradimensional task-switching, indicating that these medial PFC subregions may only be engaged in other forms of cognitive flexibility, and not in reversal learning specifically (Ragozzino et al., 2003). Most evidence suggests the medial PFC is only recruited for tasks involving a higher attentional demand and performance monitoring that require a shift in the strategy or rule (rather than the contingency) required to complete a task (Laubach et al., 2015). Seemingly, the OFC represents expected outcomes during reversal learning, possibly by utilizing value information stored in the region and/or deriving outcome information from subcortical networks tracking the reward environment (Cai and Padoa-Schioppa, 2014; Wassum and Izquierdo, 2015). The OFC projects to both the limbic and associative striatum, receiving reciprocal input via the mediodorsal nucleus of the thalamus, suggesting either area could work in concert with the OFC to direct reversal learning (Middleton and Strick, 1996; Schilman et al., 2008).

Studies in non-human primates have revealed that the striatum and OFC primarily modulate reversal learning via dopamine and serotonin signaling, respectively (Groman et al., 2013). Depleting dopamine in the OFC of

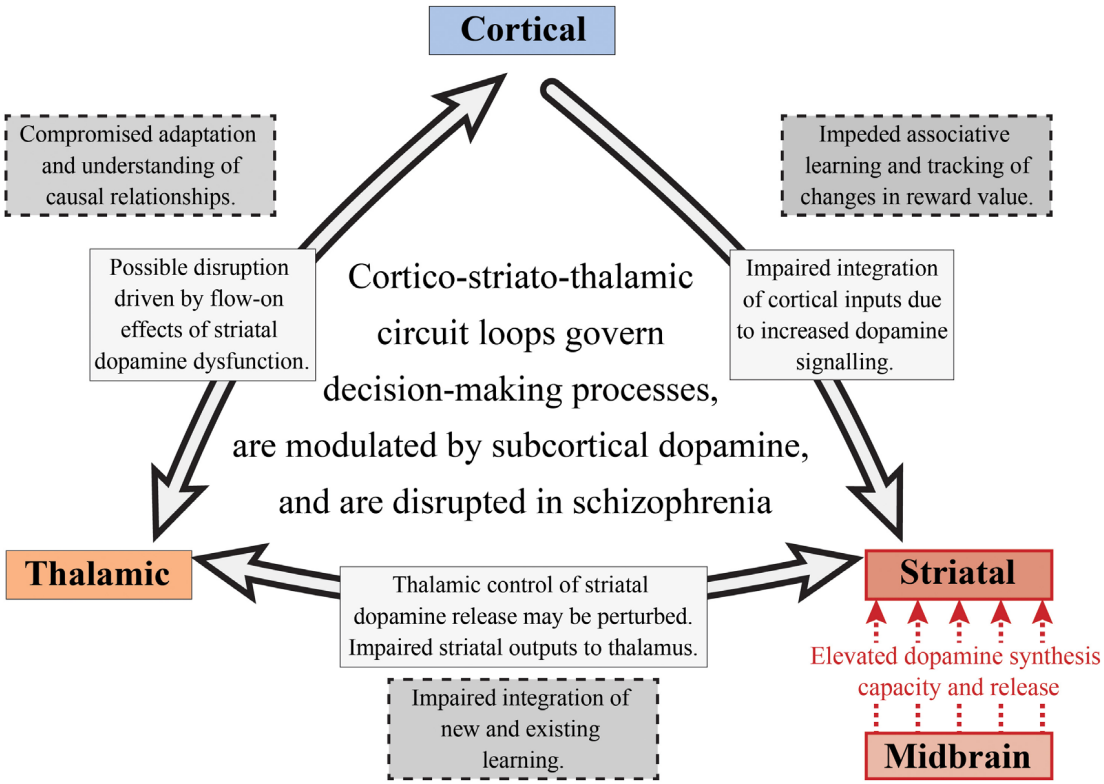

FIGURE 5 | Subcortical dopamine, cognition and schizophrenia. This is a simplified diagram of the cortico-striato-thalamic circuit loop that is disrupted in schizophrenia. Increased striatal dopamine signaling, as well as the impaired integration of cortical inputs into the striatum, may affect a number of cognitive components involved in decision-making including those linked with goal-directed and flexible behavior. The nigrostriatal pathway (red) is responsible for the increased dopamine synthesis and release in the associative striatum. This could result in a perturbation of the thalamostriatal pathway, impacting striatal dopamine release and impairing the integration of new and existing learning. The corticostriatal pathway is also affected in schizophrenia as there is compromised integration of cortical inputs into the striatum, potentially impacting on associative learning and value tracking processes. Finally, this may have flow on effects for the thalamocortical pathway which would result in an inability to understand the consequences of actions and to appropriately adapt behavior.

non-human primates had no effect on reversal learning, whereas depleting dopamine in the striatum led to a non-perseverative reversal learning deficit (Clarke et al., 2007, 2011). In contrast, reducing serotonin signaling in the OFC impairs reversal performance by increasing perseveration (Clarke et al., 2004). Perseveration is the repetition of a behavior that occurs in the absence or cessation of a stimulus. So non-perseverative reversal learning deficits indicate that dopamine signaling in the associative striatum is not critical for the immediate adjustment to a reversal, but rather the subsequent acquisition and maintenance of a selected strategy in response to a reversal. It has been suggested that an optimal balance of dopamine D2 receptor function is required for ideal reversal learning performance (Izquierdo et al., 2012). This is supported in studies across mice, monkeys and humans that show low dopamine D2 receptor availability correlates with poorer reversal learning performance (Jocham et al., 2009; Groman et al., 2011; Laughlin et al., 2011). Lesions of the dorsomedial striatum (associative) also impair serial reversal learning but do not effect initial discrimination learning (Featherstone and McDonald, 2005; Ragozzino, 2007). This suggests that examining the serial reversal learning deficits in schizophrenia specifically (Brunelin et al., 2013), may allow us to better understand dopaminergic alterations in the associative striatum.

Does Increased Associative Striatal Dopamine Function Compromise Cortico-Striato-Thalamic Circuits in Schizophrenia?

It has been hypothesized that perturbations in cortico-striato-thalamic circuits play a major role in the pathogenesis of psychosis, which may also have implications for the global cognitive deficit observed in the disorder as well (Dandash et al., 2017). This hypothesis and its link with psychosis is often implied in the pathophysiological models of the disorder as the activity of these circuit loops are heavily modulated by dopamine (Robbins, 1990; Pantelis et al., 1992). As described in **Figure 5**, these loops generally act in a way that relays information from the cortex, through the basal ganglia, thalamus and then back to the cortex (Alexander et al., 1986). These circuits can act both independently and inter-dependently, whereby inputs from one loop can modify the output of other loops, allowing for the flexible modulation of internally generated and externally

aroused behavioral responses to the environment (Haber, 2003). Based on information examining the specific neural circuits that mediate dopamine dysregulation, the circuit loop of greatest interest to schizophrenia research in cognition should be the dorsal "associative" loop. This loop relays information from the cortex to the associative striatum, then onto the pallidum and substantia nigra, and then finally onto the mediodorsal and ventral anterior nucleus of the thalamus, that then relays the information back to the cortex (Dandash et al., 2017).

Elevated dopamine function in schizophrenia is observed in both the substantia nigra dopamine cell bodies and their associative striatal terminals. Thus, altered dopamine transmission may be one of the fundamental mechanisms driving the disruption of the cortico-striato-thalamic circuit involved in decision-making (see **Figure 5**). Given that pathology in one part of a circuit rarely remains isolated, this will also affect the functions of interconnected systems (Fornito et al., 2015). Therefore, if we choose to examine cognitive processes that are selective for the associative striatum, such as goal-directed action and serial reversal learning, we will not only be able to understand the cognitive effects of subcortical dopamine alterations in schizophrenia, we will also be able to examine the effects on other components of cortico-striato-thalamic circuit loops. We suggest that in schizophrenia, impairments in goal-directed behavior and serial reversal learning may be due to perturbations in multiple components of the cortico-striatal-thalamic circuit loop. These disruptions may be driven by elevated dopamine synthesis and release from the midbrain into the associative striatum, which can hinder the maintenance and execution of decision-making processes. Impaired integration of cortical inputs into the striatum as a consequence of altered dopamine signaling may also be observed. This dysfunctional cortico-striatal pathway may then lead to impeded associative learning and an inability to track changes in reward value. For the thalamostriatal component of this circuit, thalamic control of striatal dopamine release may be disturbed as well as striatal outputs to the thalamus, impairing the integration of new and existing learning.

CONCLUSION

Altered decision-making processes lead to inappropriate choices that further disadvantage people with schizophrenia through functional impairments and reduced quality of life (Kiwanuka et al., 2014). Antipsychotic medication is not effective in ameliorating these cognitive symptoms and there are currently no approved treatments, highlighting the need for novel investigative approaches (Kasper and Resinger, 2003; Lally and MacCabe, 2015). Emerging evidence suggests that dysfunction in the associative striatum, be it dopamine or otherwise, could precipitate the cognitive phenotypes observed in schizophrenia. This could occur due to direct changes in the associative striatal outputs or by impairing the integration of cortical inputs during decision-making. The complexity of these circuit loops, and decision-making processes in general, emphasizes that further research is required if we are to gain a better understanding of the underlying neurobiology of schizophrenia. We contend that research should now shift focus toward a better understanding of the role of specific striatal pathways in cognition, using tools that allow researchers to discretely manipulate circuitry in animal models and examine the effects through outcomes measured on sensitive cognitive tasks. For example, examining the role of the dopaminergic nigrostriatal pathway on goal-directed action could help us better understand the cognitive consequences of the increased dopamine function in the associative striatum observed in schizophrenia. In contrast, as serial reversal learning is relatively selective for cortico-striatal function, probing this process in animals could allow us to better understand the effects that altered associative striatal connectivity and circuit dynamics have on cognition in schizophrenia. For this reason, a detailed evaluation of the consequences of increased associative striatal dopamine function on cortico-striatal-thalamic circuitry and decision-making processes in preclinical models, is paramount.

AUTHOR CONTRIBUTIONS

K-AC, TB, and JK all contributed to the writing of the manuscript.

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REVIEW
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Negative Symptoms of Schizophrenia and Dopaminergic Transmission: Translational Models and Perspectives Opened by iPSC Techniques

Ginetta Collo[1], Armida Mucci[2], Giulia M. Giordano[2], Emilio Merlo Pich[3,4†] and Silvana Galderisi[2†]*

[1] Department of Molecular and Translational Medicine, University of Brescia, Brescia, Italy, [2] Department of Psychiatry, University of Campania "Luigi Vanvitelli", Naples, Italy, [3] Research & Development, Alfasigma Schweiz, Zofingen, Switzerland, [4] Department of Brain Sciences, Imperial College London, London, United Kingdom

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***Correspondence:**
Armida Mucci
armida.mucci@gmail.com

[†] These authors share last authorship

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Negative symptoms (NS) represent a heterogeneous dimension of schizophrenia (SCZ), associated with a poor functional outcome. A dysregulated dopamine (DA) system, including a reduced D1 receptor activation in the prefrontal cortex, DA hypoactivity in the caudate and alterations in D3 receptor activity, seems to contribute to the pathogenesis of NS. However, failure to take into account the NS heterogeneity has slowed down progress in research on their neurobiological correlates and discoveries of new effective treatments. A better neurobiological characterization of NS is needed, and this requires objective quantification of their features that can be applied in translational models, such as animal models and human inducible pluripotent stem cells (iPSC). In this review we summarize the evidence for dopaminergic alterations relevant to NS in translational animal models focusing on dysfunctional motivation, a core aspect of NS. Among others, experiments on mutant rodents with an overexpression of DA D2 or D3 receptors and the dopamine deficient mice are discussed. In the second part we summarize the findings from recent studies using iPSC to model the pathogenesis of SCZ. By retaining the genetic background of risk genetic variants, iPSC offer the possibility to study the effect of de novo mutations or inherited polymorphisms from subgroups of patients and their response to drugs, adding an important tool for personalized psychiatry. Given the key role of DA in NS, we focus on findings of iPSC-derived DA neurons. Since implementation of iPSC-derived neurons to study the neurobiology of SCZ is a relatively recent acquisition, the available data are limited. We highlight some methodological aspects of relevance in the interpretation of *in vitro* testing results, including limitations and strengths, offering a critical viewpoint for the implementation of future pharmacological studies aimed to the discovery and characterization of novel treatments for NS.

Keywords: schizophrenia, negative symptoms, dopamine, animal models, iPSC

INTRODUCTION

Schizophrenia Dimensions and Subtypes

Schizophrenia (SCZ) is often a chronic, disabling mental disorder that affects about 1% of the world's population. Pharmacological treatments have proven effective but have not substantially improved the functional outcome for the majority of people with SCZ, as they are not effective on dimensions which account for most of the functional impairment of these people (Fleischhacker et al., 2014; Galderisi et al., 2014, 2016, 2018b). Several observations concerning heterogeneity of risk factors, clinical picture, course, response to treatment, biological correlates, and functional outcome suggest heterogeneity of pathophysiological mechanisms (Kirkpatrick et al., 2001, 2017; Kirkpatrick and Galderisi, 2008).

Both dimensional and categorical approaches have been adopted to reduce the heterogeneity of the syndrome. The dimensions relevant to the phenomenology of primary psychotic disorders, most commonly reported by factor analytic studies (Liddle, 1987; Peralta and Cuesta, 2001; Rosenman et al., 2003; Demjaha et al., 2009), include: the positive (delusions and hallucinations), disorganization (including formal thought disorder, inappropriate affect, and disorganized behavior) and negative dimensions. However, the number of psychopathological dimensions and the symptoms included in each of them are still controversial (Peralta and Cuesta, 2001; Rosenman et al., 2003; Demjaha et al., 2009).

In particular, the negative symptom (NS) dimension is not unitary and can be subdivided in Avolition-apathy, including avolition, anhedonia and asociality, and Expressive deficit, including blunted affect and alogia, or in five distinct domains: avolition, anhedonia, asociality, blunted affect, and alogia (Kirkpatrick et al., 2006, 2011, 2017; Galderisi et al., 2013, 2018a; Mucci et al., 2015b, 2019; Ahmed et al., 2019; Strauss et al., 2019a,b). Secondary NS (i.e., those symptoms secondary to psychosis, depression, extrapyramidal side effects of antipsychotic drugs or environmental deprivation) represent a further source of heterogeneity. Recent meta-analyses have shown that available antipsychotic (AP) drugs, including clozapine, are not effective in the treatment of NS (Fusar-Poli et al., 2015). None of the included studies have distinguished between primary and secondary NS (Leucht et al., 2017). Only one manufacturer sponsored study showed the efficacy of cariprazine – a dopamine D3 partial agonist – on NS (Nemeth et al., 2017).

As to categorical approaches, two SCZ subtypes are still regarded as deserving investigation, though not included in classification systems: the Deficit Schizophrenia and the Treatment Resistant Schizophrenia.

Deficit Schizophrenia is characterized by the presence of primary and enduring NS; it shows different risk factors, signs and symptoms, course, response to treatment, and functional outcome, with respect to the non-deficit subtype of SCZ (Kirkpatrick et al., 2001, 2017; Kirkpatrick and Galderisi, 2008; Mucci et al., 2017). Subjects with Deficit Schizophrenia, as compared with those without the deficit features, have specific risk factors (i.e., male gender and summer birth), worse early premorbid social and academic adjustment, poorer general cognitive abilities (as assessed by intelligence quotient), more neurological soft signs and poorer functional outcome and response to treatment (Kirkpatrick et al., 2001, 2017; Galderisi et al., 2002; Kirkpatrick and Galderisi, 2008; Peralta et al., 2014; Bucci et al., 2016; Mucci et al., 2017). Treatment Resistant Schizophrenia (25–35% of subjects with SCZ) is identified *post hoc* after several attempts to treat the subject and might be related to different pathophysiological mechanisms with respect to treatment responsive SCZ. Thus, the identification of treatment-resistant cases might be useful to reduce heterogeneity of hypothesized pathophysiological mechanisms (Howes and Kapur, 2014; Howes et al., 2017a).

MAIN HYPOTHESES ON PATHOPHYSIOLOGICAL MECHANISMS OF SCHIZOPHRENIA

The Dopamine (DA) hypothesis is rooted in the knowledge of the role of DA transmission in controlling behavior across species, in the association between DA D2–D3 receptor blockade and clinical efficacy of AP on positive symptoms as well as in the psychotogenic and in the psychotomimetic effects produced by DA enhancing substances.

However, about one third of subjects with SCZ does not respond to AP as reported above; furthermore, cognitive deficits and primary NS are not improved by AP, while they predate the onset of psychosis and are probably related to neurodevelopmental abnormalities, which might represent core aspects of the pathophysiology of SCZ.

A complex hypothesis attempts to reconcile these observations, in which a subcortical DA excess (with enhanced transmission at the postsynaptic D2–D3 receptors) is associated to prefrontal DA hypofunction (at the D1 receptor, which is the most diffuse receptor type in the cortex). Cortical hypoDA function has been related to NS and cognitive deficits (Weinberger, 1987; Howes and Kapur, 2009; Laruelle, 2014; Howes et al., 2017b). However, a meta-analysis of imaging correlates of psychopathological dimensions has not confirmed a relationship between dorsolateral prefrontal cortex hypofunction and NS (Goghari et al., 2010). The "revised" DA hypothesis of SCZ (Howes and Kapur, 2009) is based on the association between severity of psychosis and increased DA transmission in the dorsomedial, associative striatum more than in the ventral striatum/nucleus accumbens. This is at odds with the first formulation of the hypothesis that highlighted the role of the ventral striatum or limbic striatum for psychosis and of the dorsal striatum for extrapyramidal side effects (Laruelle, 2014). The SCZ pathophysiology would include a reduced DA transmission in the nucleus accumbens, related to NS; an increased DA transmission in the associative striatum related to psychosis, and a reduced DA transmission in the neocortex related to cognitive deficits (Howes and Kapur, 2009; Laruelle, 2014; Howes et al., 2017b).

Dopamine transmission alterations might also derive from a cortical glutamatergic dysfunction involving the NMDA receptor (NMDA-R, which is modulated in opposite directions by D1 and D2 receptors). Glutamatergic signaling deficits are regarded as one of the earliest neurodevelopmental abnormalities associated with SCZ, with primary deficits in NMDA-R signaling, particularly in layer 3 pyramidal neurons in prefrontal cortex (Jardri et al., 2016). These deficits lead to an excitatory/inhibitory imbalance and are thought to underlie working memory deficits; they seem to impair recurrent excitation and thus the maintenance of information in the working memory. However, the mechanisms by which excitatory/inhibitory imbalance at the cellular level might relate to clinical symptoms remain unclear.

Finally, in consideration of the well-known role in motivation and incentive behavior, the DA hypothesis provides a reasonable neurobiological substrate to the salience hypothesis (Howes and Kapur, 2009; Howes and Nour, 2016; Howes et al., 2017b) and, at the same time a conceptual framework that links risk factors, including pregnancy, obstetric complications, early life stress, trauma, drug use, and gene variants, that are known to increase presynaptic striatal DA transmission.

Dopaminergic Dysfunctions Relevant to Negative Symptoms

As outlined above, NS are heterogeneous. Investigation of neurobiological correlates of primary vs. secondary NS and motivation-related vs. expression-related symptoms has not been systematic (Kirkpatrick et al., 2017; Galderisi et al., 2018a). However, some hypotheses can be formulated based on the existing literature. In particular, motivation impairment, underlying avolition, asociality, and possibly anhedonia, can derive from different DA pathophysiological mechanisms, involving either the salience or the positive-valence system (Galderisi et al., 2018a). The pattern of motivational deficit found more often in SCZ includes a reduced anticipation of reward (which is still discussed as a possible correlate of depression), enhanced effort discounting and reduced valuation of action, in the presence of a preserved hedonic response (Barch et al., 2016; Galderisi et al., 2018a). Motivation-related deficits in SCZ seem to recognize mechanisms different from those more often observed in depression, in which the poor motivation is associated with a reduced sensitivity to reinforcers and pleasure. Several brain imaging studies found in SCZ alterations of the DA-dependent response of the ventral striatum to reward anticipation and an association with NS (Radua et al., 2015). However, in other studies, the same alterations were found to correlate with positive symptoms, and not with NS (Nielsen et al., 2012), and discrepant findings (i.e., no significant deviation of the DA-dependent response in the ventral striatum) were observed in subjects treated with second generation antipsychotics (SGA) (Juckel et al., 2006; Schlagenhauf et al., 2008; Mucci et al., 2015a). Furthermore, studies in which an association between reduced ventral striatum response and NS was found, generally used the negative subscale total of the Positive and Negative Syndrome Scale (PANSS), which includes ratings of cognitive deficits and a poorly sensitive assessment of Avolition-apathy. When a better assessment of Avolition-apathy was achieved with the Schedule for Deficit Syndrome (SDS), subjects with SCZ and high Avolition-apathy severity showed reduced fMRI BOLD response of associative striatum and normal response of the nucleus accumbens to reward anticipation, when compared to both healthy controls and subjects with SCZ and low Avolition-apathy severity; the same pattern was observed in Deficit Schizophrenia subjects. The reduced imaging bold signal in associative striatum also correlated with reduced real-life motivation (Mucci et al., 2015a). These subjects, characterized by high Avolition-apathy severity, showed also abnormalities of structural and functional connectivity in the motivation-related circuits (Amodio et al., 2018; Giordano et al., 2018). Reduced associative striatum activation during a devaluation task was observed in subjects with SCZ and was found to be associated with the Scale for the Assessment of Negative symptoms (SANS) Avolition-apathy subscale (Morris et al., 2015).

Impaired Motivation Due to Reduced DA Transmission in the Limbic or Associative Striatum: Translational Animal Models

Because of the importance of effort-related dysfunctions in SCZ, animal tests of effort-based decision-making have recently been used to develop formal models of motivational symptoms. In rodents, disconnection studies have shown that a distributed circuit is involved in effort discounting: nucleus accumbens, amygdala (basolateral), prefrontal and anterior cingulate cortex, and basolateral pallidal neurons. D1/D2 antagonists or DA depletion in nucleus accumbens bias behavior toward effort discounting, leaving intact the hedonic reaction and learning of reward-stimuli associations; the same is obtained by inactivation of nucleus accumbens core neurons by local $GABA_{A/B}$ receptor blockade (Salamone et al., 2016b). Experiments involving a progressive effort-reward ratio task showed high variability of performance (i.e., of engagement on progressive higher effort to obtain valued outcomes, up to the break point when effort exceeds outcome); in fact, some rats engaged in motivated lever press behavior very little (low responders), while others did much more (high responders), suggesting differential DA system involvement. The D1-dependent signal transduction marker pDARPP-32(Thr34) (i.e., DARPP-32 phosphorylated at the threonine 34 residue) was found significantly more expressed in the nucleus accumbens core in high responders compared to low responders (Salamone et al., 2016b). In addition, several neurotransmitters interact with the DA signaling in order to regulate effort-related functions. For instance, DA D2R interacts with adenosine A_{2A} receptors to regulate effort discounting; due to this interaction, antagonists of A_{2A} receptors reverse DA D2R antagonism on effort discounting. Intra-NAcc injection of pilocarpine, a muscarinic agonist, interferes with DA transmission and produces effort discounting. Reduced selection of high effort choices in rodents can be induced by the administration of tetrabenazine (TBZ) which inhibits the vesicular monoamine transporter type 2 (VMAT-2). The inhibition of VMAT-2, encoded by Slc18a2, results in reduced

vesicular storage and depletion of monoamines, and produces the same pattern of preference for low-effort option, without interfering with the devaluation test (which indicates integrity of outcome valuation) (Salamone et al., 2016a). The VMAT-2 inhibitor TBZ produces the low-effort bias without impairing the sensitivity to reinforcers (in a maze test when no barrier is used, treated rats will get the valued food in the correct arm), memory or orientation (Salamone et al., 2016a; Yohn et al., 2016). TBZ effects on motivation can be reverted by bupropion and by A_{2A} receptor antagonists (clinically used as antiparkinsonism agents). The A_{2A} receptors are co-localized on enkephalin-positive medium spiny neurons in both dorsal striatum and nucleus accumbens (Salamone et al., 2016a; Yohn et al., 2016). The models above seem related to motivational deficits relevant to depression (reverted by bupropion) and secondary NS related to AP (reverted by antiparkinsonism drugs).

Animal models of anhedonia and asociality have focused mainly on NMDA-R activity, using, for instance, NMDA-R antagonists such as phencyclidine (PCP), MK-801 and ketamine. The exposure of rats to PCP, an antagonist of NMDA-R, mimics cognitive deficits and NS. In particular, it has been observed that the anhedonic effect of the NMDA antagonisms appears at doses greater than those that produce other effects such as asociality and cognitive deficits and also induces neural pathology distinct from that observed in SCZ. In addition, this anhedonic effect does not contribute to disentangle the two aspects of anhedonia (anticipatory and consummatory) (Neill et al., 2014). In rodents, the acute and chronic administration of MK-801 led to social interaction deficits. The sub-chronic administration of ketamine induced NS-related behaviors, while findings concerning the acute administration, which induce rapid and transient psychotomimetic effects, have been less consistent (Lee and Zhou, 2019). The postnatal NR1 knockout mutant mice, a model which produces early postnatal inhibition of NMDA-R activity in corticolimbic GABAergic interneurons, contributes to the onset of SCZ-like abnormalities (novelty-induced increase in locomotion, mating and nest-building difficulties, anxiety and anhedonia-like behaviors) in adult rodents. These behavioral abnormalities are exacerbated by social isolation and associated with deficits of social memory, spatial working memory and prepulse inhibition (Belforte et al., 2010).

Animal models of poor motivation of SCZ are represented by the DA deficient mice induced, for example, with local neurotoxic injection with 6-OHDA in nucleus accumbens, mutant rodents with an overexpression of DA D2R (DR2-OE) or an overexpression of DA D3R (DR3-OE).

Accumbal DA deficient mice have no impairment in hedonic reactions but prefer low-effort options, showing no willingness to work to reach valued outcome (Salamone et al., 2016b). In these models reward valuation and motivation are separable processes and value computations seem to be intact. Motivation to work for large rewards can be restored improving DA transmission in the dorsomedial striatum (Palmiter, 2008).

Overexpression of DA D2R in striatal medium spiny neurons specifically induced by a viral vector strategy in adult mice leads to increased behavioral activation and effort expenditure (Trifilieff et al., 2013), while the same overexpression during development leads to the opposite effect with effort discounting (Ward, 2016). Mutants with constitutive DR2-OE show effort discounting, without reduced sensitivity for rewarding outcome (if no effort is required, they prefer the valued outcome). However, these rodents also show an impaired valuation of response outcome, as if a disconnection of value and action were present. Overall, the deficit in effort-related choices in constitutive DR2-OE mice seem to be due to reduced sensitivity to the value of different response options. Upregulation of D3 receptors in constitutive DR3-OE was associated with a downregulation of striatal D1R and led to an impairment of the motivation and incentive behavior, with preserved basic behaviors and cognitive functions (Simpson et al., 2014). However, given the pre- and postsynaptic expression of D3 receptors in different limbic structures, these data cannot be conclusive, pointing to the importance to test the effects of pharmacological agents acting on D3R receptors on motivational impairment (Neill et al., 2016; Sokoloff and Le Foll, 2017), as described in the following paragraph.

Overall, these animal models have improved knowledge on motivation impairment that characterize NS, but they did not significantly contribute to clarify pathophysiological mechanisms underlying the other NS dimensions (i.e., alogia) and their interrelationships.

The Clinical Relevance of D3 Receptor in Targeting Negative Symptoms

Recent advances in the studies of D3 receptor structure, function and properties have provided a boost in the search and discovery of D3 selective ligands (Platania et al., 2012; Leggio et al., 2016; Maramai et al., 2016; Sokoloff and Le Foll, 2017; Torrisi et al., 2017). These receptors are predominantly located in areas that are relevant to psychotic symptoms, such as ventral mesencephalon, striatum, thalamus, hippocampus, and frontal cortex (Leggio et al., 2016; Maramai et al., 2016; Sokoloff and Le Foll, 2017).

D3 receptors have a higher affinity for DA than D2 receptors; their ligand binding does not lead to the classical D2 receptor subtype side effects (e.g., extrapyramidal symptoms) and in preclinical models results in improvement of cognitive impairment and NS (Leggio et al., 2016; Maramai et al., 2016; Sokoloff and Le Foll, 2017). As reported above, the overexpression of D3 receptor in striatum disrupts motivation, suggesting that changes in D3 receptor may be involved in NS (Simpson et al., 2014). On the contrary, null mutation (KO) of D3 receptors or administration of D3 receptor antagonists are associated with the attenuation of enhanced motivation produced by the excessive DA release induced by psychostimulants (Song et al., 2012), suggesting a complex interplay between presynaptic and postsynaptic D3 receptors (Merlo and Collo, 2015). Therefore, D3 partial agonist/antagonism would be a valuable approach for the treatment of NS.

Most AP, both first and second generation, do not display selectivity for D3 over D2 receptors, but few compounds,

including aripiprazole, blonanserin, and cariprazine, show some D3 selectivity (Leggio et al., 2016). Among these AP, only cariprazine, a DA D3-preferring D3/D2 receptor partial agonist and serotonin 5-HT1A receptor partial agonist, showed an impact on NS. In animal models of rats exposed to chronic mild stress, cariprazine attenuated the decrease in sucrose intake, which represents a model of anhedonia (Leggio et al., 2016). To date, in human, cariprazine is the only AP that has proven more effective than other SGA (i.e., risperidone) in improving NS in subjects with SCZ with predominant and persistent NS (Nemeth et al., 2017; Fleischhacker et al., 2019). Compared to cariprazine, aripiprazole, which is a D2/D3 and 5-HT1A partial agonist, has a lower affinity at D3 receptor. In rodents, aripiprazole does not lead to improvement in social behaviors (Leggio et al., 2016). Further effort is needed in order to improve our knowledge in the involvement of D3R in NS for development of effective treatments.

INDUCIBLE PLURIPOTENT STEM CELLS

Inducible pluripotent stem cells (iPSC) from human donors are a relatively new tool in translational research for disease modeling, as well as for the discovery of novel pharmacologic therapeutics. Different robust protocols have been developed in order to generate iPSC from somatic cells of human donors that are reprogrammed into a pluripotent state through the forced expression of a set of transcription factors (Oct3/4, Sox2, Klf4, c-Myc) that affects their epigenetic state (Takahashi et al., 2007; Aasen et al., 2008; Staerk et al., 2010; Petit et al., 2012). Differentiating human iPSC into specific central nervous system (CNS) phenotypes (e.g., neurons, glia cells) with acceptable fidelity compared to *in vivo* brain neurons, offers the possibility to generate what has been called "disease models in a dish" (Inoue and Yamanaka, 2011; Marchetto et al., 2011; Penney et al., 2019). One critical aspect of iPSC-derived neurons is that the genome of the donor is represented in each iPSC clone, while the epigenetic features of the tissue of origin are almost completely erased. When a standardized and highly controlled process of differentiation is applied *in vitro*, the resulting difference of iPSC-derived neurons from healthy donors should mainly depend on the original genetic makeup related to the disorders, allowing the assessment of the modulatory effects of genetic mutations on cellular functions (Deflorio et al., 2017; Penney et al., 2019). Given the role that DA system plays in NS, in this review we focus on the evidence supporting the use of human DA neurons derived from iPSC as a potential translational approach for a better understanding of the molecular and cellular mechanisms associated to SCZ with persistent and primary NS. Since implementing iPSC-derived neurons to study the neurobiology of psychiatric disease is a relatively recent acquisition, the available data are limited. An initial translational approach was proposed using iPSC-derived forebrain neurons from donors with SCZ (reviewed in Soliman et al., 2017; Balan et al., 2019), while pharmacological studies using neuroactive drugs known to produce psychotomimetic effects were performed in iPSC-derived mesencephalic DA neurons (Cavalleri et al., 2018). In the following paragraphs these findings will be summarized (see also **Table 1**) and some research about future directions will be proposed.

The Target Tissue: Midbrain DA Neurons and Their Embryologic Development

In adult brain the midbrain DA (mDA) neurons are mainly localized in the ventral part of the mesencephalon, harbored within the substantia nigra pars compacta (SN, A9) and, more medially, into the ventral tegmental area (VTA, A10). The nigrostriatal DA projections originated from the SN preferentially innervate the dorsolateral part of the striatum through the nigrostriatal pathways, while the mesolimbic pathway and mesocortical pathway originated from the VTA innervate the ventral part of the striatum and the infralimbic prefrontal cortex, respectively. During development, mDA neurons arise from the midbrain-hindbrain junction sending their terminals to various regions of the developing forebrain. In this period, at the midbrain-hindbrain boundary, DA neuron precursors express the transcription factors OTX2, known to play a critical role during the differentiation (Maxwell and Li, 2005). Later, DA cells develop in the ventral midbrain mostly due to the upregulation and release of sonic hedgehog (SHH) from the notochord, and Fgf8 from the isthmus. Other factors implicated in early development of mDA neurons include Wnt1, En1, En2, Nurr1, Pitx3, Lmx1B, Foxa2, and Pax5 (Maxwell and Li, 2005). Ultimately, maturation of mDA neurons is marked by the expression of tyrosine hydroxylase (TH) and dopamine transporter (DAT) in dendrites and soma, VMAT and SYN1 expression in the vesicle of synaptic terminals, the evidence of functional release and uptake of DA and the repetitive burst firing, a signature of electrophysiological DA neuron phenotype (Weihe et al., 2006).

iPSC Differentiation Into Human DA Neurons

Several protocols capable to differentiate neurons with developmental trajectory resembling that of ventral mDA neurons have been described (Chambers et al., 2009; Kriks et al., 2011; Fedele et al., 2017; Marton and Ioannidis, 2019). Accordingly, ventral mDA neurons are identified by the co-expression of developmental markers. At first, iPSC are driven towards a phenotype that is equivalent to the progenitor status, defined using appropriate combinations of markers for forebrain/midbrain, i.e., OTX2, FOXA2, and LMX1A expression. Intermediate zone-like progenitors are then identified by FOXA2 co-expression with midbrain markers NURR1 and engrailed1 (EN1). Mantle zone neurons are identified by co-expression of FOXA2 with TH, the key enzyme for production of DA, and by the ventral mDA neuronal identifier PITX3. Terminal differentiation has further supported by the co-expression of functional markers such as DAT, D3R, GluR1/2, NR2A/B (Kriks et al., 2011; Cavalleri et al., 2018; Collo et al., 2018), by neurochemical properties, i.e., release and uptake of DA, and by electrophysiological signature, e.g., the burst firing typical of native DA neurons of the mammal ventral mesencephalon

TABLE 1 | Pharmacological studies using iPSC derived neurons from subjects with schizophrenia.

Donors	Neuronal types	Differentiation time (days)	Relevant phenotypic changes	Effective pharmacological agents	Non-effective pharmacological agents	References
Schizophrenia patients from high risk families: one childhood onset schizophrenia; two affected siblings (one schizophrenia and one schizoaffective disorder); one adult schizophrenia patient	Glutamatergic (70%), GABAergic (30%), dopaminergic less than 10%	1–3 months	Reduced dendritic number, neuronal connectivity and dendritic spines. Lower expression of glutamate receptors subunits GRIK1, GRIN2A, GRM7. Defective Wnt (e.g., decreased WNT7A) and cAMP signaling (e.g., increased PDE4D, PDE7B)	Loxapine (10 μM) increases neuronal connectivity	Clozapine (5 μM) Olanzapine (1 μM) Risperidone (10 μM) Thioridazine (5 μM)	Brennand et al., 2011
Members of a DISK 1 mutation family: one schizophrenia patient, one patient with ajor depression and two unaffected subjects. Isogenic iPSc lines	Glutamatergic (90%), very few GABAergic and dopaminergic	Up to 6 weeks	Reduced synaptic vesicle protein SV2 density, reduced vesicle release (FM1-43) and spontaneous ESP. Increase expression of SYN2 SYN3, SYP, SYNPR, NRXN1, VAMP2. Increase expression of transcription factor MEF2C.	Rolipram, reverses synaptic deficit		Wen et al., 2014, 2017
Monozygotic twin patients with treatment resistant schizophrenia, one responder to clozapine and one non-responder	Tuj1 positive neurons	2 weeks following Ngn2 overexpression	Reduced homophilic cell adhesion molecules (e.g., *CDH8*, *DSC*) protocadherin genes.	Clozapine (1 μM), differential gene expression between responder and not responder to clinical treatment		Nakazawa et al., 2017
Nine patients with treatment resistant schizophrenia and nine healthy donors	GABAergic cortical interneurons	6–8 weeks	Reduced ND2 and ND4L NADH dehydrogenases (complex I) Decrease mitochondrial function (maximal respiration and reserve capacity) Increased oxidative stress	Acetyl-l-carnitine, significant increases maximal respiration and reserve capacity Ameliorate arborization deficits	Omega-3 fatty acids, coenzyme Q10, N-acetyl cysteine, α-tocopherol	Ni et al., 2019

(Devine et al., 2011; Kriks et al., 2011; Collo et al., 2018). Recent transplantation experiments in rodents and monkeys using human iPSC derived DA precursors from healthy and parkinsonian donors have demonstrated their capacity to survive and integrate in the host tissue, showing newly innervation to striatum after neurotoxic lesions and improving defective motor activity via functional integration (Hallett et al., 2015; Kikuchi et al., 2017a,b).

Fidelity of iPSC Derived Neurons *in vitro* Models: 2D Culture and 3D Culture-Organoids

In vitro iPSC differentiation has been preferentially performed using 2D cultures protocols, but recent technological improvements allow also 3D cultures, as exemplified by the successful development of human brain organoids (Lancaster et al., 2013; Arlotta, 2018).

The cellular phenotypes obtained by these methodologies reach a variable level of identity between iPSC-derived DA neurons vs. the mDA neurons in the brain when probed using transcriptomics and electrophysiological profiling. For example, using iPSC-derived DA neurons differentiated in 2D cultures up to 50 days, global differences in gene expression were found, in particular for genes related to the level of neuronal maturation (Xia et al., 2016). By using human midbrain-like organoids cultured up to 2 months, these differences were much reduced (Jo et al., 2016); a comparison of the genes expressed by these midbrain-like organoids with genes expressed by human DA neurons grown in 2D cultures (Lin et al., 2016a) and with available data from human mDA neurons (GTEx Consortium, 2015) showed an overlap with the gene expression profile of prenatal midbrains. The major difference with the 2D cultures was the presence of markers for astroglia and oligodendrocytes usually detected at late stages or not detected at all in 2D cultures (Jo et al., 2016). An important contribution regarding the critical role of the culture heterogeneity, i.e., the relative amount of different cell types presents in the samples analyzed for transcriptomics, was recently provided by Sandor et al. (2017). Using a TH-tagged intracellular marker, the transcriptomic profiles of iPSC-derived DA neurons revealed high similarity to the profile of post-mortem human mDA neurons. When DA neurons from Parkinson's disease patients carrying LRRK2 G2019S genetic variants were compared to control individuals, functional convergence of differentially expressed genes was observed. However, no functional convergence amongst differentially expressed genes was found when non-purified iPSC-derived DA neurons were used. These data indicate that cellular heterogeneity observed in iPSC-derived neuronal cultures, i.e., the presence

of variable number of non-DA neurons and astrocytes, could be a major confounder in assessing transcriptomic profiles, possibly more relevant to the difference of individual genotypic background (Sandor et al., 2017). Moreover, iPSC-derived DA neurons possess electrophysiological and synaptic properties similar to those of native mDA neurons (Kriks et al., 2011; Hartfield et al., 2014; Fedele et al., 2017). These single-cell functional features are at the basis of the development of networks of actively firing neurons that can generate bursting activity typical of functional circuits assessed *in vivo*.

Two main approaches for growing human brain organoids were proposed. The first approach, exemplified by Quadrato et al. (2016), allows human iPSC to form a layer of stem cells, the neuroepithelium, from which neuronal precursors differentiate and self-organize into multiple brain-like sub-regions. The authors provided the gene expression profiling in over 80,000 individual cells isolated from 31 human brain organoids after 9 months in culture and they identified several neuronal types (GABAergic, glutamatergic and also DAergic) interconnected in circuits via mature synapses, visualized using electron-microscopy, and organized in sub-regions capable to detect and react to external stimuli (Quadrato et al., 2016). In the second approach, exemplified by Birey et al. (2017) and Xia et al. (2016), a series of signaling factors was used to control the patterning of neuroepithelium development so to define specific brain sub-regions, e.g., forebrain or hypothalamus. This technique is presently hampered by the limited understanding of the signaling factors required for leading the development of several specific sub-regions. Notwithstanding these limitations, both approaches have so far enriched our understanding of the development of human brain circuit.

Initial studies on midbrain DA-like neurons were performed on human organoids derived from embryonic stem cells (Jo et al., 2016) or from regionally patterned neuroepithelial stem cells (Simao et al., 2015; Monzel et al., 2017) after 50–120 days of culture. These multicellular organoids contained spatially organized groups of DA neurons expressing markers typically found *in vivo* in A9 mDA (e.g., GIRK2, DAT) or A10 mDA (e.g., Calcineurin), as well as anatomically mature synapses, synaptic vesicle trafficking, release of DA in response to depolarizing stimuli, polarized membrane potential (Vm = −70 mV), voltage-dependent potassium currents, spontaneous calcium transients, spontaneous electrophysiological peacemaker activity that was reactive to the D2/D3-preferential DA agonist quinpirole via D2/D3 autoreceptor stimulation (Simao et al., 2015; Jo et al., 2016; Monzel et al., 2017). In addition, in human organoid cultures but not in mouse organoids or 2D cultures, neuromelanin-like granules were found accumulating in DA neurons over time (Jo et al., 2016).

In conclusion, the current human iPSC differentiation protocols provide consistent degree of fidelity with the original *in vivo* DA neuron phenotypes, generally higher in 3D organoids vs. 2D cultures. The molecular and morphological phenotypes remind a late-fetal or early-infantile phenotype, probably better positioned for modeling developmental disorders occurring in children or adolescent individuals, e.g., autism or SCZ (Quadrato et al., 2016; Soliman et al., 2017), rather than disorders emerging later in life.

PHENOTYPE OF HUMAN iPSC-DERIVED NEURONS FROM DONORS WITH SCHIZOPHRENIA

In SCZ, the most common cellular pathology observed in post-mortem studies includes reduced dendritic complexity, reduced synaptic spine number and neuronal size of cortical neurons. This pathology was proposed to be due to a neurodevelopmental liability that results in a greater elimination of dendritic spine ("pruning") during infancy and adolescence, but its molecular mechanisms are still unclear (Moyer et al., 2015). Intriguingly, the phenotypes obtained by most investigators using iPSC-derived neurons from donors with SCZ show reduced dendritic spine and reduced synaptic functions, hinting to a neurodevelopment-related impairment (Ahmad et al., 2018; **Figure 1**). Most of these data were obtained in cortical-like glutamatergic neurons, while only few articles were dedicated to study the DA neuron phenotype (Balan et al., 2019).

The first evidence of defective phenotype in iPSC-derived glutamatergic neurons from subjects with SCZ was published by Brennand et al. (2011). After 3 months in culture with astrocytes, glutamatergic cortical neurons from subjects with SCZ were morphologically different from those of healthy controls, displaying a reduced number of dendritic spines, reduced neuronal connectivity (as measured with rabies virus tracing assay), lower expression of some glutamate receptor subunits (GRIK1, GRM7, GRIN2A) and defective Wnt (e.g., decreased WNT7B) and cGMP/cAMP signaling (e.g., increased PDE4). These data are in line with the postmortem observation of reduced dendritic spines in cortical pyramidal neurons and reduced gray matter volume in SCZ, features that are not affected by AP or SGA treatments (Konopaske et al., 2014); instead, they were associated with NS (Roth et al., 2004). Defective dendritic and synaptic phenotypes of iPSC-derived neurons were confirmed by several laboratories (Ahmad et al., 2018; Balan et al., 2019). This defective phenotype may be related to down-regulation of key neurodevelopmental genes. For example, evidence of consistent down-regulation of NPTX2, a protein involved in excitatory synapse, was obtained when transcriptomics datasets from post-mortem brain and from iPSC-derived neurons were meta-analytically compared between SCZ and matched healthy subjects (Manchia et al., 2017). However, at present, no iPSC-derived neurons were generated from donors with SCZ with prevalent or persistent NS. Indirect information can be collected from studies on iPSC donated from treatment-resistant SCZ patients under current clozapine treatment (see **Table 1**), clozapine being used in subjects with prevalent NS (Brar et al., 1997).

Our knowledge of defective phenotypes of iPSC-derived DA neurons are based on the observations of Robicsek et al. (2013) reporting a delayed differentiation and maturation with reduced neurite length and number, low expression of TH

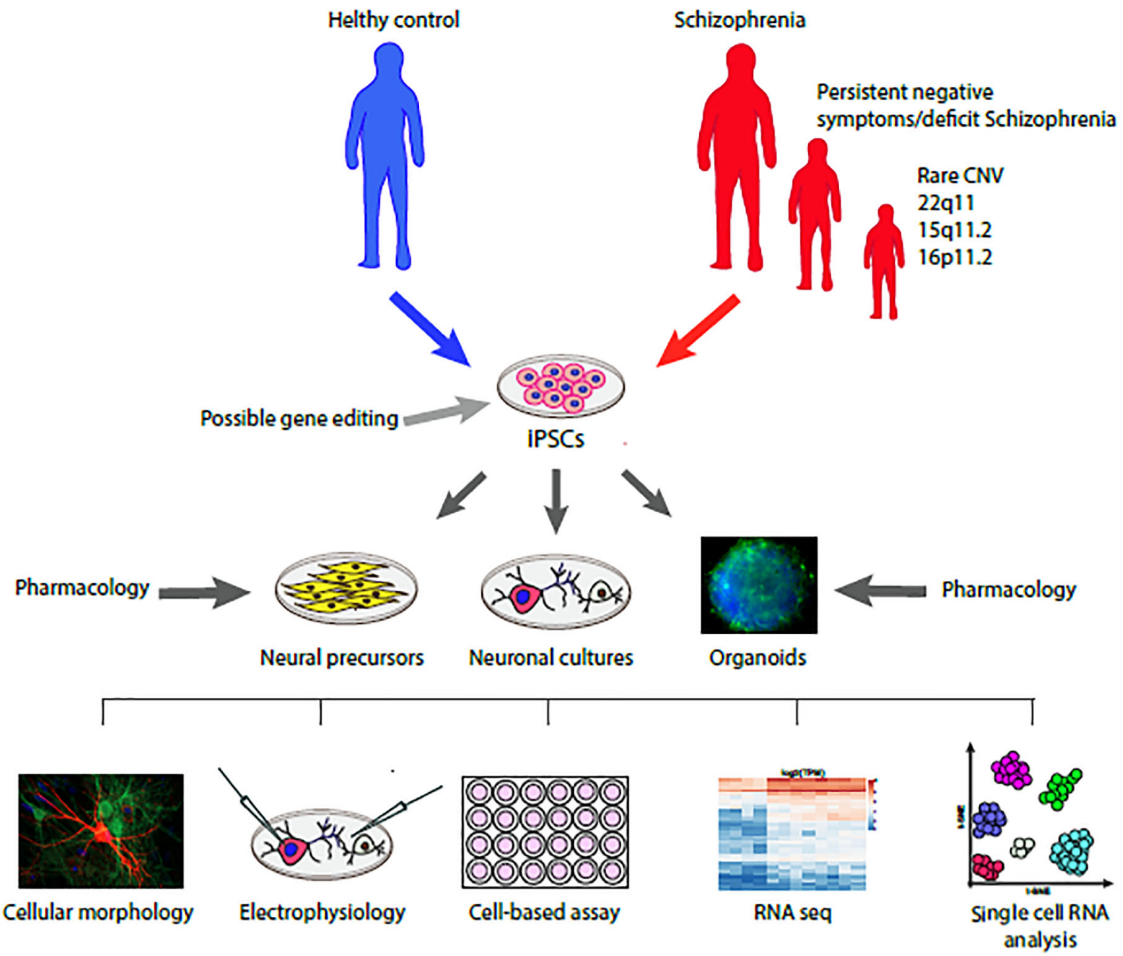

FIGURE 1 | Schematic representation of the translational model to study the cellular and molecular phenotype of iPSC derived neurons from donors with schizophrenia compared with healthy controls and their response to pharmacological agents. Further evolution of this working model will include a precision medicine approach driven by patient stratification based on specific clinical descriptors (e.g., negative symptoms) or genetic liabilities (e.g., rare CNVs) associated to possible gene editing intervention.

and β3-tubulin and no DAT expression. In these cultures, glutamatergic neurons showed also a defective maturation, displaying reduced synaptic contacts. These results are in keeping with the defective development of cortical neurons and the prefrontal hypofunction of the corticolimbic DA system in SCZ patients (see paragraph 2). Subsequently, Hook et al. (2014) observed a relatively unaffected maturation of DA neurons, but reported an increased DA production and release, featuring the same key aspect of an overactive subcortical DA system observed in basal ganglia and ventral mesencephalon (Howes and Nour, 2016). Methodological differences between the two studies were highlighted by Hartley et al. (2015). Using a protocol that enhanced FOXA2 expression to drive the midbrain phenotype, they obtained DA neurons that did not display major differentiation and maturation defects. These data suggest a critical role of specific protocols, possibly favoring the development of different subpopulations of DA neurons with different functional phenotypes. However, this interpretation is still hypothetical given the limited amount of published data, the unchecked reproducibility of the procedures and the genetic heterogeneity of donors.

iPSC-Derived Neurons in Genetically Profiled Subjects With Schziophrenia

Since iPSC retain the genetic characteristics of the donors and the heritability for SCZ is high, about 80% in twin studies (Hilker et al., 2018), it has been proposed that iPSC could reliably reproduce some critical aspect of the cellular biology associated to risk gene variants. A landmark meta-analysis, the Schizophrenia Working Group of the Psychiatric Genomics Consortium (2014) profiled common SNP variants from about 36 K subjects with SCZ and identified 128 common variant associations covering 108 independent loci. These loci include regions containing genes relevant for neurodevelopmental, synaptic function and plasticity, glutamatergic and DA neurotransmission, neuronal calcium and G-protein coupled receptor signaling and ion channels. A recent meta-analysis performed in about 67 K cases

by the Psychiatric Genetics Consortium in Schizophrenia-3, confirmed the 108 independent loci and added additional 152 novel associations (O'Donovan, 2019). The strongest association was with complement C4 gene variants, proposed to be involved in the enhanced synaptic pruning observed during neurodevelopment in children and adolescents. Using an *in vitro* model featuring iPSC-derived microglia and cortical neurons from SCZ donors, Sellgren et al. (2019) demonstrate an increased rate of synapse engulfment and elimination. In this model, the effects on DA neurons were not studied.

Approximately 1.4% of the cases in genome-wide significant association are CNVs preferentially observed in 1q21.1, 2p16.3, 3q29, 7q11.2, 15q11.2, distal and proximal 16p11.2 and 22q11.2 (Marshall et al., 2017). The resulting syndromes generally affect young individuals and include comorbidities of SCZ, intellectual disabilities, autisms and epilepsy. Several recent studies profiled the phenotype of iPSC-derived neurons from donors with SCZ associated with CNVs, whose deficits were most likely produced by the genetic mutation. Among the neurons obtained from iPSC most were glutamatergic, few were GABAergic and none were DA neurons (Hoffmann et al., 2018). Delayed development was observed in iPSC-derived glutamatergic neurons from donors with 22q11 microdeletion (Pedrosa et al., 2011). More recently, RNA-seq analysis was used to investigate gene expression in iPSCs-neurons from donors with SCZ and autism spectrum disorders due to 22q11.2, showing downregulation of 753 genes, the majority being included in apoptosis, cell cycle, cell survival and MAPK signaling Gene Ontology, with a particular attention to the sub-network modulated by PRODH (Lin et al., 2016b). Studies on iPSC-derived neurons from subjects with 15q11.2 mutation also showed defective synaptic and dendritic development (Das et al., 2015). Intriguingly, different results were obtained with iPSC-derived neurons from subjects with 16p deletion, showing neuronal hypertrophy with increases in soma size and dendrite arborization, whereas 16p duplication iPSC derived neurons showed the opposite phenotype, with a reduction of dendritic arborization, especially in excitatory neurons (Deshpande et al., 2017).

A series of interesting studies were conducted using iPSC-derived forebrain neurons obtained from donors affected from a familial form of SCZ and major psychiatric disorders that co-segregate with a mutation of the disrupted-in-schizophrenia-1 (DISC1) gene (Wen et al., 2014; Ye et al., 2017; Wang et al., 2019). DISC1 is a scaffold protein acting as a key regulator of neuronal intracellular trafficking of vesicles and proteins, interacting also with DA D2 receptor (Su et al., 2014). In the first article deficits in synaptic number and synaptic vesicle release was observed in iPSC-derived neurons from carriers with a DISC1 frameshift mutations. The synaptic deficits were reverted by the correction operated via DISC1 gene editing in the isogenic cells (Wen et al., 2014). In a more recent article, using the same iPSC-derived neurons, these authors showed that the phenotypic synaptic impairments were related to the transcriptional dysregulation due to the defective interaction between the Activating Transcription Factor 4 (ATF4) and DISC1 (Wang et al., 2019). In another study, iPSC-derived neurons and organoids developed from the same DISC1 mutants were successfully used to investigate the role of the disrupted DISC1-Ndel1 complex on defective neurogenesis, a critical neurodevelopmental phenomenon in SCZ (Ye et al., 2017).

PHARMACOLOGIC STUDIES IN iPSC-DERIVED NEURONS

As recently reviewed (Ahmad et al., 2018; Balan et al., 2019), the learning from iPSC derived neurons has a great potential in unraveling critical mechanisms for novel therapeutics for SCZ (**Figure 1**). However, so far, few studies have been dedicated to harness the potential of *in vitro* pharmacology in human iPSC-derived neurons. In **Table 1** we summarize the studies using pharmacological approaches in iPSC-derived neurons of relevance for SCZ research.

As mentioned in the previous paragraphs, Brennand et al. (2011) were among the first to assess the effects of AP and SGA such as clozapine, olanzapine, risperidone, thioridazine, and loxapine. They exposed the iPSC-derived neurons to AP or SGA for 3 weeks, showing no effect on the phenotype, with exception of 10 μM loxapine, that was able to partially revert the impaired neuronal connectivity. However, the *in vitro* concentration probably was too high to explain a possible clinical effect (Chakrabarti et al., 2007). This lack of effects on cellular structural parameters and neuroplasticity by most AP and SGA was somewhat expected, since antipsychotics are considered to deliver a symptomatic treatment rather than a disease-modifying treatment. The reversal of functional synaptic deficits in iPSC-derived neurons from members of a family carrying DISC1 mutations previously discussed (Wen et al., 2014), was reported with incubation with the PDE4 inhibitor rolipram, a pharmacologic agent known to affect cAMP/cGMP signaling. Interestingly, increased PDE4 expression was reported by loss of function of DISC1 or ATF4 (Soda et al., 2013), supporting a rationale for pharmacological PDE4 inhibition. One exploratory study (Nakazawa et al., 2017) included iPSC-derived neurons from a pair of monozygotic twins with diagnosis of treatment-resistant SCZ and different response to clozapine. The authors found that the neuronal cultures from the iPSC-derived neurons of the clozapine-responder tween when exposed to 1 μM clozapine showed differential higher expression levels of 167 genes and lower expression levels of 95 genes, when compared to the RNA profile obtained from the clozapine-non-responder tween. Since several overexpressed genes are part of the functional group of homophilic cell adhesion molecules, the authors concluded that response to the clozapine treatment may be related to an enhancement of cell adhesion gene expression relevant for neuronal plasticity that are defective in non-responders.

More recently, evidence of a reversible dysfunctional bioenergetic signature based on profiling acyl-carnitines and mitochondrial functions was observed in subjects with SCZ (Cao et al., 2019). This study corroborates the results obtained in iPSC-derived cortical interneurons from 9 donors with SCZ, showing a consistent mitochondrial bioenergetic deficit that was reverted by *in vitro* treatment with acyl-l-carnitine (Ni et al., 2019). No information is currently available on DA neurons. These results are in line with cortical dysfunctional metabolic

activities observed in subjects with SCZ (Duarte and Xin, 2019). Intriguingly, prefrontal hypometabolism has been related to NS (Wolkin et al., 1992) in the same cortical regions affected by reduced DAergic input.

Among the pharmacological models of psychosis used in humans, exposure to low dose ketamine and to psychostimulant, such as amphetamine, cocaine or methamphetamine, have been considered with interest since they mimic several aspects of the psychotic symptoms in SCZ.

Ketamine, a dissociative anesthetic that blocks central NMDA-R, produces transient increases of DA and acute psychotomimetic effects, including NS (Pomarol-Clotet et al., 2006) and it was used to explore the hypo-glutamatergic and DA cortical hypotheses of SCZ (Krystal et al., 1994; Pomarol-Clotet et al., 2006; Thiebes et al., 2017). Ketamine was recently tested on iPSC-derived DA neurons from healthy donors, resulting in increased dendritic arborization dependent upon AKT-mTOR pathway activation that requires a viable D3 receptor DA neurotransmission (Cavalleri et al., 2018). These effects remind the Akt-mTOR dysfunction reported in postmortem SCZ brain (Ryskalin et al., 2018). Similar effects, also mediated by a D3 receptor-dependent Akt-mTOR pathway, were observed in human and mouse DA neurons exposed to direct D3 receptor-preferential DA agonists, such as pramipexole or ropinirole (Collo et al., 2018) or in mouse primary DA neurons exposed to psychostimulants or D3 receptor-preferential DA agonists (Collo et al., 2012). Intriguingly, the effects of ketamine, amphetamine and of D3 receptor-preferential DA agonists were prevented by DA-D3 receptor blockers, supporting a possible mechanistic interpretation of the clinical effects on NS produced by D3-prevalent partial agonist AP, such as cariprazine (Sokoloff and Le Foll, 2017).

CONCLUSION AND PERSPECTIVES

Schizophrenia is a chronic, neurodevelopmental, disabling mental disorder with a severe impact on functional outcome. It is also a heterogeneous disorder, including a various blend of positive symptoms, NS and cognitive impairment. Pharmacological treatments have proven effective mostly on positive symptoms, but have not substantially improved the functional outcome for the majority of people with SCZ, as they are not effective on dimensions which account for most of the functional impairment of these people, such as NS and cognitive impairment. The remarkable heterogeneity and complexity of the disorder poses a tremendous challenge in identifying pathophysiological mechanisms in order to find novel and targeted treatments.

Translational animal models have improved the knowledge about some pathophysiological mechanisms underlying SCZ. With regard to NS, some animal models provided a better understanding of the neurobiological mechanisms of deficit in motivation, by recapitulating critical circuit impairments that are highly conserved among mammals. Unfortunately, this circuit-based model can only partially clarify the pathophysiological mechanisms of those NS which are not related to motivation, such as blunted affect and alogia. In addition, we have to take into account that animal models have some important limitations. Indeed, rats or mice exhibit profound differences with respect to humans in brain anatomy and functions, as well as some aspects of social behavior; thus how these models reflect complex symptoms, such as NS, is debatable.

The discovery of iPSC has opened the door for new translational strategies to better characterize the pathophysiological mechanisms of SCZ. Indeed, this technique provides an opportunity to study the cellular neuropathology of specific subjects with SCZ. Neuronal cultures differentiated from iPSC share the same genetic background of their donors, ideally linking a specific cellular biology with the specific clinical condition of the donors. Studies from neurological disorders associated to single gene mutations show that iPSC-derived neurons could generate precious information about the molecular mechanisms involved in driving the clinical symptoms (Avior et al., 2016). Taking into account the documented implication of DA dysfunctions in NS, a reasonable translational approach is represented by iPSC-derived DA neurons, which could provide disease-relevant phenotypes at transcriptomic and cellular levels. To date iPSC translational research is at the beginning and much more effort is needed. However, this method has the potential to characterize the heterogeneity of the disorder by effectively implement a Precision Medicine approach (**Figure 1**). In a near future this method might provide a meaningful characterization of SCZ subtypes e.g., subjects with primary and persistent NS, thus fostering the development of specific treatments. The clinical characterization will be essential for the identification of subtypes (or clusters) of patients aimed to iPSC donation. A suggested approach for future application of this methodology would be to characterize specific abnormalities in iPSC-derived DA neurons from subjects with prevalent or persistent NS, and use them to test novel pharmacologic agents/AP aimed to revert these changes *in vitro*. The selected pharmacologic agents/AP will be then assessed in animal translational models to further characterize effects on motivation and incentive behavior *in vivo*. Finally, the selected agents, if safe and well tolerated, will be tested in subjects with NS, assessing changes in brain circuits engaged in motivation and measuring changes of clinical scores for NS using NS assessment instruments which overcome the limits of the PANSS (e.g., The Brief Negative Symptom Scale or the Clinical Assessment Interview for Negative Symptoms). Overall, integrating a circuit-based approach to study motivation in preclinical mammals with the information coming from human iPSC-derived cellular models should provide a strong translational paradigm for testing novel potential treatments in subjects with SCZ.

In conclusion, due to the potentiality of the iPSC method to provide meaningful data also in a limited number of subjects, the classification of subjects using both categorical and dimensional perspectives (e.g., Deficit Schizophrenia with avolition or with

blunted affect) might pave the way for innovative translational studies inspired to the Precision Medicine paradigms.

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All authors contributed to critically revising the content of the manuscript, and approved the final manuscript for submission to Frontiers in Neuroscience.

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REVIEW
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Disrupted Sleep and Circadian Rhythms in Schizophrenia and Their Interaction With Dopamine Signaling

*Anna Ashton and Aarti Jagannath**

Sleep and Circadian Neuroscience Institute, Nuffield Department of Clinical Neurosciences, University of Oxford, Oxford, United Kingdom

Sleep and circadian rhythm disruption (SCRD) is a common feature of schizophrenia, and is associated with symptom severity and patient quality of life. It is commonly manifested as disturbances to the sleep/wake cycle, with sleep abnormalities occurring in up to 80% of patients, making it one of the most common symptoms of this disorder. Severe circadian misalignment has also been reported, including non-24 h periods and phase advances and delays. In parallel, there are alterations to physiological circadian parameters such as body temperature and rhythmic hormone production. At the molecular level, alterations in the rhythmic expression of core clock genes indicate a dysfunctional circadian clock. Furthermore, genetic association studies have demonstrated that mutations in several clock genes are associated with a higher risk of schizophrenia. Collectively, the evidence strongly suggests that sleep and circadian disruption is not only a symptom of schizophrenia but also plays an important causal role in this disorder. The alterations in dopamine signaling that occur in schizophrenia are likely to be central to this role. Dopamine is well-documented to be involved in the regulation of the sleep/wake cycle, in which it acts to promote wakefulness, such that elevated dopamine levels can disturb sleep. There is also evidence for the influence of dopamine on the circadian clock, such as through entrainment of the master clock in the suprachiasmatic nuclei (SCN), and dopamine signaling itself is under circadian control. Therefore dopamine is closely linked with sleep and the circadian system; it appears that they have a complex, bidirectional relationship in the pathogenesis of schizophrenia, such that disturbances to one exacerbate abnormalities in the other. This review will provide an overview of the evidence for a role of SCRD in schizophrenia, and examine the interplay of this with altered dopamine signaling. We will assess the evidence to suggest common underlying mechanisms in the regulation of sleep/circadian rhythms and

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***Correspondence:**
Aarti Jagannath
aarti.jagannath@ndcn.ox.ac.uk

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the pathophysiology of schizophrenia. Improvements in sleep are associated with improvements in symptoms, along with quality of life measures such as cognitive ability and employability. Therefore the circadian system holds valuable potential as a new therapeutic target for this disorder.

Keywords: circadian, sleep, schizophrenia, dopamine, clock, SCRD

INTRODUCTION

Schizophrenia is a severe psychiatric disorder, it is a leading cause of disability affecting nearly 1% of the global population (Vos et al., 2017; Moreno-Küstner et al., 2018). It is heterogeneous in nature, characterized by a combination of positive symptoms, including hallucinations and disorganized speech, negative symptoms such as social withdrawal, and cognitive symptoms. The neural mechanisms underlying it are unclear, but the most widely accepted hypothesis is aberrant dopamine (DA) signaling. Sleep and circadian rhythm disruption (SCRD) is a common feature of the disorder and is closely associated with symptom severity and patient quality of life (Cosgrave et al., 2018a). It is now considered to be more than symptomatic and is thought to contribute to the pathophysiology of schizophrenia (Manoach et al., 2016; Cosgrave et al., 2018b).

Circadian rhythms are oscillations in physiology and behavior of around 24 h, having evolved across phylogeny to enable an organism to anticipate daily changes in the external environment, such as the light/dark cycle. The endogenous circadian clock drives these rhythms. In mammals, this consists of a molecular transcriptional-translational feedback loop (TTFL) in which the transcription factors CLOCK and BMAL1 induce expression of the clock genes *Per1/2* and *Cry1/2*, which then feedback to repress CLOCK and BMAL1 transcriptional activity (**Figure 1A**; Reppert and Weaver, 2002). This core clock machinery is present in almost every cell throughout the body (Dibner et al., 2010). Peripheral clocks are aligned by the master circadian pacemaker in the suprachiasmatic nuclei (SCN) of the hypothalamus, resulting in a coordinated network of cell autonomous circadian oscillators driving rhythmic outputs (**Figure 1B**). The SCN receives light input directly from the retina, which acts as the primary time cue, or "zeitgeber," for entrainment of the clock to changes in the external environment. The sleep/wake cycle is one such rhythm under the control of the circadian clock, but it is also driven by homeostatic sleep pressure, which accumulates during wakefulness and dissipates with sleep (Borbely and Achermann, 1999). Numerous neurotransmitters are involved in the regulation of sleep, including DA, which is an established regulator of the sleep/wake cycle, exerting a potent wake-promoting activity (Eban-Rothschild et al., 2018). There is also evidence that DA has roles in the regulation of circadian rhythms, such as through modulation of SCN entrainment and behavioral oscillators.

The mechanisms underlying SCRD in schizophrenia are currently unclear. However the involvement of DA in both schizophrenia pathogenesis and the sleep and circadian systems suggests that it may be involved. Here, we examine the interplay of SCRD with DA in schizophrenia, assessing the role of DA in the regulation of sleep and circadian rhythms, as well as the circadian influence on DA itself.

SLEEP AND CIRCADIAN RHYTHM DISRUPTION IN SCHIZOPHRENIA

Sleep and circadian rhythm disruption is widespread and well documented in schizophrenia. Indeed sleep disruption has been noted to be associated with mental health problems for over a century and the prevalence of SCRD in schizophrenia was reported to be as high as 80% (Wirz-Justice et al., 2001; Chouinard et al., 2004; Afonso et al., 2011; Bromundt et al., 2011). These showed that patients with schizophrenia had disrupted sleep/wake cycles as measured by wrist actigraphy and melatonin and cortisol profiles, which are markers of endogenous rhythmicity in patients with schizophrenia. However, as most of these studies lacked suitable control subjects, it was difficult to draw conclusions about confounding effects from medication and socio-economic factors including employment status. Wulff et al. (2012), were amongst the first to systematically evaluate circadian disruption in a cohort with schizophrenia against healthy unemployed controls and showed all of the 20 patients assessed had significant SCRD, but none of the controls. Importantly, the patients showed wide heterogeneity in phenotypes, including advanced/delayed phase, non-24 h rhythms that were not entrained by the light/dark cycle and fragmented and irregular sleep patterns, perhaps reflecting the heterogeneity in the disease itself. The nature and extent of SCRD in schizophrenia have been covered in previous reviews and we point the reader to these for further information (Wulff et al., 2010; Jagannath et al., 2013; Chan et al., 2017; Cosgrave et al., 2018b).

Causal Role of Sleep and Circadian Rhythm Disruption

Recent studies provide evidence that sleep disruption may indeed play a causal role in the development of psychosis. It is clear that SCRD can precede the appearance of psychosis on the one hand, and exacerbate negative outcomes on the other. Fragmented sleep and blunted circadian rhythmicity of behavior have been observed in adolescents and young adults at risk for psychosis (Castro et al., 2015) and SCRD predicted the severity of psychosis symptoms and psychosocial impairment at 1 year follow-up in adolescents with clinically high risk for psychosis (Lunsford-Avery et al., 2017). With similar findings in bipolar disorder (Ng et al., 2015), now often considered to be on the same spectrum as schizophrenia, further long-term studies are required to assess the predictive power of SCRD in early diagnosis. Even

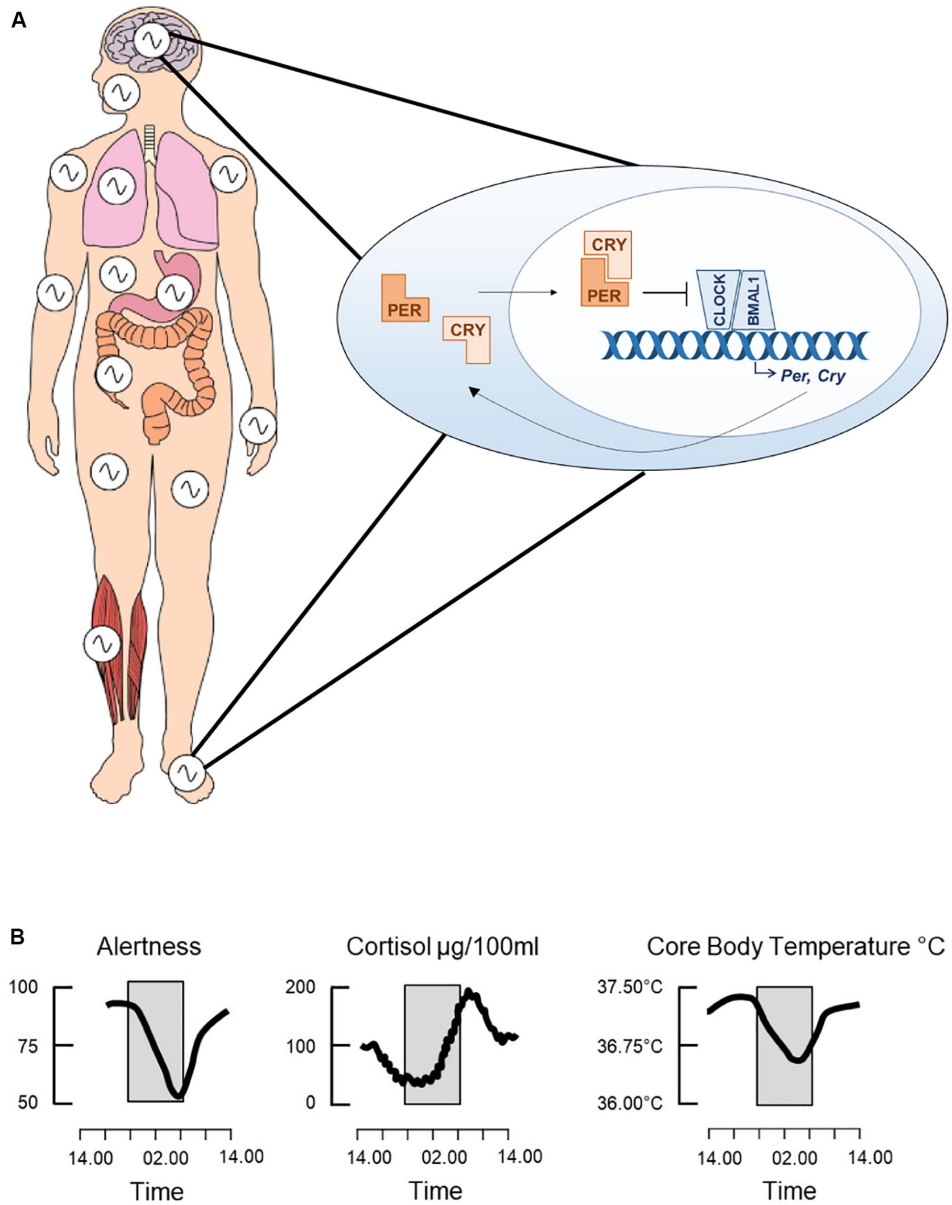

FIGURE 1 | The circadian clock. The clock consists of a molecular transcriptional-translational feedback loop whereby the transcription factors, CLOCK and BMAL1, heterodimerize and induce expression of the core clock genes, *Per* and *Cry* **(A)**. PER and CRY then feedback onto CLOCK and BMAL1 by inhibiting their transcriptional activity. This feedback loop cycles with a period of around 24 h and is present in cells throughout the body, thus driving both the central circadian clock and peripheral clocks. Collectively these drive daily rhythms in physiology and behavior, such as changes in alertness levels, secretion of the stress hormone cortisol and core body temperature **(B)**. Adapted from Jagannath et al. (2013), used with permission.

in healthy subjects, psychotic episodes can be predicted by poor perceived sleep quality in combination with reduced sleep duration (Cosgrave et al., 2018b). Further, sleep deprivation, or restricted sleep to mimic insomnia in healthy subjects leads to symptoms associated with schizophrenia such as reduced prepulse inhibition, increased delusions and hallucinations, and psychotic episodes (Petrovsky et al., 2014; Reeve et al., 2018).

Disrupted sleep in schizophrenia is associated with poor quality of life, higher rates of relapse and suicide (Hofstetter et al., 2005; Pompili et al., 2009; Palmese et al., 2011), and the stabilization of sleep can have beneficial effects. A controlled trial administering cognitive behavioral therapy (CBT) to reduce insomnia in a cohort of over 3000 university students had the concomitant outcome of reducing the appearance of paranoid delusions and hallucinations in those subjects whose insomnia was reduced (Freeman et al., 2017). This approach has also been used in patients with schizophrenia where improvements in sleep are associated with reduced persecutory delusions and hallucinations (Myers et al., 2011; Waite et al., 2016). Pharmacotherapy for

sleep stabilization includes traditional sedative-hypnotics such as benzodiazepines, and second generation sedating antipsychotics such as quetiapine, olanzapine and risperidone improve sleep quality, and pharmacotherapy for sleep also correlates with an improvement in negative symptoms (Kajimura et al., 1995; Yamashita et al., 2004; Kluge et al., 2014). Pharmacotherapy is administered with caution however, as many sedative-hypnotics impair slow-wave sleep and REM sleep patterns, sleep parameters which may already be impaired in schizophrenia.

Clinical studies examining melatonin use as an add-on therapy in schizophrenia suggest that it may also be effective at ameliorating sleep disruption in this disorder (reviewed in Bastos et al., 2019). Melatonin is a key hormonal output of the central clock, its rhythmic release acts to signal circadian time to the brain and peripheral tissues. Administered alongside antipsychotic medication, it results in improved sleep measures including sleep efficiency and duration (Shamir et al., 2000; Kumar et al., 2007). Furthermore, animal studies have demonstrated that it reduces schizophrenia-like behaviors in mice, suggesting that it may also be effective at treating symptoms such as cognitive impairment and social withdrawal (da Silva Araújo et al., 2017; Onaolapo et al., 2017). However, studies which have looked at the effect of melatonin on the psychotic symptoms in patients, although limited, show conflicting results. A reduction in symptom severity has been reported with add-on treatment of both melatonin (Modabbernia et al., 2014) and the melatonin receptor agonist, ramelteon (Mishra et al., 2020). In contrast, Romo-Nava et al. (2014) did not detect any improvement resulting from melatonin administration over placebo, although this study also included patients with bipolar disorder. Inconsistencies in melatonin efficacy may be due to deficiencies in its target regions or its receptors. Indeed, a polymorphism in the MT1 melatonin receptor gene is associated with schizophrenia (Park et al., 2011), indicating that the receptor expression may be altered in some patients.

Together the evidence from these studies suggests SCRD could indeed play a causal role in the development of schizophrenia and that the stabilization of SCRD has beneficial outcomes. As an extension, these findings suggest the SCRD could be a manifestation of a dysfunctional circadian system, which could underlie aspects of the pathophysiology of schizophrenia.

Genome-wide association studies have associated mutations in circadian clock genes with an increased risk of schizophrenia; single nucleotide polymorphisms (SNPs) in *CLOCK*, *PER2*, *PER3*, *RORB*, and *TIMELESS* have been associated with the disorder as assessed within relatively small groups of patients, numbering a few hundred (Takao et al., 2007; Mansour et al., 2009; Zhang et al., 2011). Whilst these associations with clock genes have not been replicated in larger studies and meta-analyses (Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014; Pardiñas et al., 2018; Rees et al., 2019; Wang et al., 2019), these larger studies have implicated SNPs in genes with a role in dopaminergic/glutamatergic neurotransmission, mitochondrial function and immunity. All of these pathways also directly feed into the regulation of circadian rhythms or are regulated by the clock; highlighting multiple points of convergence with circadian rhythms in the pathophysiology of schizophrenia. For example, dopaminergic regulation of the clock is described in detail below and there is a growing body of literature on elevated neuroinflammation in the prefrontal cortex in schizophrenia. This has now been linked to increased nuclear factor-κB (NF-κB) transcriptional activation (Volk et al., 2019), and NF-κB function is regulated by CLOCK, with *Clock* deficient mice showing significantly reduced NF-κB activation in response to immunostimuli (Spengler et al., 2012).

Circadian disruption could result from aberrant synaptic networks and connectivity within the brain, which characterize both schizophrenia and bipolar disorder (Chai et al., 2011), or from a deficit at the level of clock gene expression. There is evidence for both. Blind-drunk (*Bdr*) is a mouse model of synaptosomal-associated protein (Snap)-25 exocytotic disruption that displays schizophrenic endophenotypes. Their circadian rhythms are fragmented and phase advanced, and there is some evidence that this arises from disruption of synaptic connectivity within the SCN, thus disrupting rhythmic outputs (Oliver et al., 2012). On the other hand, skin fibroblasts isolated from patients with chronic schizophrenia lose rhythms in the expression of the clock genes *CRY1* and *PER2* when compared with healthy controls (Johansson et al., 2016), and *PER1/2/3* and *NPAS2* mRNA levels were reported to be altered in white blood cells from schizophrenia patients (Sun et al., 2016). These studies show a deficit at the level of the molecular clock in schizophrenia that is cell autonomous, much like the clock itself. This has been replicated recently by a study analyzing gene expression in the human dorsolateral prefrontal cortex in schizophrenia and control subjects (Seney et al., 2019). By factoring time-of-death into transcriptome analysis, Seney et al. (2019) showed diurnal rhythms in gene expression of very different sets of genes in two groups, with circadian signaling showing strong rhythmic patterns in the control participants but not the schizophrenia patients. In contrast, mitochondrial signaling genes were rhythmic in the patients, but not the controls. The implications of these findings are as yet unclear, but point toward a mechanistic role for circadian rhythm, and consequently circadian disruption in schizophrenia.

MECHANISMS UNDERLYING SLEEP AND CIRCADIAN DISRUPTION IN SCHIZOPHRENIA: ROLE OF DOPAMINE?

The mechanisms underlying SCRD in schizophrenia are currently unclear. However, there is evidence from numerous studies that DA plays a role in the regulation of both sleep and the circadian system. Given that aberrant DA signaling is the longest standing hypothesis for schizophrenia pathogenesis, this may underlie the SCRD in the disorder.

Dopamine in Wake and Sleep

Dopamine is one of the major regulators of sleep and wakefulness. Evidence from numerous studies spanning mammalian species and non-mammalian models, demonstrates the importance of DA in sleep control across phylogeny (Eban-Rothschild et al., 2018). The dopaminergic neurons in

the ventral tegmental area (VTA) and dorsal raphe nucleus (DRN) are key components of the neuronal circuitry for the regulation of sleep and wake states in mammals, owing to their potent wake-promoting activity (Lu et al., 2006; Eban-Rothschild et al., 2016, 2018; Cho et al., 2017; Oishi et al., 2017; Yang et al., 2018). Selective optogenetic stimulation of VTA DA neurons in mice can induce behavioral arousal responses during anesthesia (Taylor et al., 2016), and induce wakefulness during NREM sleep, even following sleep deprivation when homeostatic sleep pressure would be high (Eban-Rothschild et al., 2016). Similarly, activation of DA neurons in the DRN induces wakefulness during NREM and REM sleep, while their chemogenetic inhibition reduces wakefulness and increases NREM sleep duration (Cho et al., 2017).

Dopamine neurons in both these regions, the VTA and DRN, exhibit differential firing patterns between sleep/wake states, although there are differences in the activity patterns between the two regions. In the DRN, DA populations are primarily wake-active, and interestingly the net increase in their activity at wake onset positively correlates with the duration of the subsequent wake episode (Cho et al., 2017), as it does in the striatum (Dong et al., 2019). Therefore DA levels at wake onset may determine the length of the following wake period, and subsequently influence sleep/wake cycles. While in the VTA, there is also higher DA activity during wake, with an increase in population burst firing compared to NREM sleep, however the activity of these neurons is further increased during REM sleep compared to wake (Dahan et al., 2007; Eban-Rothschild et al., 2016), suggesting distinct roles of these two DA regions in arousal. The increase in firing of VTA neurons is accompanied by an increase in extracellular DA levels in their projection target regions, the nucleus accumbens and prefrontal cortex (Léna et al., 2005), leading to sleep/wake-state dependent changes in DA levels.

However, not all DA populations are arousal-inducing, there is also evidence for a sleep-promoting role. Selective lesions of DA neurons in the substantia nigra, which project to the dorsal striatum, leads to increased wakefulness, reduced NREM and REM sleep, and fragmentation of sleep/wake states in rats, while optogenetic stimulation of these neuron terminals leads to increased NREM sleep (Qiu et al., 2016). Furthermore, low doses of D_2 receptor (D_2R) agonists have been reported to induce sedative effects and increase sleep in humans and rodents (Monti et al., 1989; Hamidovic et al., 2008; Laloux et al., 2008). Although this may be due to activation of pre-synaptic autoreceptors and the subsequent inhibition of DA neurons in arousal-promoting regions (Monti and Monti, 2007).

Further evidence for the role of DA in arousal is provided by the effects of stimulants (Boutrel and Koob, 2004). Genetic and pharmacological studies have demonstrated that the primary mechanism underlying the wake-inducing action of the stimulant modafinil is an increase in DA (Wisor et al., 2001; Qu et al., 2008). This is the preferred treatment for hyper-somnias such as narcolepsy, and is thought to act through inhibition of DA reuptake. Similarly, selective DA reuptake inhibitors have also been shown to promote wakefulness in rodents and dogs (Nishino et al., 1998; Luca et al., 2018).

Collectively these studies have demonstrated that DA signaling has a key role in the regulation of sleep/wake states. Generally it serves a wake-promoting role, however its effects are bidirectional and can promote both wakefulness or sleep depending on the brain region and receptor subtypes involved. Its role in sleep/wake control is therefore complex, and the precise molecular and neuroanatomical mechanisms through which it acts are still unclear, yet it is evident that normal DA signaling is important for stable sleep/wake cycles.

Dopamine and the Circadian Clock

The sleep/wake cycle is also under circadian control to ensure that its timing is aligned with the external light/dark cycle, and the two systems are closely linked. There is growing evidence that DA is also an important modulator of the circadian system.

Dopamine signaling is involved in the functioning of two key components of the circadian system: the retina and the SCN. In the retina, it is necessary for functions including light adaptation and visual acuity (Jackson et al., 2012). Importantly, it is also one of the main modulators of retinal circadian activity (Green and Besharse, 2004) and can influence the core circadian clock, controlling the amplitude of rhythmic clock gene expression (Yujnovsky et al., 2006). There is also evidence that DA via D_2R drives rhythmic expression of the photopigment melanopsin in intrinsically photosensitive retinal ganglion cells (ipRGCs; Sakamoto et al., 2005), which are the primary photoreceptors responsible for light input to the SCN (Peirson and Foster, 2006). Therefore retinal DA is a key modulator of non-visual light detection, and may be important for photic entrainment of the master clock. Indeed, Doi et al. (2006) demonstrated that genetic deletion of D_2R in mice leads to a deficient masking response to light, whereby light suppresses locomotor activity during the dark period when the mice are usually active. However, the D_2R null mice did exhibit normal entrainment to a light/dark cycle, and photic responses in the SCN and pineal gland were intact. These data suggest that D_2R is required for behavioral responses to light, but not for other non-visual photic responses such as photic entrainment of the SCN. Although in this study D_2R was deleted throughout the brain, so while retinal DA signaling may be involved, the involvement of DA signaling in other regions cannot be ruled out.

In the SCN itself, there is a high number of dopaminergic neurons, with *Drd1*-expressing neurons comprising approximately 60% of cells in rodents (Smyllie et al., 2016). Early studies demonstrated that DA is required for entrainment of the developing SCN, acting to synchronize fetal-maternal circadian rhythms (Mendoza and Challet, 2014). More recently, studies have suggested that DA modulation of the central clock persists into adulthood (Jones et al., 2015; Landgraf et al., 2016; Smyllie et al., 2016; Grippo et al., 2017). Studies report that *Drd1*-expressing SCN neurons are able to entrain and set the period of circadian rhythms in mice (Jones et al., 2015; Smyllie et al., 2016). However, this does not provide direct evidence for a role of DA itself, as the *Drd1*-positive cells overlap with the majority of arginine vasopressin (AVP) and vasoactive intestinal peptide (VIP) neurons, other key SCN subpopulations involved in entrainment.

However, Grippo et al. (2017) did demonstrate DA modulation of the adult SCN clock via D_1R signaling. They showed that DA signaling in the mouse SCN is required for normal re-entrainment following a jet lag-like phase shift in the light/dark cycle (Grippo et al., 2017). Retrograde tracing suggested that this was mediated by direct DA innervation from the VTA, with enhancement of this DA input leading to accelerated re-entrainment. Interestingly, this facilitation of resynchronization by DA required light input, in line with previous studies that showed that administration of D_1R agonist alone during constant conditions does not elicit any phase shifts in hamster (Duffield et al., 1998; Grosse and Davis, 1999). This suggests that direct midbrain DA innervation of the SCN can modulate the light-responsiveness of the master clock, enabling quicker re-entrainment. Importantly, this study also demonstrates that the level of DA tone can have a direct impact on the central clock, and therefore provides a mechanism whereby aberrant DA signaling, such as an inappropriately timed elevation in DA tone, could influence the master clock. However, this study did not detect changes in several circadian parameters following modulation of SCN DA signaling, such as the period length during constant conditions and the phase angle of entrainment. Therefore, it appears that while DA via D_1R has a modulatory role of the light-responsiveness of the clock, it is not necessary for its normal functioning. Studies looking at additional measures of the circadian clock other than locomotor activity are required to elucidate the involvement of DA in the clock, given the essential role of DA in motor function (Ryczko and Dubuc, 2017).

There is also evidence for a role of DA in clock regulation outside of the SCN. As previously discussed here, DA is a major modulator of the retinal clock, in addition to this it also acts on the striatal clock. The dorsal striatum is heavily innervated by dopaminergic projections from the substantia nigra. Evidence from *in vivo* pharmacological and lesioning studies suggest that DA signaling via D_2R is required for an intact rhythm in *Per2* expression in the dorsal striatum (Hood et al., 2010). Extracellular DA levels were found to oscillate here over 24 h with a peak during night, preceding the peak in *Per2* expression. However, this was measured in rats maintained under a light/dark cycle, so it is unclear whether this is an endogenous circadian rhythm that persists under constant darkness or whether it requires light input.

There is also evidence that DA can regulate circadian rhythms in hormone production and behavior. It is thought to be necessary for the rhythmic release of prolactin in the arcuate nucleus of the hypothalamus (Bertram et al., 2010), and exerts an inhibitory modulation of melatonin synthesis in the pineal gland (González et al., 2012). Melatonin is an important output of the SCN for the regulation of circadian and seasonal rhythms throughout the body, and also feeds back to the SCN itself to modulate the central clock. DA is also thought to control rhythms in activity; lesioning of dopaminergic regions leads to a lengthening in the period of wheel-running and drinking activity during constant conditions (Isobe and Nishino, 2001; Tanaka et al., 2012). However, as DA itself is under circadian control (Chung et al., 2014) as discussed below, it may be that in these cases DA is an intermediate signal between the circadian clock and clock-controlled processes, rather driving the circadian rhythms itself. Nevertheless, it appears to be an important component in the regulation of circadian-controlled processes.

However, DA is in fact able to act independently of the master clock to regulate rhythms in behavior through DA-driven pacemakers which are entrainable by two non-photic stimuli: food and methamphetamine. The DA-enhancing psychostimulant, methamphetamine, can induce circadian rhythmicity in locomotor activity in the absence of an SCN, via the methamphetamine-sensitive circadian oscillator (MASCO; Tataroglu et al., 2006). This finding led to the identification of a 'dopamine ultradian oscillator' (DUO), whereby ultradian oscillations in DA drive an ultradian rhythm in locomotor activity, which persists in *Bmal1* null mice and is therefore independent of the circadian clock (Blum et al., 2014). Interestingly, this study used *Slc6a3-/-* mice to model a hyper-dopaminergic phenotype, due to their deletion of DA transporter (DAT) for DA re-uptake, a potential model for schizophrenia. Elevated DA in these mice led to aberrant ultradian activity rhythms with fragmented daily activity, which mirrors the disruptions to the rest/activity cycle observed in patients with schizophrenia (Wulff et al., 2012), suggesting a causal role for DA in these symptoms. This highlights the central role that DA has in the regulation of activity rhythms, and importantly, demonstrates a potential mechanism through which aberrant DA can lead to abnormalities in rest/activity rhythms seen in schizophrenia.

Dopamine is also necessary for the activity of the food entrainable oscillator (FEO). In the absence of an SCN this drives food anticipatory activity (FAA), whereby an increase in locomotor activity precedes mealtimes by 1 to 3 h (Liu et al., 2012; Gallardo et al., 2014). DA signaling via D_1R in the dorsal striatum mediates this rhythmic activity, suggesting that DA is required for daily rhythms in feeding activity. While the underlying mechanisms are still being elucidated, these SCN-independent oscillators likely feed into the SCN to regulate rhythmic behavior in concert with the central circadian clock.

Interaction of Dopamine With Other Neurotransmitter Systems

There is evidence that DA interacts directly with other neurotransmitter systems that are involved in the regulation of sleep and the circadian clock, namely adenosine and glutamate via NMDA receptors. There is an antagonistic interaction between DA and the G-protein-coupled adenosine receptors via both D_1R and D_2R. The D_1R heteromerize with the A_1R, resulting in an inhibitory action of adenosine to dampen DA receptor response (Ciruela et al., 2011). Whereas D_2R form a complex with the A_{2A} subtype of adenosine receptors, resulting in a reciprocal antagonistic interaction whereby D_2R activation can also dampen the $A_{2A}R$ response (**Figure 2A**). Activation of D_2R is thought to inhibit $A_{2A}R$ activation of the cAMP-PKA pathway, therefore leading to inhibition of adenosine-induced protein phosphorylation and gene expression, such as

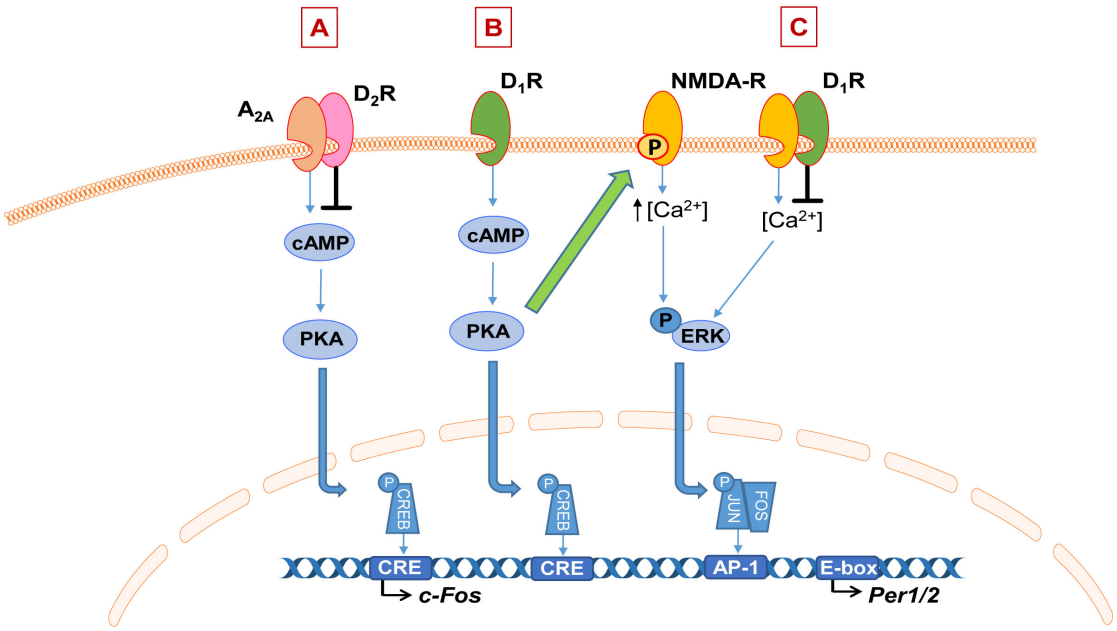

FIGURE 2 | Schematic of dopamine interaction with adenosine and NMDA signaling. **(A)** Dopamine (DA) D2 receptors (D_2R) heteromerize with adenosine 2A receptors ($A_{2A}R$) to inhibit their downstream signaling via the cAMP-PKA pathway, impacting gene expression such as immediate early genes. **(B)** Example of DA-mediated enhancement of NMDA receptor (NMDA-R) signaling. Activation of the dopamine D1 receptor (D_1R) activates the cAMP-PKA pathway which leads to phosphorylation of the NR2B subunit of the NMDA receptor, which is thought to enhance NMDA-dependent calcium influx and therefore downstream responses such as ERK phosphorylation and subsequent transcriptional activation. On the other hand, heteromerization of D_1R or D_2R with NMDA receptors inhibits NMDA-mediated currents **(C)**.

of immediate-early genes (IEGs; Ferré et al., 1997; Svenningsson et al., 2000). Adenosine is involved in sleep/wake control; the accumulation of extracellular adenosine during wake and its dissipation in sleep is thought to underlie homeostatic sleep drive, or "sleep pressure" (Landolt, 2008). Furthermore, the stimulant caffeine acts primarily through antagonism of $A_{2A}R$ (Huang et al., 2005). Therefore, DA-mediated modulation of adenosine signaling through receptor heteromerization is a potential mechanism through which DA can modulate sleep and wakefulness, although the role of this interaction in sleep control is yet to be elucidated.

It is well-established that DA modulates NMDA receptor signaling; the two receptors can form functional heteromers and their downstream signaling pathways also interact at multiple levels (Perreault et al., 2014; Klein et al., 2019). Their interaction is complex; DA can have both a potentiating and inhibitory effect on the NMDA receptor response. Activation of D_1R can enhance NMDA-mediated responses (Cepeda et al., 1993, 1998; Levine et al., 1996). Evidence suggests this is through D_1R-dependent phosphorylation of the NR2B subunit of the NMDA receptor, which is thought to enhance NMDA-dependent calcium influx and therefore downstream responses such as ERK signaling (**Figure 2B**; Pascoli et al., 2011; Murphy et al., 2014). On the other hand, heteromerization of both the D_1R and D_2R with NMDA receptors leads to inhibition of NMDA currents (**Figure 2C**; Perreault et al., 2014). NMDA receptor activation is one of the primary mediators of light input into the SCN and therefore a key mechanism for photic entrainment of clock gene expression in the master clock (Golombek and Rosenstein, 2010). Together, these represent intriguing pathways for future investigation through which aberrant DA signaling may impact neurotransmitter systems that are central to the regulation of the sleep and circadian system.

EFFECTS OF ANTIPSYCHOTIC MEDICATION ON SLEEP AND CIRCADIAN RHYTHMS

Given the influence of DA on sleep and the circadian clock, it would be expected that antipsychotic drugs which act to antagonize DA receptors will also influence these systems. Generally both typical and atypical antipsychotics can improve sleep, although their effects on sleep architecture in patients are not yet fully understood. Studies using both subjective measures and polysomnographic assessments have reported an increase in total sleep time and sleep efficiency, in parallel with clinical improvements (reviewed in detail in Cohrs, 2008). However there are variable effects of different medications on specific sleep parameters, such as the percentage of REM sleep. This is likely due to their different pharmacological profiles; they each act on multiple neurotransmitter systems in addition to dopamine, including serotonin, noradrenaline, and histamine (Miyamoto et al., 2005). Rodent studies have shown that antipsychotic drugs with different receptor selectivity have differential effects on sleep/wake states (Ongini et al., 1993; Gould et al., 2016).

Atypical antipsychotics generally have higher affinity for serotonin receptors, which may underlie their overall higher efficacy at improving sleep over typical antipsychotics (Cohrs, 2008). Studies on the effects of antipsychotic medication in healthy subjects have also demonstrated sleep-promoting effects, indicating that this activity is independent of schizophrenia pathology. However, in a study of subjective sleep quality in hospitalized patients, sleep disturbances were found to persist in the majority of patients undergoing antipsychotic treatment (Waters et al., 2012), demonstrating limited efficacy of antipsychotics at improving sleep in all cases. Although most of the patients in this study were on additional medication such as antidepressants or anticonvulsants, leading to potential confounds.

In addition to influencing sleep, there is also some evidence that antipsychotics affect the circadian system, with differential effects of the typical and atypical classes. The atypical antipsychotic clozapine appears to improve abnormalities in rest/activity rhythms in schizophrenia patients, in comparison to typical antipsychotics such as haloperidol (Wirz-Justice et al., 1997, 2001). These studies lacked pre-treatment baseline data however, therefore it is unclear whether haloperidol itself had a role in driving the arrhythmic rest/activity patterns in these cases. Indeed, there is evidence from studies in healthy mice to suggest that haloperidol can influence the circadian clock. Acute administration of haloperidol can induce *Per1* expression in the mouse SCN via NMDA receptors and CREB signaling (Viyoch et al., 2005). Whereas chronic treatment can suppress *Per1* expression across various brain regions (Coogan et al., 2011), but has no effect on *Bmal1* in most of these regions, suggesting that alterations in the negative arm of the core clock TTFL did not impact on the positive arm here. On the other hand, clozapine was not found to influence clock genes in a small sample study analyzing their expression in white blood cells from patients following an 8 week treatment course (Sun et al., 2016). There were no improvements in the abnormal rhythmic clock gene expression, despite the improvements in circadian rhythms in response to clozapine treatment previously reported. Overall, the actions of antipsychotic medication on the circadian system are unclear from these limited studies. Nevertheless, these findings do demonstrate the importance of appropriate timing of drug administration to correspond with the endogenous clock and sleep/wake cycle, in order to ensure that patients' are not sleeping at the wrong time of day and to prevent further sleep and circadian disturbance.

INFLUENCE OF THE CIRCADIAN CLOCK AND SLEEP ON DOPAMINE SIGNALING

Studies detailed above demonstrate that DA is able to modulate the entrainment of the central circadian clock and its outputs, as well drive behavioral oscillators and modulate circadian clocks outside of the SCN. Interestingly the link between DA and the circadian clock is bidirectional, with DA also being under the control of the clock.

Several elements of DA signaling have a diurnal rhythm, including expression of DA receptors and tyrosine hydroxylase (TH), the rate-limiting enzyme in DA synthesis (Castañeda et al., 2004; Weber et al., 2004; Ferris et al., 2014; Ozburn et al., 2015; Sidor et al., 2015). Numerous rodent studies have reported diurnal changes in extracellular DA levels in various brain regions such as the prefrontal cortex and nucleus accumbens, with generally higher levels at night when rodents are active (Smith et al., 1992; Hood et al., 2010; Menon et al., 2019). Similarly, circulating DA levels in men peak during the day (Sowers and Vlachakis, 1984).

Evidence suggests that these diurnal rhythms are driven by direct regulation of DA signaling components by the circadian clock. The synthetic enzyme TH is under direct transcriptional control by both CLOCK (Sidor et al., 2015) and REV-ERBα (Chung et al., 2014); their genetic ablation leads to elevated DA signaling, with an increase in active TH and firing of dopaminergic VTA neurons (McClung et al., 2005; Chung et al., 2014). Furthermore NPAS2 directly regulates expression of the DA receptor gene *Drd3*, and is required for its rhythmic expression in the nucleus accumbens (Ozburn et al., 2015). In addition, the enzyme monoamine oxidase A (MAOA), which is required for DA metabolism, is also under circadian control; *Per2* is required for its diurnal rhythm in expression, and *Per2* mutant mice exhibit reduced MAOA activity and increased DA levels in the striatum (Hampp et al., 2008). Collectively, these studies demonstrate that DA signaling itself is rhythmic and is under direct circadian control.

There are also numerous lines of evidence that the DA system is modulated by melatonin. The MT1 melatonin receptors are expressed in dopaminergic regions of the rodent and human brain, including the VTA and nucleus accumbens where they exhibit diurnal changes in expression (Uz et al., 2005; Lacoste et al., 2015). Generally, studies have demonstrated an inhibitory effect of melatonin on the dopaminergic system, with an inhibition of DA release widely reported across species and brain regions (Zisapel and Laudon, 1982; Zisapel et al., 1982, 1985; Dubocovich, 1983; Boatright et al., 1994; Exposito et al., 1995), as well as an inhibition of dopaminergic neuronal firing (Domínguez-López et al., 2014). However at the molecular level, studies appear to indicate an enhancement of DA levels, with melatonin reported to increase TH activity (Alexiuk et al., 1996; McMillan et al., 2007), and decrease DA degradative enzyme activity (Esquifino et al., 1994; Stefanovic et al., 2016), as well as increase expression of vesicular monoamine transporter 2 (VMAT2; Stefanovic et al., 2016), which mediates synaptic release of DA. The mechanisms underlying the inhibitory action of melatonin are therefore unclear and its effects are likely to be dependent on both region and circadian time. Zisapel et al. (1985) demonstrated that melatonin inhibits dopamine in a time-dependent manner, suggesting that there is a circadian rhythm in its sensitivity to melatonin. Nevertheless, melatonin clearly influences DA signaling and therefore abnormalities in the melatonin rhythm, such as has been observed in schizophrenia, may disturb the DA system.

There are also changes in extracellular DA levels across the spontaneous sleep/wake cycle. The fluctuations vary between brain regions, but generally levels are higher during wakefulness (Léna et al., 2005; Dong et al., 2019; Menon et al., 2019). In the mouse striatum, DA levels also exhibit dynamic changes within periods of wakefulness and at sleep/wake state transitions (Dong et al., 2019). Given the potent wake-promoting activity of DA, it is currently unclear whether this correlation of DA levels with sleep/wake state is dependent on the sleep/wake cycle or whether elevated DA precedes wakefulness onset. However, there is evidence that sleep deprivation leads to an increase in DA in the nucleus accumbens (Murillo-Rodríguez et al., 2016), suggesting that sleep can modulate DA levels.

BIDIRECTIONAL RELATIONSHIP BETWEEN DOPAMINE AND SLEEP AND CIRCADIAN SYSTEMS

Overall, it is clear that DA and the sleep and circadian systems interact at multiple levels, and this reciprocal relationship may be a key element of schizophrenia pathophysiology. Numerous studies outlined here have demonstrated the involvement of DA in the regulation of both sleep and circadian rhythms. It is therefore implicated that alterations to DA signaling, such that occur in schizophrenia, would lead to disruptions to sleep and circadian rhythms. Indeed, elevation of DA signaling through genetic and pharmacological approaches leads to disrupted sleep and fragmented rest/activity rhythms (Wisor et al., 2001; Monti and Monti, 2007; Blum et al., 2014). Furthermore, circadian rhythm disturbances and disrupted sleep/wake cycles are common in Parkinson's disease, in which degeneration of nigrostriatal dopaminergic neurons leads to altered DA signaling (Videnovic et al., 2014; Grippo and Güler, 2019). Therefore dysregulation of the DA system can lead to disruption to sleep and circadian rhythms, and so this may be a key mechanism underlying the SCRD in schizophrenia.

On the other hand, DA is also under the control of the circadian clock and exhibits sleep-dependent changes, therefore it is likely that SCRD will lead to dysregulation of the DA system. SCRD is a key feature of schizophrenia pathophysiology, it largely occurs independent of medication status or disease stage (Karatsoreos, 2014) and there is compelling evidence for a causal role of SCRD in schizophrenia. Indeed, studies outlined above have demonstrated that an intact clock is required for normal levels of DA activity and rhythmic expression of DA signaling components, with alterations to clock gene expression causing elevations in dopaminergic tone. Furthermore, destabilization of the sleep/wake cycle can cause aberrant DA signaling, with sleep deprivation resulting in increased DA activity (Volkow et al., 2008; Zant et al., 2011; Murillo-Rodríguez et al., 2016).

Therefore, overall the DA system and the sleep and circadian systems are key modulators of each other. The two have a bidirectional relationship whereby dysregulation of one will lead to disturbances to the other, this will likely lead to a positive feedback loop in which the two exacerbate each other (**Figure 3**). Given that both aberrant DA signaling and SCRD are implicated in schizophrenia pathogenesis, this interplay may be an important process in the development of the disorder.

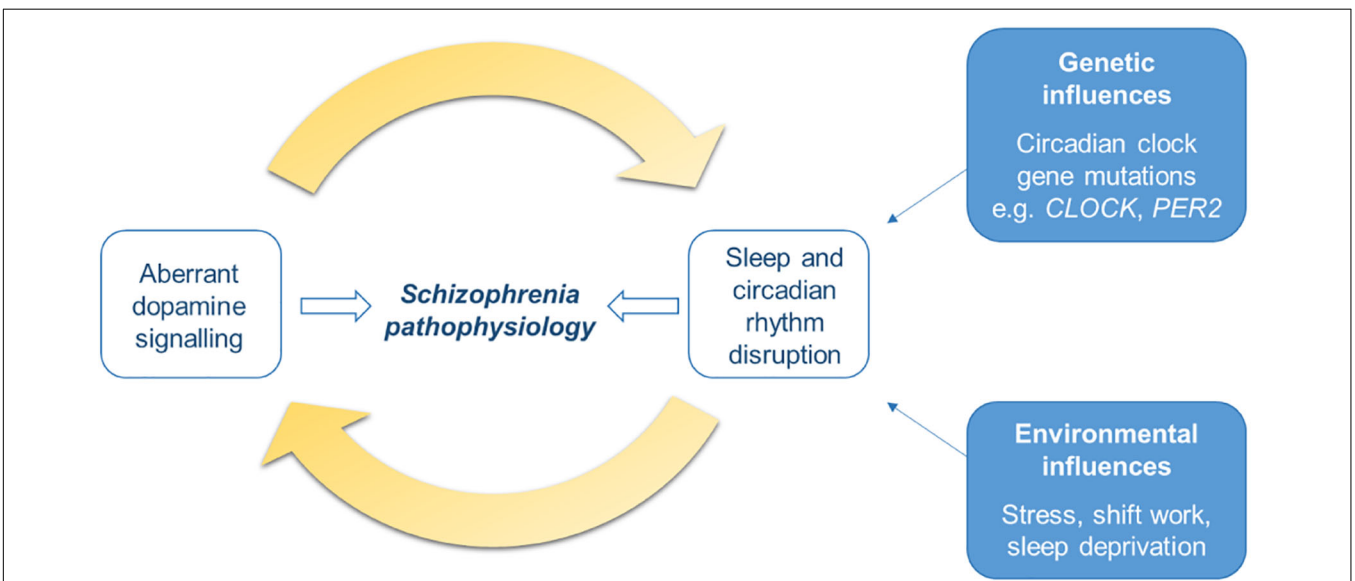

FIGURE 3 | Schematic of the bidirectional relationship of dopamine signaling with sleep and circadian rhythm disruption (SCRD). The dopamine (DA) system is involved in sleep-wake control and circadian rhythms, therefore aberrant DA signaling will result in disruption to sleep and circadian rhythms. On the other hand, DA is also under the control of the circadian clock and its activity varies with sleep stage, therefore SCRD, arising from dysregulation of the DA system itself, or genetic or environmental factors, will aberrantly impact DA signaling. Dysregulation to each of these systems will exacerbate each other; disturbances to one will worsen abnormalities in the other, forming a positive feedback cycle. This process may be an important mechanism underlying schizophrenia pathogenesis and progression of the illness.

Studies assessing the outcome of early intervention to address sleep and circadian disturbances in the prodromal stages will help to elucidate the causal role of this bidirectional relationship in schizophrenia.

CONCLUSION

Sleep and circadian rhythm disruption is a key feature of schizophrenia, with deficits present from the level of the molecular clock up to behavioral rhythms. Studies discussed here demonstrate that DA signaling is an important modulator of sleep and circadian rhythms, and therefore constitutes a common mechanism underlying both sleep and circadian rhythm regulation, and schizophrenia pathophysiology. On the other hand, DA is also influenced by the circadian clock and sleep, and the close reciprocal relationship of the two systems appears to be important in the development of schizophrenia. Whilst further understanding of the mechanisms underlying this complex interaction is needed, there are already several potential pathways where dysregulation of DA can lead to disturbances to sleep and circadian rhythms, suggesting that aberrant DA may lead to SCRD in schizophrenia. Nevertheless, targeting sleep and the circadian system holds valuable therapeutic potential to improve symptoms and quality of life for patients.

AUTHOR CONTRIBUTIONS

AA and AJ have both contributed to the writing of the manuscript. All authors contributed to the article and approved the submitted version.

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REVIEW
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Dysregulation of Midbrain Dopamine System and the Pathophysiology of Schizophrenia

Susan F. Sonnenschein[1], *Felipe V. Gomes*[2] *and Anthony A. Grace*[1]*

[1] Departments of Neuroscience, Psychiatry and Psychology, University of Pittsburgh, Pittsburgh, PA, United States,
[2] Department of Pharmacology, Ribeirao Preto Medical School, University of Sao Paulo, Ribeirao Preto, Brazil

Dysregulation of the dopamine system is central to many models of the pathophysiology of psychosis in schizophrenia. However, emerging evidence suggests that this dysregulation is driven by the disruption of upstream circuits that provide afferent control of midbrain dopamine neurons. Furthermore, stress can profoundly disrupt this regulatory circuit, particularly when it is presented at critical vulnerable prepubertal time points. This review will discuss the dopamine system and the circuits that regulate it, focusing on the hippocampus, medial prefrontal cortex, thalamic nuclei, and medial septum, and the impact of stress. A greater understanding of the regulation of the dopamine system and its disruption in schizophrenia may provide a more complete neurobiological framework to interpret clinical findings and develop novel treatments.

Keywords: dopamine, ventral tegmental area, hippocampus, amygdala, thalamus, prefrontal cortex, medial septum

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Francesca Managò, Italian Institute of Technology (IIT), Italy

***Correspondence:**
Anthony A. Grace
GraceAA@pitt.edu

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INTRODUCTION

Dopamine (DA) modulates circuit reactivity based on environmental stimuli and prior experience and thus plays a central role in functions including reward processing, reinforcement, and habit formation (1–3). Midbrain DA neurons have also been shown respond to novel or aversive stimuli in the absence of reward (4) and it has been proposed that DA signaling may more generally influence sensory processing, such as weighting the salience (5) or certainty (6) of perceived stimuli. Dysregulation of the DA system has been fundamental to many models of the pathophysiology of schizophrenia (7, 8). It is implicated particularly in psychotic symptoms, which involve profound perceptual disturbances (hallucinations) and fixed beliefs resistant to contradictory evidence (delusions). Hallucinations and delusions tend to co-occur and are thus proposed to manifest due to a common pathophysiological mechanism (6, 9). Psychotic symptoms can be attenuated by D_2 receptor blocking drugs (10, 11) that reduce the abnormal increased DA neuron activity (12–14), but the underlying cognitive processes likely involve complex connections between numerous brain regions that remain dysfunctional. This article will discuss some of the circuits that regulate DA neuron activity and how dysfunction in these upstream circuits may influence the DA system in schizophrenia.

DOPAMINE DYSFUNCTION IN SCHIZOPHRENIA

Clinical imaging studies have provided strong support for the DA hypothesis of schizophrenia. Imaging studies that measured radioligand displacement from DA receptors as a measure of DA activity have shown that patients with schizophrenia display increased DA release in response to low-dose amphetamine, compared to healthy controls (15–17), which correlates with transient worsening of psychotic symptoms (17). Patients also demonstrate increased baseline levels of synaptic DA in the striatum, measured in a DA depletion paradigm (18), which has been shown to correlate with their amphetamine-induced DA release (19). Both measures are observed in antipsychotic drug-naive patients and drug-free patients with prior APD treatment, and both predict treatment response of psychosis to antipsychotic drugs (18–20). Elevated striatal DA synthesis capacity, measured by fluorodopa uptake into DA terminals, is also consistently observed in patients and shown to correlate with psychotic severity (21). Numerous studies have found increased response capacity of the DA system in individuals at clinical high risk (CHR) for psychosis, which correlates with greater severity of prodromal symptoms (22–25). Longitudinal studies have further shown that there is a progressive increase in striatal DA function as CHR patients transition to full syndrome expression (24), which has been shown to predict conversion to psychosis (23, 25). Elevated DA synthesis capacity is a less consistent finding in chronic patients in remission, shown to be significantly elevated compared to healthy controls in some studies (26–29), though not all (30–32), suggesting that increased DA function most clearly signals active psychosis. The elevation in DA is limited to striatal projections (33, 34). In contrast, mesocortical projections, particularly to the dorsolateral PFC, display reduced DA release compared to healthy controls, which may contribute to impaired prefrontal-dependent cognitive processes (35). It is currently unknown what accounts for these coexisting differences in DA regulation. Together, these findings point to dysregulation of the DA system as central to the development and expression of psychotic symptoms.

DOPAMINE NEURON PROJECTIONS TO THE STRIATUM

Midbrain DA neurons can be subdivided with respect to their location, projection target, and functional significance (36, 37). The striatum is one of the primary targets of DA signaling and receives dense projections from DA neurons following a topological gradient. In rodents, more medial DA neurons of the ventral tegmental area (VTA) innervate more reward-related ventral striatal regions, including the nucleus accumbens (38). More lateral DA neurons of the substantia nigra project to the dorsomedial and dorsolateral striatum, which are relevant to habit formation and motor function, respectively (39, 40). DA neurons that are located at the transition from lateral VTA to substantia nigra project to the rostral caudate, or associative striatum, which is most implicated in measures of increased presynaptic DA function and demonstrates the strongest correlation to psychotic symptoms in patients with schizophrenia (33, 34). In primates, the relative position of VTA DA neurons shift, but their topological organization is retained. Whereas rodents have a prominent VTA that is located medial to the substantia nigra, in the primate the DA neurons are shifted, with the rodent VTA projection to limbic and associative striatum now becoming the dorsal tier of the substantia nigra and the rodent substantia nigra that projects to the dorsal striatum now comprising the primate ventral tier substantia nigra neurons (41, 42).

ACTIVITY STATES OF MIDBRAIN DOPAMINE NEURONS

DA neurons exhibit two patterns of activity, known as tonic and phasic states, that have different functional implications and are regulated by distinct afferent systems. In vitro in the absence of inputs, DA neurons maintain a basal activity state through the generation of a pacemaker conductance (43–45). However, in vivo recordings in normal rats have shown that not all DA neurons are showing spontaneous activity; instead, approximately half of midbrain DA neurons are not spontaneously active, and instead exist in a hyperpolarized state (45–47) due to inhibitory input from the ventral pallidum (48), an area that is regulated by a pathway that arises from the ventral subiculum of the hippocampus (vHipp). When the vHipp is activated, it provides a glutamatergic drive of GABAergic projection neurons in the nucleus accumbens, which in turn inhibits the ventral pallidum and increases the proportion of VTA DA neurons that are spontaneously active (i.e. "population activity"; **Figure 1**).

Spontaneously active DA neurons, in vivo, can display an irregular tonic firing pattern and rapid, phasic burst firing (45, 49, 50). Burst firing is dependent on glutamatergic afferents from the pedunculopontine tegmentum via activation of NMDA receptors (**Figure 1**) (48, 51). In DA neurons that are nonfiring, NMDA fails to activate NMDA receptors due to a magnesium block that is present at hyperpolarized membrane potentials (52). Thus, only DA neurons that are depolarized (spontaneously active) have the potential to exhibit burst firing. DA neurons exhibit burst firing when exposed to a behaviorally salient stimuli, such as a potential threat or reward (53, 54). Therefore, the number of neurons firing can control the amplitude of the behaviorally salient phasic burst response; when there are more DA neurons firing (i.e., greater population activity), NMDA will cause a greater number to exhibit phasic bursts, thus amplifying the phasic response to stimuli (45, 55). In other words, the vHipp-nucleus accumbens-ventral pallidum (vHipp-NAc-VP) circuit allows the baseline level of responsivity of the DA system, which is dependent on population activity, to be adjusted based on the context in which the stimuli are presented.

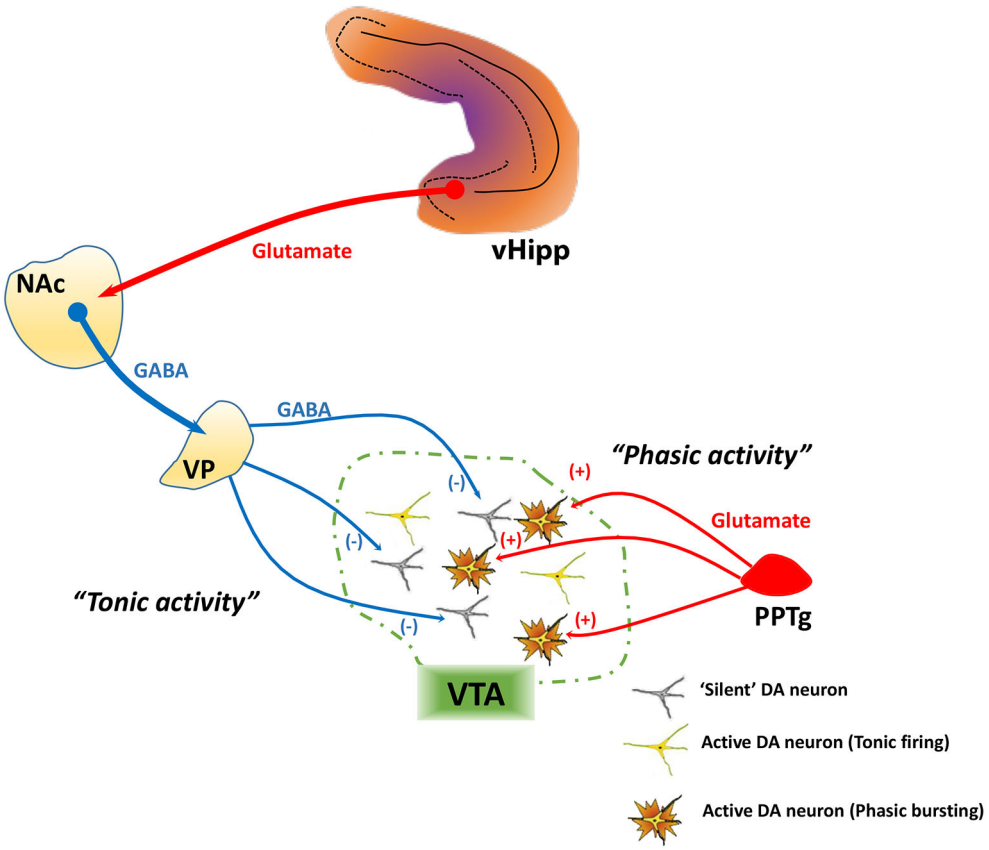

FIGURE 1 | Tonic and phasic dopamine (DA) neuron activity are regulated by distinct afferent systems. DA neurons generate their own activity through a pacemaker conductance. However, a substantial population of DA neurons is not firing spontaneously, being held in a hyperpolarized state by a GABA-mediated inhibitory input from the ventral pallidum (VP). The VP, in turn, is controlled by a pathway originating from the ventral hippocampus (vHipp). The vHipp projects to the nucleus accumbens (NAc), which inhibits the VP. By contrast, phasic burst firing is driven by glutamatergic inputs arising from several areas, primary among these being the pedunculopontine tegmentum (PPTg). This afferent system regulates firing states within the population of spontaneously active DA neurons, because only neurons that are firing spontaneously can burst fire—NMDA channels on hyperpolarized ("silent") DA neurons are under magnesium block and won't change state. Therefore, the PPTg provides the rapid, behaviorally salient phasic signal, whereas the VP, by controlling the number of DA neurons firing, determines the gain of the phasic signal.

CIRCUITS THAT INFLUENCE VTA DOPAMINE NEURON POPULATION ACTIVITY THROUGH THE vHIPP-NAc-VP PATHWAY

Elevated DA system activity in schizophrenia results from dysfunction in a larger hippocampal-midbrain-striatal circuit, with a primary locus of pathophysiology that appears to develop in the vHipp. Deficits in the structure and function of the hippocampus are consistently observed in imaging and post-mortem studies of schizophrenia patients (56). Imaging studies show that the anterior hippocampus, which is homologous to the limbic vHipp in rodents (57), is hyperactive in individuals with schizophrenia (58). Most studies report increased hippocampal glutamate levels in both first-episode and chronic patients, independent of medication status (59), and changes in hippocampal metabolism and blood flow are associated with more severe psychotic symptoms in patients (60–62) and those at CHR (63, 64). Increased cerebral blood volume (CBV) has been reported specifically in the CA1 and subiculum of the hippocampus in patients with schizophrenia (65). Increased CBV is also present during the prodromal stage and predicts conversion to psychosis (66, 67) and hippocampal atrophy (68). Multiple lines of evidence have suggested that the hippocampal hypermetabolism is due to reduced parvalbumin (PV)+ GABA interneuron regulation of pyramidal neuron activity, secondary to excitotoxic degeneration of PV+ interneurons (69, 70). NMDA receptor antagonists, such as PCP and ketamine, may similarly exacerbate or mimic psychosis by blocking NMDA receptors on PV+ interneurons and thus disinhibiting pyramidal neurons (71, 72). This can lead to increased levels of glutamate and loss of PV+ interneurons following chronic NMDA receptor antagonist administration (68, 73–75).

One can induce an analogous disruption of hippocampal physiology in animal models based on developmental

disruption, including the methylazoxymethanol acetate (MAM) neurodevelopmental rat model (76, 77). The MAM model involves administration of the mitotoxin MAM to pregnant dams on gestational day 17, which correlates with the vulnerable timepoint of the 2nd trimester in humans to adverse events such as maternal infection (78). The offspring of MAM-treated dams ("MAM rats") develop region-specific disruption of neuronal maturation that results in adult phenotypes relevant to schizophrenia, in contrast to the offspring of dams that receive a saline injection, ("SAL rats") (76, 79, 80). Adult MAM rats display loss of PV+ interneurons in the vHipp (81), resulting in a baseline hyperactive state from loss of inhibitory control of pyramidal cell activity (82). The increased vHipp drive results in an increase in DA neuron population activity through the vHipp-NAc-VP circuit and inactivation of vHipp in MAM rats can normalize the DA neuron activity and related aberrant behavior (48, 82). Taken together, these data suggest a that a loss of PV+ interneurons in the hippocampus leads to increased DA neuron population activity and a hyper-responsive DA state, in line with clinical evidence of increased presynaptic DA function (21, 42).

Several brain regions can enhance VTA DA system activity through interactions with the vHipp-NAc-VP pathway. Here we discuss evidence indicating the involvement of the medial prefrontal cortex (mPFC), thalamic nuclei, and medial septum on the VTA DA system and how changes in the activity of these regions may lead to a hyperdopaminergic state as seen in schizophrenia.

Medial Prefrontal Cortex and the Regulation of the DA System

Dysfunction within the mPFC plays a central role in the pathophysiology of several psychiatric illnesses, including schizophrenia. For instance, contrary to the increased presynaptic striatal DA synthesis and release (83), it has been found that DA transmission is decreased in the PFC of schizophrenia patients (35). This cortical hypodopaminergic state is thought to be associated with impairments in cognitive and executive function in schizophrenia (35, 84). Also, a reduced PFC activity has been associated with elevated striatal DA function in schizophrenia patients and at-risk individuals (28, 85).

The mPFC is thought to be a major regulator of the DA system but with the outcome, either inhibitory and excitatory responses, reflecting the specific anatomy of mPFC afferents to the VTA. Two major mPFC subdivisions, the infralimbic (ilPFC) and the prelimbic (plPFC) cortices, send direct projections to the VTA (86) as well as to other regions linked with control of the midbrain DA system, such as the NAc (87). The ilPFC, in particular, seems to regulate the DA system activity through its modulation of the activity of the vHipp and basolateral amygdala (BLA). It was showed that the ilPFC exerts a bidirectional control over VTA DA system via the BLA and vHipp. Whereas the inactivation of the ilPFC increases VTA DA neuron population activity in a vHipp-dependent manner, the activation of the ilPFC decreased VTA DA neuron population activity (88). Compared with the ilPFC, the inactivation of the plPFC produced opposite effects on VTA DA neurons. Whereas the activation of the plPFC had no effect, the plPFC inactivation decreased VTA DA neuron population activity (88). This is consistent with the opposite manner that the ilPFC and plPFC impacts behavioral responses (89, 90). The mechanism by which the plPFC affects VTA DA system is still not completely understood, but it may involve the removal of plPFC attenuation of vHipp activity and/or removal of the inhibitory influence of the plPFC over the ilPFC.

Whereas vHipp activation upregulates DA responsivity, the amygdala decreases tonic DA neuron firing. Activation of the BLA has been shown decrease DA neuron population activity in the medial affect-related regions of the rat VTA, which is proposed to be due to a glutamatergic projection to the ventral pallidum, because blocking glutamate in the ventral pallidum prevents BLA activation-dependent down-regulation of DA neuron firing (91). Furthermore, the decrease in DA neuron population activity observed following activation of the ilPFC depends on an intact amygdala (88). Therefore, the opposing modulatory actions of the vHipp and the amygdala are determined by ilPFC activity.

It is worth noting that the mPFC does not project directly to the vHipp (92). Thus, the effect of ilPFC inactivation on increases in VTA DA neuron population activity, that was prevented by removal of the vHipp influence, may involve other brain regions such as the entorhinal cortex and thalamic nucleus reuniens (93, 94) since both receive direct excitatory projection from the ilPFC (92) and in turn provide powerful excitatory influence over the vHipp (92, 95, 96). Therefore, both the entorhinal cortex and nucleus reuniens could be a relay between the ilPFC and the vHipp that could potentially affect activity of DA neurons in the VTA.

Thalamic Nuclei and Regulation of the DA System

The thalamus has long been implicated as a potential node of dysfunction in schizophrenia (97) mainly due to its heavily reciprocal connectivity with the hippocampus and prefrontal cortex (98–100), thus serving as a critical mediator of communication between these brain regions. Reductions in resting-state functional connectivity between the thalamus and the hippocampus and prefrontal cortex have been reported at both the chronic and early stages of schizophrenia. It has also been reported in at-risk individuals and may predict conversion to psychosis in this group (101–104). Thalamic dysconnectivity patterns consistent with those seen in schizophrenia were also observed in healthy individuals after receiving ketamine to model psychosis (105). Also, a reduction in sleep spindles, which are non-rapid eye movement sleep oscillations generated by the thalamic reticular nucleus, has been consistently reported in schizophrenia patients, and the magnitude of this reduction was inversely correlated with the severity of psychotic symptoms (106, 107). These findings suggest that the thalamus may serve as a hub of wide-scale network dysfunction in schizophrenia.

Recent rodent studies have similarly indicated circuit abnormalities underlying and resulting from thalamic dysfunction (108). The thalamus is composed of multiple nuclei, each with their

distinct afferent and efferent projections (109). Our group has focused on the nucleus reuniens, a thalamic midline nucleus, since it is bidirectionally connected to the hippocampus and prefrontal cortex (98, 99, 110). The nucleus reuniens, in rodents, forms the primary route of communication between the prefrontal cortex and vHipp and is essential for behaviors involving coordinated action of these two regions, such as spatial navigation and fear memory (111–113). Regarding corticothalamic projection to nucleus reuniens, pyramidal neurons from layers 5 and 6 of the medial prefrontal cortex send direct projections to the nucleus reuniens (114, 115) and some neurons from layer 6 of the ilPFC neurons send collaterals to the antero-medial portion of the thalamic reticular nucleus (116), the same subregion of the thalamic reticular nucleus that projects to reuniens (117).

We showed that activation of the nucleus reuniens increases DA neuron population activity in the VTA via its projection to the vHipp, since it was prevented by vHipp inactivation (93). Also, as described above, the inactivation of the ilPFC increases DA neuron population activity in the VTA, an effect that was dependent on the vHipp (88). The mPFC, however, does not send direct projections to the vHipp (92). Besides sending dense projections to the vHipp (96), the nucleus reuniens drives vHipp activity (118). The ilPFC inactivation enhances VTA DA system activity via vHipp likely by disinhibiting the nucleus reuniens since the inactivation of the nucleus reuniens prevented these changes (88). These findings suggest that 1) the ilPFC potently regulates the vHipp via nucleus reuniens and 2) the ilPFC inhibition leads to disinhibition of nucleus reuniens, likely due to deactivation of the thalamic reticular nucleus, which in turn, via its excitatory projections to the vHipp, enhances VTA DA system activity. The ilPFC was found to modulate several aspects of the firing pattern of neurons in the nucleus reuniens (119). Thus, the nucleus reuniens may mediate the regulation of the VTA DA system activity by the ilPFC. Overall, these findings suggest that a loss of top-down prefrontal regulation via disruption of corticothalamic communication, as has been observed in schizophrenia, could contribute to hippocampal overdrive and, consequently, to the hyperdopaminergic state characteristic of the disorder (**Figure 2**).

Another thalamic nucleus recently implicated in the regulation of the VTA DA system is the paraventricular nucleus of the thalamus. It was observed that the pharmacological activation of the paraventricular nucleus of the thalamus enhanced VTA DA neuron population activity, which was completely prevented by the inactivation of either vHipp or NAc (120). Both the paraventricular nucleus of the thalamus and vHipp send extensive glutamatergic innervation to the NAc (121, 122). Interestingly, the inactivation of the paraventricular nucleus of the thalamus attenuated the increased VTA DA neuron population activity induced by the vHipp activation (120). Moreover, this regulation seems to simultaneously require activity in both the vHipp and paraventricular nucleus of the thalamus. The inactivation of the paraventricular nucleus of the thalamus reverses vHipp-induced increases in VTA DA neuron population activity. Similarly, vHipp inactivation reverses the paraventricular nucleus of the thalamus-induced increases (120). Together, these findings suggest that convergent glutamatergic inputs from the vHipp and paraventricular nucleus of the thalamus to the NAc work in concert to regulate VTA DA neuron activity. In addition, the inactivation of the paraventricular nucleus of the thalamus reverses the abnormal increase in VTA DA neuron population activity exhibited by MAM rats (120), similar to what is observed

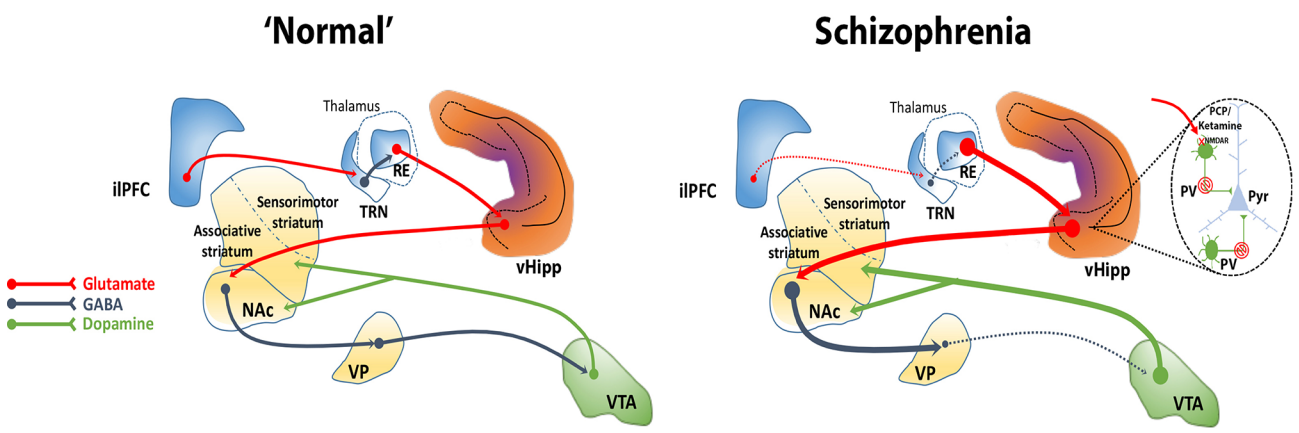

FIGURE 2 | The ventral hippocampus (vHipp) regulates midbrain DA system activity through a polysynaptic circuit. The vHipp excites neurons in the nucleus accumbens (NAc) that, in turn, inhibit ventral pallidal (VP) activity. Given that the VP provides an inhibitory tone to VTA DA neurons, activation of the vHipp results in an enhance VTA DA neuron activity. In schizophrenia, a PV+ interneuron cell loss combined with a disruption of corticothalamic projections contributes to the hyperactivity of glutamatergic pyramidal (Pyr) neurons in the vHipp that drives an increase in active DA neurons projecting to the associative striatum that underlies the emergence of psychotic symptoms in schizophrenia. NMDA receptor antagonists, such as PCP and ketamine, may similarly exacerbate or mimic psychosis by blocking NMDA receptors on PV+ interneurons and thus disinhibiting Pyr neurons. Corticothalamic-hippocampal abnormal interactions can induce a hyperdopaminergic state, for instance, through a dysfunction of the medial prefrontal cortex (mPFC) that could disinhibit the nucleus reuniens (RE), possibly via loss of feedforward inhibition from the reticular nucleus of the thalamus (TRN).

after the inactivation of the vHipp in MAM rats (81). These findings indicate that aberrant thalamic activity may contribute substantially to the hyperdopaminergic state seen in schizophrenia.

Medial Septum and the Regulation of the DA System

Another brain region that may influence vHipp activity and, in turn, regulate midbrain DA system activity is the medial septum. The medial septum sends dense cholinergic and GABAergic projections to several hippocampal regions (123, 124), including the vHipp (125). These projections are critical for hippocampal theta oscillation (126, 127), a major operational mode of the hippocampus, which is thought to be indicative of cognitive processing of environmental information (128).

The GABAergic projections from the medial septum synapse primarily on PV+ interneurons in the hippocampus (124, 126, 129), which is the interneuron subtype associated with the hippocampal hyperactivity and downstream hyperdopaminergic state present in schizophrenia (8, 42). On the other hand, the cholinergic projections provide slow depolarization of their target pyramidal neurons (126). Thus, the GABAergic and cholinergic projections from the medial septum can differently impact the excitatory-inhibitory balance in the vHipp which could ultimately lead to changes in the VTA DA system. In this context, our group recently found that pharmacological activation of the medial septum by a local infusion of NMDA increased the number of spontaneously active DA neurons in the VTA (130). An opposite effect was found in the substantia nigra. These effects induced by medial septum activation on both the VTA and substantia nigra depend on the vHipp since they were prevented by the inactivation of this brain region (130). Moreover, the effects of medial septum activation on VTA DA neuron population activity were also prevented by the infusion of the muscarinic receptor antagonist scopolamine into the vHipp, suggesting that medial septum cholinergic inputs to the vHipp may be involved in these effects (130). In addition, the inactivation of the anterior portion of the VP blocked the increased VTA DA neuron population activity induced by medial septum activation (130). On the other hand, inactivation of the posterior portion of the VP blocked the suppression of substantia nigra DA neuron population activity by medial septum activation. This suggests that there are topographically organized parallel circuits by which medial septum activity can bi-directionally affect DA neurons. Also, these findings indicate that medial septum seems to modulate midbrain DA system activity via the vHipp-NAc-VP pathway.

These opposite actions on VTA and substantia nigra DA neurons mediated by medial septum activation were recently associated with an enhancement of cognitive flexibility (131), a process profoundly attenuated in schizophrenia (132). The concept is that activation of the VTA causes the subject to think about the action, while attenuation of the substantia nigra prevents action until after weighing options. Interestingly, the regulation of the midbrain DA system activity by the medial septum in the MAM model of schizophrenia is different from that observed in normal rats (133). Whereas medial septum activation increases VTA DA neuron population activity and inhibits the substantia nigra in the normal rat (130, 133), an activation of the substantia nigra and a reduction of the abnormal increased VTA DA neuron population activity in MAM rats to baseline levels was observed (133). A possible explanation for these findings is that, in MAM rats, medial septum activation leads to an increase in the pyramidal neuron inhibition which would mitigate the vHipp hyperactivity (81). For example, the medial septum activation in normal rats leads to the release of GABA from the medial septum GABAergic projections to vHipp (123, 125). Since interneurons tend to be more sensitive to GABA than pyramidal neurons (134, 135), the released GABA would activate $GABA_A$ receptors on interneurons in the vHipp. This would inhibit interneurons, which in turn leads to the disinhibition of pyramidal neurons. On the other hand, in MAM rats, GABA released in the vHipp induced by the medial septum activation would be more likely to reach pyramidal neurons due to the loss of interneurons in the vHipp. These changes combined with the loss of cholinergic activation of a parallel set of GABAergic projections to vHipp pyramidal neurons that impact the substantia nigra (123, 124) would increase vHipp drive of the VTA while increasing vHipp inhibition of the substantia nigra. Therefore, in contrast to the control condition, in the MAM rats the excitation of the substantia nigra combined with inhibition of the VTA would cause the subject to act before thinking, or causing impulsive behavior (133). Overall, the findings observed in MAM rats indicate that the medial septum-vHipp pathway as a potential target to reverse the hyperdopaminergic state in schizophrenia patients.

IMPACT OF STRESS ON VTA DOPAMINE NEURON REGULATION

A diathesis-stress model proposes that schizophrenia develops due to stress exposure acting on a pre-existing vulnerability (136). Indeed, a large body of work highlights the importance of stress as a risk factor in the development of schizophrenia (43, 137–139). Early life stress and chronic social stressors, in particular, have been shown to increase the risk of schizophrenia (140, 141). Acute stress can trigger psychotic symptoms (142) and impaired stress tolerance is associated with prodromal symptoms (143). The correlation between early life stress and severity of positive symptoms (144) may partially be due to the interaction between stress, the hippocampus, and the DA system (145, 146).

The vHipp, which is integral in regulating context-dependent responses (147, 148), also shows marked vulnerability to stress across many psychiatric conditions. This may in part be due to a high expression of glucocorticoid receptors to respond to activation of the hypothalamic-pituitary-adrenal (HPA) axis (149). While an elevation in glucocorticoids is essential to respond to perceived threat, chronic elevation can result in impaired function and hippocampal atrophy (150, 151). This would be exacerbated by stress-induced activation of amygdala-hippocampal glutamatergic projections that target PV+ interneurons (152). Prolonged stressors can lead to dendritic shrinkage and neuronal loss in the hippocampus (149), including

a loss of PV+ interneurons (153). It has thus been hypothesized that vHipp dysfunction may contribute to the diathesis in prodromal patients that puts them at risk for developing psychosis in response to stress (154).

Both CHR individuals and schizophrenia patients demonstrate elevated DA release in response to stress compared to healthy controls (155, 156). In adult rats, prolonged stressors, such as restraint stress (157) or repeated footshock (158), increase DA neuron population activity and the level of DA in nucleus accumbens (159). The increase in DA neuron activity can be normalized by inhibiting the vHipp (157, 158). However, at later timepoints, there is a compensatory reduction in DA neuron population activity, referred to as an opponent process (160), and shown to be dependent on the BLA (146, 161). In contrast, during puberty, prolonged stress exposure in rats has been shown to result in a long lasting increase DA neuron activity in adulthood, suggesting that stress before or during puberty is particularly impactful to the responsivity of the DA system (162, 163).

Heightened stress responsivity, insufficient prefrontal inhibition activity in the amygdala (152, 164, 165), and general loss of corticothalamic communication, may contribute to vHipp dysfunction and the emergent hyperdopaminergic state. Extreme stress, or a failure of the PFC to mitigate the impact of stress, could lead to loss of PV+ interneurons in the hippocampus in late adolescence or early adulthood. This in turn would lead to hippocampal hyperactivity and DA system dysregulation. We have shown previously that peripubertal administration of the benzodiazepine diazepam, can prevent the increased anxiety-like behavior and BLA hyperactivity, and normalize hyperdopaminergic activity typically present in adult MAM rats (166–168). These studies suggest that increased stress responsivity, particularly at crucial developmental stages, could lead to the emergence of psychosis in adults and that decreasing stress or other means of reducing vHipp activity during peripubertal period has the potential to circumvent the pathological processes that leads to DA system dysregulation (8). Evidence from animal studies indicate that sex differences should be taken into account since female rodents appear to show greater resilience to schizophrenia-like traits resulting from developmental stress (169). These findings may be associated with the delayed onset and lesser severity of schizophrenia in females (170, 171).

CONCLUSION

The DA system has long been implicated in the expression and treatment of psychotic symptoms in schizophrenia. Study of the circuits that drive DA dysfunction can provide greater and more integrative understanding of a system-wide pathophysiology. Disruption of these circuits through developmental insults and pathological stressors can lead to DA system dysregulation. Ultimately, a greater understanding of the circuits that drive DA system dysfunction in schizophrenia can provide a neurobiological basis for interpreting clinical studies and potential targets for the treatment and prevention of schizophrenia and related psychotic disorders.

AUTHOR CONTRIBUTIONS

All authors contributed to the article and approved the submitted version.

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Mark David Tricklebank, King's College London, United Kingdom

Reviewed by:
Jason Uslaner, University of Michigan, United States
Damiana Leo, University of Mons, Belgium

***Correspondence:**
Rouba Kozak
rouba.kozak@takeda.com
Tamás Kiss
kiss.t@wigner.hu

†Present addresses:
Rouba Kozak, Takeda Pharmaceuticals International Co., Cambridge, MA, United States
Tamás Kiss, Department of Computational Sciences, Wigner Research Centre for Physics, Budapest, Hungary
Keith Dlugolenski, Alnylam Pharmaceuticals, Inc., Cambridge, MA, United States
Kyle Kuszpit, AstraZeneca, Washington, Washington, DC, United States
Brian D. Harvey, Translational In-Vivo Sciences, Biogen Worldwide Medical, Cambridge, MA, United States
Polina Stolyar, Disease Area Exploratory, Novartis Institutes for Biomedical Research, Cambridge, MA, United States
Stacey J. Sukoff Rizzo, Department of Neurobiology, Aging Institute, University of Pittsburgh School of Medicine, Pittsburgh, PA, United States
Dmitri Volfson, Takeda Pharmaceuticals International Co., Cambridge, MA, United States
Mihaly Hajós, Cognito Therapeutics, Inc., Cambridge, MA, United States
David L. Gray, Cerevel Therapeutics, Inc., Boston, MA, United States

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Characterization of PF-6142, a Novel, Non-Catecholamine Dopamine Receptor D1 Agonist, in Murine and Nonhuman Primate Models of Dopaminergic Activation

Rouba Kozak[1†], Tamás Kiss[1*†], Keith Dlugolenski[1†], David E. Johnson[1], Roxanne R. Gorczyca[1], Kyle Kuszpit[1†], Brian D. Harvey[1†], Polina Stolyar[1†], Stacey J. Sukoff Rizzo[1†], William E. Hoffmann[1], Dmitri Volfson[1†], Mihaly Hajós[1,2†], Jennifer E. Davoren[1], Amanda L. Abbott[2], Graham V. Williams[2], Stacy A. Castner[2] and David L. Gray[1†]*

[1] Global Research and Development, Pfizer Inc., Groton, CT, United States, [2] Department of Comparative Medicine, Yale School of Medicine, New Haven, CT, United States

Selective activation of dopamine D1 receptors remains a promising pro-cognitive therapeutic strategy awaiting robust clinical investigation. PF-6142 is a key example from a recently disclosed novel series of non-catechol agonists and partial agonists of the dopamine D1/5 receptors (D1R) that exhibit pharmacokinetic (PK) properties suitable for oral delivery. Given their reported potential for functionally biased signaling compared to known catechol-based selective agonists, and the promising rodent PK profile of PF-6142, we utilized relevant *in vivo* assays in male rodents and male and female non-human primates (NHP) to evaluate the pharmacology of this new series. Studies in rodents showed that PF-6142 increased locomotor activity and prefrontal cortex acetylcholine release, increased time spent in wakefulness, and desynchronized the EEG, like known D1R agonists. D1R selectivity of PF-6142 was supported by lack of effect in D1R knock-out mice and blocked response in the presence of the D1R antagonist SCH-23390. Further, PF-6142 improved performance in rodent models of NMDA receptor antagonist-induced cognitive dysfunction, such as MK-801-disrupted paired-pulse facilitation, and ketamine-disrupted working memory performance in the radial arm maze. Similarly, PF-6142 reversed ketamine-induced deficits in NHP performing the spatial delayed recognition task. Of importance, PF-6142 did not alter the efficacy of risperidone in assays predictive of antipsychotic-like effect in rodents including pre-pulse inhibition and conditioned avoidance responding. These data support the continued development of non-catechol based D1R agonists for the treatment of cognitive impairment associated with brain disorders including schizophrenia.

Keywords: prefrontal cortex, schizophrenia, working memory, pro-cognitive therapeutics, Parkinson's disease

INTRODUCTION

D1 receptors (D1Rs) play a central role in important domains of cognitive function including spatial learning and memory, reversal, extinction, and incentive learning (Huang and Kandel, 1995; Rascol et al., 1999; Goldman-Rakic et al., 2000; Seamans et al., 2001; Williams and Castner, 2006; Takahashi et al., 2012; Wass et al., 2013) and D1R expression or signaling are compromised in a variety of psychiatric, neurological, and endocrine disorders including schizophrenia, drug addiction, and Parkinson's disease (Haney et al., 1998; Wang et al., 1998; Mailman et al., 2001; Rosell et al., 2015; Papapetropoulos et al., 2018). Studies conducted by Sawaguchi and Goldman-Rakic using both agonists and antagonists (Sawaguchi et al., 1990; Sawaguchi and Goldman-Rakic, 1994) indicated that the modulation of working memory processes by mesocortical DA in primates is primarily mediated by D1Rs. Local administration of D1R antagonists into the dorsolateral prefrontal cortex (PFC) induced deficits in a working memory task whereas blockade of D2-like receptors gave no impairment. Subsequent studies revealed that a primary function of D1R activation is to enhance and stabilize task-related activity of PFC neurons (Williams and Goldman-Rakic, 1995).

Importantly, acute treatment with D1R agonists was shown to ameliorate age-related impairments of working memory (Arnsten et al., 1994) and to restore working memory performance in states characterized by prefrontal hypodopaminergia such as chronic stress, chronic neuroleptic treatment, and following low-dose 1-metil-4-fenil-1,2,3,6-tetrahidropiridin treatment (Schneider et al., 1994; Castner et al., 2000). D1R agonist therapy may ameliorate cognitive impairment by enhancing insufficient DA tone in the PFC of patients with schizophrenia (Abi-Dargham and Moore, 2003; Goldman-Rakic et al., 2004; Williams and Castner, 2006; Granado et al., 2008).

Although collective data suggests that increased signaling at D1R may benefit cognitive function in settings with dopaminergic deficits, there is experimental evidence showing that prefrontal dopamine (DA) transmission operates within a defined working range for efficient cortical function (Goldman-Rakic et al., 2000; Seamans and Yang, 2004; Williams and Castner, 2006; Cools and D'Esposito, 2011). On one hand, hyperactivation of D1Rs in the PFC of rodents induces impaired working memory (Zahrt et al., 1997), while on the other, a series of studies showed D1R agonist-produced divergent effects on cognitive performance. These observations led to the hypothesis of an inverted U-shaped dose response curve of D1R function in working memory (Vijayraghavan et al., 2007), an idea that is further supported by clinical studies (Mattay et al., 2003).

Despite the importance of this target, there has been a notable paucity of agents available clinically (Zhang et al., 2009). To date, only a few D1R selective agonists, such as dihydrexidine and ABT-431, have been approved for clinical use. In a set of small clinical studies these compounds yielded ambiguous results such as unchanged (Girgis et al., 2016) and enhanced (Rosell et al., 2015) working memory performance, as well as unchanged (Girgis et al., 2016) and increased (Mu et al., 2007) prefrontal perfusion in schizophrenia patients, possibly due to their PK and tolerability limitations.

Recent reports describe a new series of structurally novel compounds which selectively activate D1 and D5 receptors and have favorable PK (Davoren et al., 2018; Gray et al., 2018). Compounds from this series have entered clinical study where their favorable PK was confirmed, and they demonstrated efficacy in reducing motor symptoms of Parkinson's disease (Papapetropoulos et al., 2018; Sohur et al., 2018) in single and repeated dose regimens and affects core aspects of cost-benefit decision making in humans (Soutschek et al., 2020a; Soutschek et al., 2020b). Herein, we characterize another exemplar of this new series of D1R-selective non-catechol agonists, PF-6142 (Davoren et al., 2018; Gray et al., 2018; Young et al., 2020) in preclinical assays evaluating its *in vivo* activity and further characterizing its pharmacological properties. This characterization includes studies which enable pharmacological comparison of PF-6142 to known D1R agonists in addition to behavioral and imaging paradigms which have not been previously explored with D1R agonists as summarized in **Table 1**.

TABLE 1 | Summary of experimental paradigms and doses of PF-6142 used.

Experiment	Acute dose (mg/kg)	Subchronic dose (mg/kg)	Figure
ACh level, rat	10, SC	10, SC for 5 d	1
ACh level, mouse	10, SC	10, SC for 5 d	1
LMA, mouse	0.32, 1, 3.2, 10, SC	1.78, 3.2, 10, SC	2
qEEG/PSG, rat	1.0, 5.6, SC		3
PPI, mouse	1.78, SC		4
CAR, rat	1.78, SC		4
RAM, rat	0.01, 0.056, 0.178, 0.56, SC		5
SDR, NHP	0.0015, 0.015, 0.15, SC		5
PPF, rat	0.1, 0.3, 1.0, 3.0, IV, cumulative		6

ACh, acetylcholine; FDG-PET, fluorodeoxyglucose positron emission tomography; IV, intravenous; NHP, nonhuman primate; PSG, polysomnography; LMA, locomotor activity; PPI, prepulse inhibition; CAR, conditioned avoidance response; PPF, paired-pulse facilitation; RAM, radial arm maze; SC, subcutaneous; SDR, spatial delayed response task.

MATERIALS AND METHODS

Drug Preparation Procedure

PF-6142 (synthesized in house by Pfizer Medicinal Chemistry group, Groton, CT; free base; Gray et al. (2018)) was dissolved in 5% dimethyl sulfoxide + 5% Cremophor EL + 90% sterile water or sterile saline + 0 to 3 molar equivalents of hydrochloric acid to a pH ~3–4 for subcutaneous administration both in rats and non-human primates (NHP). For intravenous (i.v.) administration, PF-6142 was dissolved in 20% (w/v) 2-hydroxypropyl-beta-cyclodextrin in sterile water for i.v. administration. Ketaset (Ford Dodge Animal Health, Iowa, USA; ketamine hydrochloride) was diluted in sterile saline. MK-801 (hydrogen maleate, Tocris Biosciences, Bristol, UK),

A-77636 (hydrochloride, Tocris), and R (+)-SCH-23390 (hydrochloride, Sigma-Aldrich, MO, USA) were corrected for the weight of the salt and dissolved in sterile water or sterile saline. Risperidone (Sigma-Aldrich) was dissolved in 1% glacial acetic acid in saline for mouse pre-pulse inhibition (PPI) studies, or in 0.3% (w/v) tartaric acid in sterile saline for conditioned avoidance responding experiments (CAR) in rats. Both risperidone solutions were adjusted to pH 4 with sodium hydroxide. Dose volumes for rats were 1 ml/kg, except for the CAR study which was 2 ml/kg. Dose volumes for mice were 10 ml/kg. Urethane was administered at 1.5 mg/kg dissolved in sterile water. PF-6142 was dissolved in 12% (w/v) sulfobutylether-beta-cyclodextrin for oral (p.o.) dosing for the polysomnography study. LY-451-646 was dissolved in 10% Cremophor EL in sterile water.

Animal Care

All animal procedures were approved by the Institutional Animal Care and Use Committee at Pfizer Inc. and conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals. All animals were at minimum 8 weeks of age at testing and were purchased from commercial vendors as follows: male C57BL/6J mice, male DRD1a wild-type (WT) and D1 DA receptor knockout mice (DRD1a-905781) from The Jackson Laboratory (Bar Harbor, ME); male CD-1 mice, male Fisher-344 rats and male Long-Evans rats from Charles River Laboratories (Kingston, NY); male Sprague-Dawley rats from Harlan Laboratories (Indianapolis, IN) for electroencephalography and polysomnography, as well as paired-pulse facilitation (PPF) studies and from Charles River Laboratories (Kingston, NY) for microdialysis studies. Rodents were group-housed in environmentally controlled animal quarters (light/dark-6:00 am/6:00 pm) and were acclimated to the facility prior to testing. Access to food and water was provided ad libitum to all rodents, except for the food restricted rats used for the radial arm maze (RAM) study and PET imaging.

An adult aged cohort of 10 male and female rhesus monkeys (Mucaca Mullata) were used for the spatial delayed response (SDR) task. They were maintained in accordance with the Yale/ Animal Care and Use Committees and federal guidelines for the care and use of nonhuman primates and were fed their full allotment of standard monkey diet (Harlan Teklad Monkey Diet, Madison, WI, USA) and fruit/vegetables prior to, during, and following the experiment described herein. Animals received their normal allotment of biscuits immediately following cognitive testing and were given species appropriate environmental enrichment such as foraging devices and safe items to play with.

Experimental Design and Statistical Analysis

Prefrontal Cortex Acetylcholine (aCh) Levels Determined *via* Microdialysis in Rat and Mouse

Surgery

Adult male Sprague-Dawley rats (280–360 g) were obtained from Charles-River Laboratories, Raleigh, NC and male C57BL/6J DRD1a WT and D1 DA receptor knockout mice (DRD1a-905781) were obtained from The Jackson Laboratories (Bar Harbor, ME). Animals were housed on a 12-h light/dark cycle with free access to food and water and allowed to acclimate for at least 5 d after arrival. Aseptic technique was used during the surgical procedure in order to prevent infection. On the day of the procedure animals were anesthetized with isoflurane (4%) and their heads shaved. The animals were then placed into a Kopf stereotaxic frame, the surgical area disinfected by swabbing with Provodine solution, and the area isolated with a sterile surgical drape. Anesthesia was maintained with isoflurane (2.5%–3%) delivered through a nose cone using a Univentor 400 anesthesia unit. Animals were given a 0.1 ml subcutaneous (s.c.) injection of Metacam (NSAID, meloxicam, 5 mg/ml, Boehringer Ingelheim) as a post-operative analgesic. Marcaine (bupivacaine, 0.5%, Hospira, Lake Forest, IL), a long acting local anesthetic, was administered s.c. at the surgical area to minimize pain and discomfort.

A 1.5–2 cm incision was made along the midline of the skull, beginning from a point just behind the eyes and running posterior. The skin was retracted with hemostats and the skull was further exposed using blunt dissection with cotton swabs. Bleeding capillaries were cauterized, and the skull dried with a sterile gauze sponge.

A microdialysis guide cannula [Bioanalytical Systems Inc (BAS), West Lafayette, IN, part # MD-2251] was placed into a guide holder on the stereotaxic frame and positioned over "Bregma". The guide cannula was positioned over the PFC (A-P, +3.2 mm; M-L, +0.7 mm, left, relative to bregma) and the location marked on the skull. Using a 0.7 mm burr, a hole was made in the skull at the cannula position. To facilitate attachment with dental cement, an additional three holes were made surrounding the cannula hole to accept bone screws. The three self-tapping bone screws were inserted, and the cannula positioned over the cannula hole then slowly lowered to a depth −1.3 mm below the surface of the dura. The guide cannula was then fixed to the skull using acrylic dental cement.

Microdialysis probes were inserted one to 2 d after guide implantation. Prior to insertion, BAS probes (part # MD2204, 4 mm) were flushed at 2 ul/min for approximately 15 min with artificial CSF (aCSF) of the following composition: 147 mM NaCL, 1.3 mM CaCl2, 2.7 mM KCl, and 1 mM $MgCl_2$. Animals were lightly anesthetized with isoflurane and the probe inserted. One to 2 h after insertion the probe flow was reduced to 0.3 µl/min and the animals allowed to recover overnight. At approximately 7:30 AM on the day after probe insertion the flow of aCSF through the probe was increased to 2 µl/min. Note- that in studies where HPLC-EC was used for **aCh** analysis, 100 nM neostigmine was added to the perfusion solution. After a stabilization period (typically around 1.5–2 h) several baseline samples were collected (15–30-min intervals) to establish an "average" basal level after which drug treatment was initiated. Samples were either collected on-line for analysis of ACh content by high performance liquid chromatography (HPLC) in conjunction with electrochemical detection (EC) or collected off-line for simultaneous determination of ACh

content by liquid chromatography tandem mass spectrometry (LC-MS/MS).

Sample Analysis

HPLC/EC. For conventional analysis, ACh was analyzed by high performance liquid chromatography (HPLC) utilizing a modification of the BAS ACh-choline assay kit (BAS part # MF-8910). Note that for this analysis procedure 100 nM neostigmine bromide was added to the probe perfusion solution to increase the detection reliability of ACh. Briefly, ACh was separated at a flow rate of 1 ml/min and a temperature of 28°C on two 10 cm ACh analytical columns (BAS part # MF-6150) connected in series, using a mobile phase containing 35 mM Na_2HPO_4, 0.1 mM EDTA, and 0.005% ProClin® and adjusted to pH 8.5 with phosphoric acid. ACh was then converted in a post-column acetylcholinesterase-choline oxidase immobilized enzyme reactor (BAS part # MF-6151) to hydrogen peroxide, which was detected electrochemically at a platinum electrode maintained at a potential of +0.5 V vs. Ag/AgCl. Chromatography data were collected and quantified by comparison to known standard concentrations using EZChrom Elite software (Agilent Technologies, Inc, Santa Clara, CA). Chromatography data for individual samples is archived on the Pfizer server \\groamrapp285 \ezchrom.

LC-MS/MS. Samples were collected off-line and dialysate ACh and histamine levels were determined using LC)-MS/MS and in the absence of locally perfused neostigmine. Microdialysates (30 µl sample volume) were collected at 15-min intervals into glass vials containing 4 µl of 10% acetic acid using a refrigerated fraction collector then stored frozen at −80°C for later analysis. Prior to analysis, deuterated Acetylcholine-1,1,2,2-d4 bromide (200 ng/ml) and deuterated Histamine-α, α, β, β-d4 dihydrochloride (1,000 ng/ml) were added to each sample as an internal standard in a volume of 70 µl. Analytes (10 µl injected sample volume) were separated on a Waters Atlantic Hilic column (100 x 2.1 mm, 3 µm particle size) at a temperature of 25 °C using a Waters Acquity Ultraperfomance liquid chromatograph (Waters Corporation, Milford, MA). Separation was achieved at a flow rate of 0.22 ml/min using a binary solvent gradient elution where solvent A consisted of 20 mM ammonium formate in 1% formic acid, pH 3.4 and solvent B was 100% acetonitrile. Each cycle began with a linear gradient running from 10% to 70% solvent A over 3 min and was then held at 70% solvent A for 1.5 min before returning to 10% solvent A in 0.5 min. The effluent from the LC column was directed at the electrospray interface of the mass spectrometer. LC-MS/MS analyses were performed using a Sciex API 3000 triple quadrapole mass spectrometer equipped with a turboionspray source (AB Sciex, Framingham, MA). The ion spray voltage was set at 1,500 V and the source temperature at 450° C. The mass spectrometer was operated in the positive ion electrospray mode with the following parameters: declustering potential, 25 V; focusing potential, 100 V; entrance potential, 5 V; collision cell exit potential, 22 V. Nitrogen was used for both the curtain and collision gas with an ion energy of 6 and 8 eV, respectively. ACh, d4 ACh, histamine, and d4 histamine were monitored using multiple reaction monitoring (MRM) mode. The MRM transitions m/z 146.2→87.1 and 150.2→91.3 were sequentially monitored for the detection of ACh and deuterated ACh, respectively. The MRM transitions m/z 112.2→95.1 and 116.1→99.0 were sequentially monitored for the detection of histamine and deuterated histamine, respectively, LC-MS/MS data were collected and analyzed by comparison to known standard concentrations using Analyst software version 1.4.1. (AB Sciex, Framingham, MA). Added details of the LC-MS/MS procedure are in E-Notebook VBN#00702189 in the Published PDF/Root/Research/Groton/E-H/Gorczyca, Roxanne R VBN#00702189/Methods/LCMS-MS protocol ACh, and HA microdialysate/PDFs/20120105-1412-v2-LCMS-MS protocol ACh and HA microdialysate.

Data Analysis

Statistical analyses were performed using Graph Pad Prism 5 software. Raw time course data was normalized for variation in basal levels among animals by converting each time point to a ratio of the response over the average baseline level (3–5 samples prior to 1st treatment) for each animal. It is referred to as fraction of baseline.

Statistical Tests

To test for significant changes from baseline, three fraction of baseline values were averaged for each treatment period. Changes from basal were evaluated using repeated measures one-way ANOVA with Dunnett's post-hoc tests. To test for significant differences in time course data a repeated measures two-way ANOVA with Bonferroni correction for multiple testing were performed.

Mouse Locomotor Activity (LMA)

Locomotor activity data were measured by an automated infrared photo-beam system in sound attenuating chambers controlled by Versamax® software, provided by Accuscan Instruments Inc. (Columbus, Ohio), which quantified beam breaks similar to the methods used previously (Xu et al., 2000). To test D1 receptor selectivity in a pharmacologic model, C57BL/6J mice were habituated to the apparatus for 90 min, followed by pretreatment with vehicle or SCH-23390 (0.01, 0.032, 0.1 0.32, s.c.) and returned to the apparatus for 30 min. After the 30-min pretreatment period mice were administered vehicle (s.c.) or PF-6142 (0.32, 1, 3.2, 10 mg/kg, s.c.) and returned to the apparatus at which time activity was measured for a 2-h period (**Figure 2A**). Data were compared against the vehicle + PF-6142 (10 mg/kg) group using a repeated measures one-way ANOVA with a Dunnett's post-test. The repeated treatment data were obtained from C57BL/6J mice that were habituated to the apparatus for 90 min, dosed with vehicle or PF-1642 (1.78, 3.2, 10 mg/kg, s.c.), returned to the chamber and measured for activity for 2 h. The same mice were treated for five consecutive days and data are presented in **Figure 2B**. Data were compared against the vehicle group using a repeated measures one-way ANOVA with a Dunnett's post-test. To test D1 receptor selectivity in a genetic model, DRD1a WT and knockout mice (D1 KO) were habituated to the apparatus for 90 min, pretreated with vehicle

or SCH-23390 (0.032 mg/kg, s.c.) and returned to the apparatus for 30 min. After 30 min mice were administered PF-1642 (10 mg/kg, s.c.) or vehicle control (s.c.) and returned to the apparatus for an additional 2 h during which time cumulative activity was recorded (**Figure 2C**). Data were compared within genotype using a two-way ANOVA with a Dunnett's post-hoc test versus vehicle treated group.

Rat Electroencephalography and Polysomnography

Model 4ET telemetry device components (Data Sciences International, St Paul, MN, USA) were bilaterally placed in subcutaneous pockets on the dorsal flank of adult male Sprague-Dawley rats (200–400 grams). Two pairs of leads were implanted superficially to burr holes drilled over the frontal cortex (stainless steel screws, Plastics One, Roanoke, VA at coordinates: A-P = +1.5 mm, M-L = 1.5 mm) and parietal cortex (coordinates: A-P = −3.7 mm, M-L = −2.2 mm), and the cerebellum bilaterally to be used as ground and reference for electroencephalographic (EEG) recordings. A stainless-steel wire (Plastics One, Roanoke, VA) was implanted into the neck muscle to monitor electromyogram (EMG). All recordings were performed inside the home cages of animals using RPC-2 telemetry receivers (Data Sciences International, St. Paul, MN) at a sampling rate of 500 Hz for data acquisition. Baseline data, while on vehicle, were obtained for 24 h prior to compound administration. PF-6142 (1.0 or 5.6 mg/kg, s.c.) was administered acutely following the baseline day.

Raw EEG traces were analyzed using custom scripts in MATLAB (The Mathworks, Natick, MA, version 7.8 (R2009a)) to evaluate spectral changes between treatment groups. Raw EEG data were read into the MATLAB software, segmented to match that of the polysomnography (PSG) data (see below) and fast Fourier transforms (FFTs) were performed. Only data collected on the parietal lead were the subject of statistical analyses. PSG analysis was applied to all EEG/EMG data and utilized an in-house algorithm developed in LabView (National Instruments, Austin TX) as previously described (Harvey et al., 2013).

Relative power data in each band aggregated over 2 h-long time bins, while on drug, were normalized to baseline levels in corresponding time bins relative to dosing. For statistical analysis the R software was used. The effects of two within factors, dose and time, on cumulative time spent in three stages, awake, REM, and NREM sleep were assessed using generalized linear mixed model. The model specification explicitly accounted for a crossover design with repeated measures by introducing auxiliary factors, day, treatment sequence, and day by time product. To account for correlations within subjects, we employed a first order autoregressive scheme, which assumes that correlations decay exponentially with the lag between the measurements. The model was fitted using the method of restricted maximum likelihood. Significant findings were followed by least significant difference tests for pairwise differences across doses and across doses at fixed times for treatment and treatment by time factors, respectively. Tukey-Kramer procedure was used to adjust for multiple hypothesis testing. For all statistical tests, $p < 0.05$ was considered significant. The same fixed factors and type of statistical model were used to explain cumulative power within EEG power bands; the power was log transformed before the analysis.

Mouse Prepulse Inhibition (PPI)

Drug and behaviorally naïve adult male C57BL/6J mice (9–11 weeks of age; n=8 per dose group) were used for PPI experiments. Subjects were tested individually in SR-Lab acoustic startle chambers (San Diego Instruments, San Diego CA, USA) equipped with a restrainer mounted atop a piezoelectric accelerometer which measured transduced movement in response to the presentation of audio stimuli presented through a speaker mounted 20 cm above the animal. Subjects were acclimated to an anteroom adjacent to the testing room at minimum 60 min prior to testing. Test sessions began with a 5 min acclimation period to background noise (65 dB) followed by presentation of six randomized repetitions of the 120 dB startle stimulus (40 ms duration) presented alone or paired in combination with a pre-pulse stimulus of 68, 72, or 74 db (20 msec in duration) presented 80 msec prior to the 120 dB startle stimulus, which was equivalent to +3, +7, and +9 db over background noise, respectively. Data were also recorded for no stimulus values to evaluate background level of response. The inter-trial interval between stimulus presentations was randomized and ranged from 10 to 20 s. Test compounds were administered 30 min (s.c.) prior to testing. For experiments evaluating both PF-6142 and risperidone, each compound was administered at a different injection site (s.c.) with PF-6142 injected immediately prior to risperidone. Percent PPI was calculated for each individual subject as the relative change in the 120 dB startle response in the presence of each prepulse intensity using the formula: 100-((prepulse-pulse)/pulse)*100 as previously described (Ralph-Williams et al., 2003).

The experiment was analyzed using a two-way mixed model ANOVA (lme4 library in R software). The model included prepulse stimulus intensity levels, treatment (as all combinations of pre-treatment and treatment), and their interaction as fixed factors and random intercept, random slope for each animal as random factors. Significant ANOVA results were followed by planned post-hoc contrasts of least squared means across treatment arms at each prepulse stimulus intensity levels, slopes with respect to prepulse stimulus intensity levels, and planned contrast between slopes. Three-way ANOVA model with pre-treatment and treatment handled as independent factors yielded the same findings for all planned comparisons (not reported here). In order to adjust for multiple hypothesis testing we used false discover rate method which controls the expected proportion of false discoveries among the rejected hypotheses. For all statistical tests, $p < 0.05$ between groups was considered significant.

Rat Conditioned Avoidance Response (CAR)

The CAR assay was performed under similar conditions previously described (Marquis et al., 2011) at WuXiAppTec Inc. (Delin Rd. #90, Waigaoquiao Free Trade Zone, Shanghai 200131, China). For this study, adult male Fisher-344 rats were trained and tested in a two-way active avoidance apparatus with MED-PC software (MED Associates, St. Albans, VT, USA).

Briefly, subjects were handled and acclimated to the shuttle boxes for 2 d prior to training sessions. During training sessions, subjects were trained to avoid an electric footshock by moving to the adjacent, non-stimulus side of the shuttle box upon presentation of tone + light stimuli which preceded the presentation of the footshock (0.6 mA, 10 s duration) by 10 s. For this assay an avoidance was defined as moving to the adjacent compartment during the tone+light presentation that preceded the shock, an escape was defined as moving to the adjacent compartment upon presentation of the shock, and an escape failure was defined as a lack of relocation to the adjacent compartment throughout the presentation of the shock. Subjects received 30 trials of training per day for 5 d. Subjects with ≥ 80% avoidance responses on two consecutive days with no escape failures, were considered qualified for testing, and were randomized across treatment groups (n=8–9 per treatment group). PF-6142 (1.78 mg/kg) and risperidone (0.1 or 0.56 mg/kg), and their respective vehicles were administered (s.c.) 60 and 30 min, respectively, prior to the start of the experiment at separate injection sites. Avoidance responding was calculated as % of the number of total trials in which an avoidance occurred. Data were calculated for each animal and compared across treatment groups using one-way ANOVA with Dunnett's post-test versus the vehicle + vehicle treated group.

Ketamine-Disrupted RAM Experiment

Two cohorts of adult male Long-Evans rats (N=30 each) were trained in a spatial working memory task on an eight-arm RAM, (Pathfinder Maze System, Lafayette Instrument Co., Lafayette, IN), using a procedure adapted from Ward et al. (1990) and described in detail in Strick et al. (2011). Briefly, rats were food-restricted to provide motivation to perform the RAM task. The task requires that the animals enter each arm to retrieve a reinforcement food pellets, using spatial cues in the room to remember which arms of the maze they have previously entered. Rats were individually placed on the maze and allowed to navigate until all eight arms were entered and the pellets were consumed or until 30 choices were made, or until 5 min had elapsed. Entry into an arm previously entered was counted as an error. If an animal failed to choose all eight arms in 5 min, the arms not chosen were also counted as errors. Training continued until all animals had reached the training criterion, defined as two or fewer errors on two consecutive days. Administration of the NMDA antagonist, ketamine, to well-trained rats consistently produces significant disruption of performance in the RAM task, resulting in a significant increase in the number of working memory errors. This study was designed to test the ability of D1 agonists to reverse ketamine-induced working memory deficits in well-trained rats. On test days, animals that met training criteria were randomly assigned to treatment groups and administered vehicle or PF-6142 (0.01, 0.056, 0.178, 0.56 mg/kg, s.c.), followed 90 min later by administration of ketamine (10 mg/kg, s.c.). Performance on the maze was evaluated 30 min later by an observer that was blinded to treatments.

The R 3.0.1 statistical software was used to compare the error rate data. The effects of treatment and the interaction on the error rate were assessed using a one-way mixed model ANOVA using generalized least squares method from lme4 library. Significant ANOVA results were followed by post-hoc pairwise comparisons of least squared means across treatment arms. In order to adjust for multiple hypothesis testing we used false discover rate method which controls the expected proportion of false discoveries among the rejected hypotheses. For all statistical tests, $p < 0.05$ between groups was considered significant.

SDR in the Nonhuman Primate

Administration of the NMDA antagonist ketamine consistently produces significant disruption of performance in the SDR task in nonhuman primates, resulting in a significant increase in the number of working memory errors. This study was designed to test the hypothesis that PF-6142 would provide significant protection versus the ketamine-induced working memory deficits in nonhuman primates.

Cognitive Testing

Rhesus monkeys were trained to stability on a variable SDR task in a sound-attenuated Wisconsin General Testing Apparatus (Roberts et al., 2010). Briefly, subjects observe while the investigator baiting one of two to seven wells with a highly preferred food reward and then covers all the wells with identical square plaques. An opaque screen is then lowered for one of five variable delays, which are pseudorandomized across trials within a session. Thus, delays are defined as 0–4 N, where "N" is a value that is animal dependent and ranges from 1 to 10 s depending upon the difficulty level of the task at which an animal reaches the criterion of stable performance. At the end of the delay period, the opaque screen is raised, and the animal must select the well that had been baited to obtain a reward. Each test session consists of 20 trials wherein both the baited well and delay length are pseudorandomized across trials. Before study initiation, all subjects were required to reach stability over a period of 10 consecutive test sessions, where stability was defined as an average of 65–75% correct. Stability was attained by varying the number of wells and the delay value for each animal. Subjects were originally trained on a two-well board with an N value of 1. The N value and the number of wells were gradually increased until the animal consistently scored within stability range. Once stability was attained for a given number of wells and N value, that combination was kept constant throughout the course of the study (Roberts et al., 2010). The range of stable performance for the 10 subjects was two to five wells and an N value of 1–7 s (median, four-well testing board, N = 5 s). Data were transformed using logarithmic function [log(x+5)] to improve normality and analyzed using ANOVA.

Drug Administration

Subjects received pretreatment with vehicle (sterile saline solution) or PF-6142 (0.0015, 0.015, 0.15 mg/kg, s.c.) 4 h before cognitive testing. They then received an intramuscular injection of either vehicle (sterile saline) or ketamine (0.7–1.7 mg/kg; Fort Dodge Co.) 0.25 h before cognitive testing. The dose of ketamine for each animal was predetermined such that all animals achieved a comparable magnitude of cognitive

impairment (e.g., a score of less than ~50% correct) relative to their pretreatment baseline performance of ~70% correct. Thus, for the present study, one monkey received 0.7 mg/kg, seven monkeys received 1.0 mg/kg, and two monkeys received 1.7 mg/kg of ketamine. The vehicle/PF-6142 and vehicle/ketamine treatments were assigned using a randomized Latin square design with a total of eight conditions. Except for the vehicle/vehicle condition, there was a minimum 2-week washout period between all acute challenges during which time animals were required to "restabilize" to baseline performance levels, which was defined as at least three consecutive testing sessions wherein cognitive performance ranged between 65% and 75% correct.

PPF and Delta Field Potential Oscillation Power Measurement in Rat

Experiments were performed on n=10 adult male Sprague-Dawley rats (275–305 g) under urethane anesthesia (1.5 g/kg, i.p.). The femoral vein was cannulated for i.v. administration of drugs. A stimulation electrode was placed in the CA1/subiculum region (coordinates: A-P = +6.3 mm, M–L = +5.2 mm, D-V = +8.0 mm) using stereotactic methods and unilateral local field potential (LFP) was recorded by a metal monopolar macroelectrode placed into the medial PFC (mPFC; coordinates: A-P = +3.0 mm, M-L = +0.6 mm, D-V = +5.0 mm). The LFP was amplified, filtered (0.1–100 Hz), displayed and recorded for on-line and off-line analysis (Spike2 program, CED, Cambridge, UK). Evoked responses to the first and the second stimuli were identified (P1 and P2, respectively) and the amount of PPF determined by the formula: (P2 amplitude/P1 amplitude). Waveform averages used to calculate PPF consisted of 60 consecutive stimuli. Five minutes were allowed between administration of each drug dose and the starting of the subsequent average of each 10-min period after each cumulative dose (0.1–1 mg/kg, IV). Disruption in power of LFP delta activity was measured as the percentage of power in low frequency (0–1.8 Hz) irregular activity in the total (0–4 Hz) delta power range. LFP power spectra were determined during periods concurrent with waveform averages and PPF calculation. Statistical significance was determined by means of two-tailed paired Student's t-test.

Plasma Protein Binding and PK Studies

Across the set of experiments, PK was either collected from satellite animals or in separate, dedicated studies. In addition to plasma PK, exposures were obtained in brain tissue for rat and mouse, and plasma protein binding and brain tissue binding were measured. Using the measured exposures, partitioning, and binding parameters, and the

$$RO(\%) = \frac{C_{b;u}(\mathrm{nM})}{C_{b;u}(\mathrm{nM}) + K_i(\mathrm{nM})} \cdot 100$$

equation, a correlated receptor occupancy estimate (RO) was calculated for each of the exposures presented in **Table 2**.

RESULTS

Acute and Sub-Chronic PF-6142 Increases ACh Levels in Rat and Mouse and the Effect Is Attenuated in the PFC of D1 Knockout Mouse

Time course data showing the ability of PF-6142 (10 mg/kg, SC) to increase ACh levels in the rat PFC after five consecutive days of treatment is presented in **Figure 1A**. An analysis of the time course data indicates that treatment with vehicle followed by PF-6142 on day 5 [vehicle (sub-chronic) + PF-6142 (acute) group on **Figure 1A**] increased cortical ACh levels in dose dependent manner post-dose as compared to 5 d of vehicle treatment [vehicle (subchronic) + vehicle (acute) group seen in **Figure 1A**, $F(\text{treatment})_{2,\ 19} = 24.74$, $p = 0.0101$, $F(\text{time})_{17,\ 323} = 17.76$, $p < 0.0001$, $F(\text{interaction})_{3,\ 42} = 6.224$, $p = 0.023$]. A comparison of the overall responses on day 5, expressed as the change in the area under the curve over the 75–180 min post-treatment time period, also shows that the PF-6142 mediated increase in cortical ACh was maintained after repeated dosing for five consecutive days ($F_{2,\ 19} = 12.09$, $p = 0.0004$; **Figure 1B**). Further, acute effects of PF-6142 and SCH-23390 in DR knockout and WT mice revealed an effect of treatment ($F_{2,\ 42} = 9.183$, $p < 0.0001$) and interaction ($F_{3,\ 42} = 3.4$, $p = 0.0263$; **Figure 1C**). Post-hoc analysis revealed that only the PF-6142 treatment in the WT group but not in the KO group had increased ACh levels when compared to vehicle.

PF-6142 Acutely and Subchronically Increases Locomotor Activity in Mice and Is Attenuated by D1 Receptor Blockade

PF-6142 dose-dependently increased horizontal activity ($F_{4,35} = 9.509$, $p < 0.0001$) and achieved significance at the 10 mg/kg dose ($p < 0.0001$). To confirm D1 selectivity of PF-6142, animals were pretreated with D1 antagonist SCH-23390 (0.01, 0.032, 0.1, and 0.32 mg/kg, s.c.) in **Figure 2A** which showed a strong effect of treatment ($F_{4,42} = 57.77$, $p < 0.0001$). SCH-23390 blocked PF-6142 (10 mg/kg; s.c.)-stimulated activity in mice ($p<0.0001$). Locomotor activity data from five consecutive days of dosing with D1 agonists are presented in **Figure 2B**. A-77636 a selective D1 receptor full agonist (3.2 mg/kg, s.c.) and PF-6142 (10 mg/kg, s.c.) increased locomotor activity in CD-1 mice [$F(\text{treatment})_{4,\ 175} = 577.2$, $p < 0.0001$, $F(\text{day})_{4,\ 175} = 1.817$, $p = 0.1276$, $F(\text{interaction})_{16,\ 175} = 2.319$, $p = 0.0040$]. PF-6142 (1.78 mg/kg) did not significantly increase horizontal activity while PF-6142 (3.2 mg/kg, s.c.) only increased activity significantly on days 4 and 5 of testing ($p = 0.0311$ and $p = 0.0309$, respectively). The daily comparison of the 3.2 mg/kg group alone did not reveal any changes between days 1–5 ($F_{4,\ 35} = 0.5469$, $p = 0.7025$). WT and D1 KO mice were treated with PF–6142 (10 mg/kg; s.c.) and data is presented in **Figure 2C** [$F(\text{treatment})_{2,\ 42} = 23.72$, $p < 0.0001$ and $F(\text{interaction})_{2,\ 42} = 10.91$, $p = 0.002$]. PF-6142 increased activity in the WT mice ($p < 0.0001$) and the effect

was attenuated by SCH-23390. The KO mice did not show hyperactivity in response to PF-6142 treatment ($p = 0.6731$).

In Vivo Freely Moving Electrophysiological Recordings

PF-6142 Significantly and Dose-Dependently Decreases Delta and Increases Beta and Gamma Oscillation Power

Freely moving animals were dosed with PF-6142 in their home cage. Electroencephalographic (EEG) data were recorded for 24 h following treatment to allow for the monitoring of long-term effects (**Figure 3A**). To remain within the expected window of treatment-related effects, time-collapsed statistical analyses of the quantitative EEG data were limited to the first 4 h. The statistical model revealed that during the first 4 h following treatment PF-6142 significantly decreased the change in delta oscillation power from its baseline value ($F_{2,\ 10} = 12.8$, $p = 0.002$). Post-hoc testing showed that the high dose (5.6 mg/kg, s.c.; $t_{15} = 5.0$, $p = 0.0004$) but not the low dose (1.0 mg/kg, s.c.; p = 0.3) resulted in significant delta power decrease relative to the vehicle treatment. Contrary to the changes in delta power, PF-6142 treatment resulted in a significant increase in beta ($F_{2,\ 10} = 5.9$, $p = 0.02$) and gamma ($F_{2,\ 10} = 21.3$, $p = 0.0003$) powers. Post-hoc tests again showed that only the high dose resulted in a significant change ($t_{15} = -2.7$, $p = 0.04$ for beta, and $t_{15} = -6.3$, $p < 0.0001$ for gamma). Power in other studied frequency bands was not found to change significantly, however the total power in the EEG signal decreased significantly ($F_{2,\ 10} = 10.3$, $p = 0.004$) with post-hoc testing confirming that only treatment with the high dose created a significant total power decrease ($t_{15} = 3.9$, $p = 0.004$).

PF-6142 Significantly and Dose Dependently Decreases the Time Spent in Sleep and Increases the Time Spent Awake

To further elucidate the effects of a D1 agonist on the vigilance state of freely moving rats housed and studied in their home cages, EEG and EMG data was subjected to PSG analysis (**Figure 3B**). Like the quantitative EEG analysis, the time-collapsed statistical analyses were limited to only the first 4 h following treatment. The statistical model revealed that treatment effects were significant for changes in the fraction of time spent in rapid eye movement (REM) sleep, ($F_{2,8} = 32.7$, $p = 0.0001$). Post-hoc pairwise comparisons *via* least squares means identified that the vehicle group had 88.2% higher fraction of REM sleep compared to the high dose group, which was significant ($t_{11} = 6.15$, $p = 0.0002$). Similarly, time spent in slow-wave sleep (SWS) was also found to significantly decrease ($F_{2,\ 10} = 56.8$, $p < 0.0001$), again with an 88.1% decrease relative to the vehicle group achieved following the administration of the high dose ($t_{15} = 10.3$, $p < 0.0001$). Since the D1 agonist suppressed sleep, it was expected that animals would spend more time awake. This was supported by the statistical analysis which confirmed that there was a significant increase of the time spent in wakefulness ($F_{2,\ 9} = 14.3$, $p = 0.001$). Animals spent significantly more time awake following the high dose (246.9% increase, $t_{15} = -5.3$, $p = 0.0003$).

TABLE 2 | Representative Exposure Data.

Species	Dose (mg/kg)	Time (h)	PF-6142 plasma concentration (ng/ml)	Calculated brain D1R occupancy estimate (%)
mouse	5.6 (SC)	1	332	~25
rat	10 (SC)	1.5	1260	~55
NHP	0.1 (SC)	0.5	31	~20

PF-6142 Does Not Impact the Effect of Risperidone on Mouse Startle and PPI

Consistent with previous published reports, vehicle treated C57BL/6J mice demonstrated prepulse dependent increases in % PPI which was dose dependently increased by pretreatment with risperidone (0.1–0.56 mg/kg) (**Figure 4A**). ANOVA revealed that the effect of prepulse stimulus intensity level was significant [$F(\mathrm{dB})_{1,\ 42} = 95.9$, $p < 2.1{\cdot}10^{-12}$], the effect of treatment was significant [$F(\mathrm{treatment})_{\ 5,\ 42} = 8.67$, $p = 1.04{\cdot}10^{-5}$], and their interaction was significant [$F(\mathrm{int})_{5,\ 42} = 3.94$, $p = 0.005$]. Post-hoc analysis showed that risperidone dose-dependently increased PPI at all pre-pulse intensities as expected, resulting in significantly higher PPI values following high dose risperidone treatment than vehicle for all decibel levels ($t_{42} = 4.79$, $p = 0.0001$ for 3 dB; $t_{42} = 3.4$, $p = 0.004$ for 7 dB; $t_{42} = 2.63$, $p = 0.029$ for 9 dB). However, consistent with the known side effect profile of risperidone, acoustic startle responses (120 dB) were dose dependently and significantly reduced (**Figure 4B**). Therefore, for the evaluation of PF-6142, experiments were conducted both with an ineffective dose of risperidone that did not alter startle responses (0.1 mg/kg) and a high dose of risperidone (0.56 mg/kg). For these combination experiments, to assess whether D1 agonism affected PPI, PF-6142 at a dose of 1.78 mg/kg was co-administered with risperidone (**Figure 4A**). This dose was selected as the highest dose of PF-6142 that in pilot experiments (**Figure 4C**) produced a modest but not significant reduction in %PPI. As expected, risperidone at only the high dose (0.56 mg/kg) but not the low dose (0.1 mg/kg) produced an increase in %PPI. In combination with risperidone, there was no effect of PF-6142 (1.78 mg/kg) on %PPI across prepulse intensities ($t_{42} = -0.74$, $p = 0.477$ for 3 dB; $t_{42} = -0.67$, $p = 0.5044$ for 7 dB; $t_{42} = -0.59$, $p = 0.5555$ for 9 dB). PF-6142 (1.78 mg/kg) produced modest impairments in %PPI which was significant relative to vehicle treated control at only the 9 dB prepulse intensity ($t_{42} = -2.43$, $p = 0.029$) which was not unexpected based modest reductions in %PPI observed in previous data (**Figure 4C**). Importantly the presence of PF-6142 did not alter risperidone's effects on PPI.

PF-6142 Has No Effect on CAR Alone or in the Presence of Risperidone in Rats.

PF-6142 was tested in the rat CAR assay for antipsychotic-like activity alone and in the presence of risperidone. PF-6142 alone (0.32–5.6 mg/kg, s.c.). did not alter % avoidance responses which relative to vehicle treated control. Mean % avoidance responses for PF-6142 were 95.93, 98.47, 95.57, and 95.75% for PF-6142 at

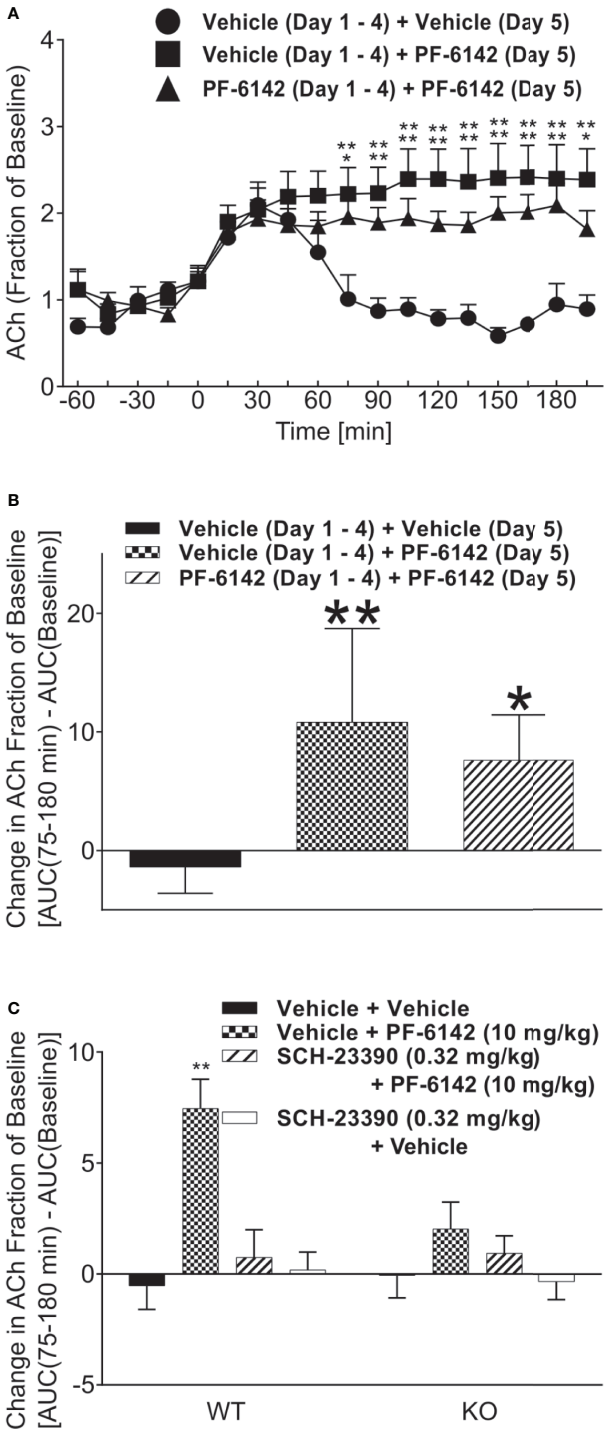

FIGURE 1 | Acetylcholine following administration of PF-6142 in rat and mouse. Acetylcholine levels in the rat prefrontal cortex (PFC) following subchronic dosing with PF-6142. **(A)** Time course data comparing the effect of vehicle or PF-6142 (10 mg/kg, SC) on ACh levels in the rat PFC after repeated dosing for 5 d. **(B)** 75–180-min total area under the curve of time course data. Points represent the mean + SEM, One-way ANOVA with Dunnett's post-test adjusted *$p < 0.05$, **$p < 0.01$ vs. vehicle. N = 7–8. **(C)** wild-type (WT) and D1 KO mouse acetylcholine levels in the PFC. 75–180-min total area under the curve (AUC) data for WT and D1 KO mice treated with PF-6142 (10 mg/kg, SC) and SCH-23390 (0.32 mg/kg, SC). Two-way ANOVA with Tukey's post-test adjusted *$p < 0.05$, **$p < 0.01$ vs. vehicle + vehicle within genotype. N = 6–7.

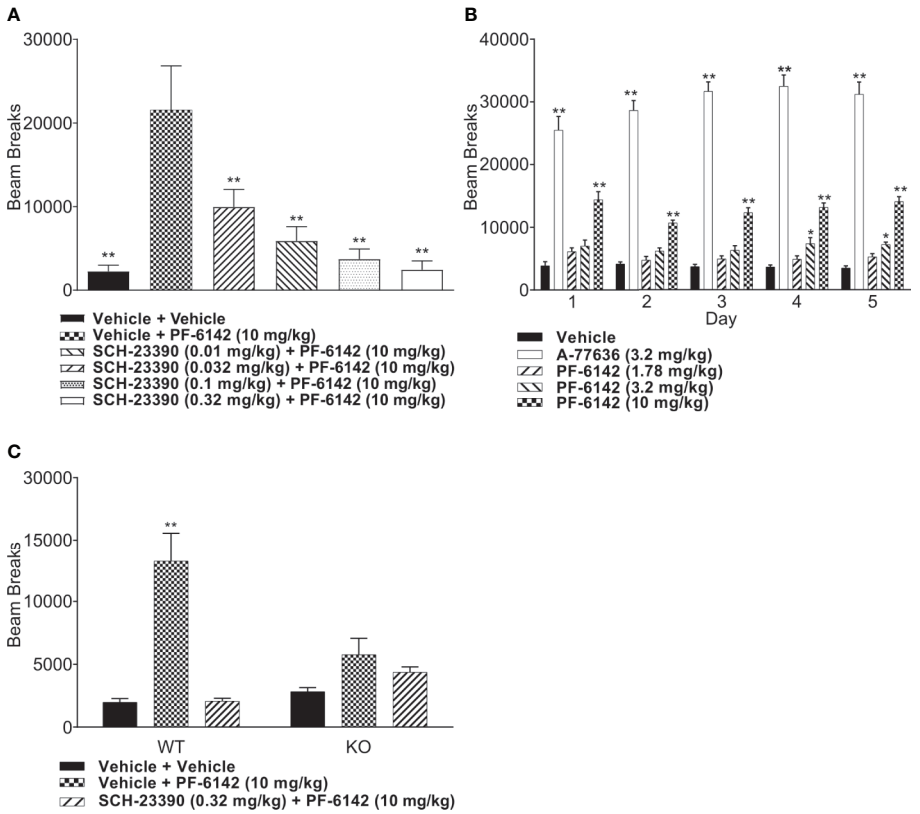

FIGURE 2 | Mouse locomotor activity (LMA) following administration of PF-6142. **(A)** Treatment with PF-6142 increases the number of beam breaks and pretreatment with the D1 antagonist, SCH-23390, effectively and dose dependently blocks the hyperactivity induced by PF-6142. **(B)** Daily administration of D1 agonists. **(C)** The hyperactive response is greatly diminished in the D1 KO mice compared to WT mice. Data are shown as the mean beam breaks + SEM. N = 8. One-way ANOVA with Dunnett's post-test adjusted *p < 0.05, **p < 0.01 vehicle + PF-6142 (10 mg/kg) **(A)**. Two-way ANOVA with Dunnett's **(B)** or Tukey's **(C)** post-test adjusted *p < 0.05, **p < 0.01 vs vehicle **(B)** or vehicle + vehicle **(C)**.

doses of 0.32, 1.78, 3.2, and 5.6 mg/kg, respectively, were analyzed with one-way ANOVA versus vehicle treated controls ($F_{4, 37} = 0.6378$, $p = 0.64$). Therefore, for combination studies, of PF-6142 with risperidone, a dose of 1.78 mg/kg PF-6142 was selected. As presented in **Figure 4D**, risperidone (0.1–0.56 mg/kg, s.c.) produced the expected dose-dependent reductions in avoidance responding consistent with an antipsychotic-like profile in this assay. Mean % avoidance responses for vehicle, 0.1, and 0.56 mg/kg risperidone were 91.88, 83.33, and 6.875%, respectively with significant reductions at 0.56 mg/kg risperidone ($p < 0.001$). PF-6142 (1.78 mg/kg, s.c.) resulted in % avoidance responding of 94.38% which was not different than vehicle. In the presence of risperidone at either dose, PF-6142 did not alter % avoidance responses produced by risperidone which were 76.25 and 8.125%, respectively. One-way ANOVA versus vehicle treated controls revealed an effect of treatment with significant reductions observed only with 0.56 mg/kg risperidone alone or with 0.56 mg/kg risperidone in combination with PF-6142 ($F_{5, 43} = 78.75$, $p < 0.0001$).

PF-6142 Reversed Ketamine-Induced Deficits in RAM Performance in Rats

Error ratio data from the rat RAM task are presented in **Figure 5A**. Ketamine (10 mg/kg, s.c.) treatment caused a robust increase in error rate (errors/choices) in rats trained to perform the RAM task. LY-451646 (0.32 mg/kg, s.c.), an α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor positive allosteric modulator, was used as a positive control and was shown to decrease the ketamine-induced error rate as expected. PF-6142 (0.01–0.56 mg/kg, s.c.) decreased the ketamine-induced error rate at all, except for the lowest dose administered ($F_{6, 48} = 5.72$, $p = 0.0002$).

PF-6142 Ameliorates Ketamine-Induced Deficits in Nonhuman Primate Spatial Working Memory

Performance data (percent correct responses) from the NHP SDR task are presented in **Figure 5B**. Treatment with ketamine

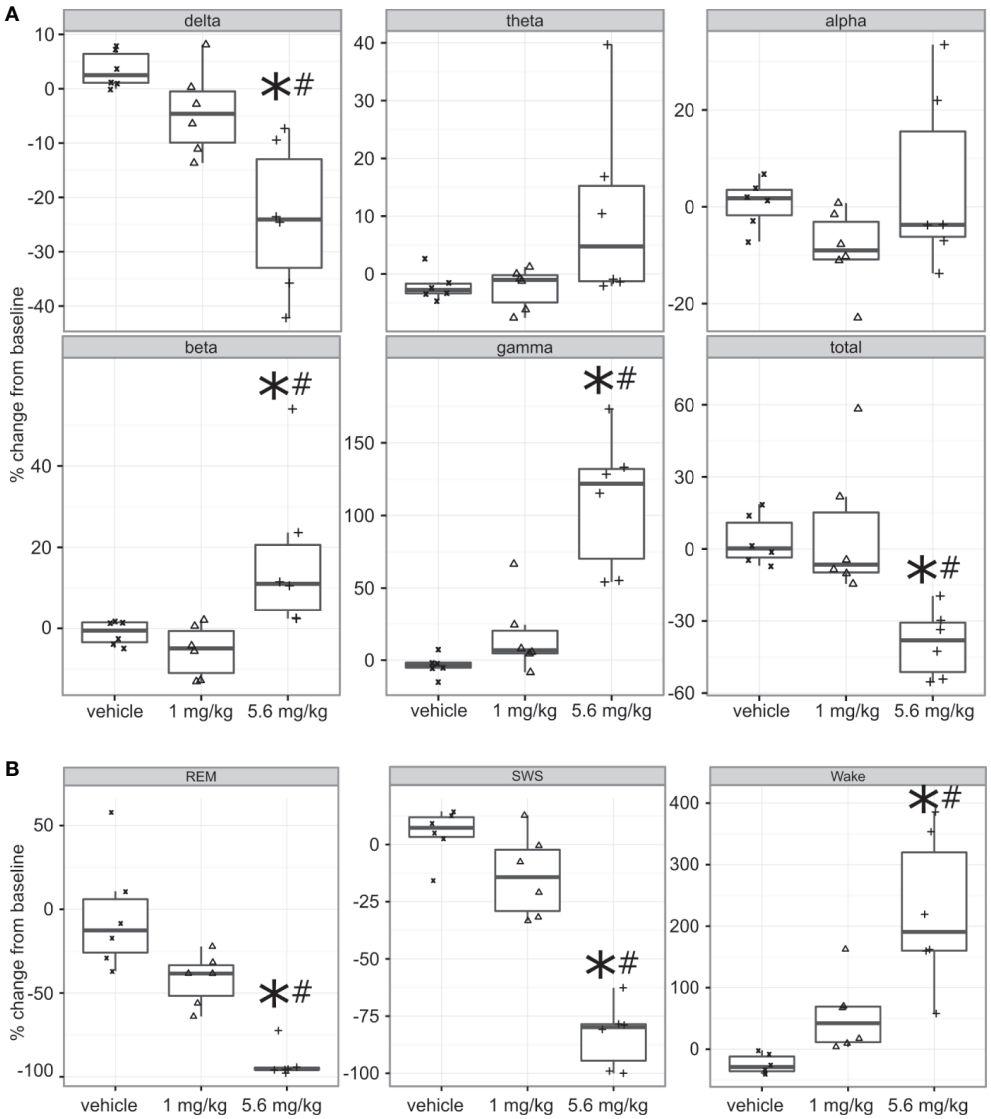

FIGURE 3 | Quantitative electroencephalography and polysomnography analysis of treatment with PF-6142. **(A)** Systemic PF-6142 treatment significantly and dose dependently modulates oscillatory power in freely moving rats. Vehicle or PF-6142 (low: 1.0 mg/kg, high: 5.6 mg/kg, SC) was administered in the morning during the inactive period of the animals in their home cage. Delta (0.5–4 Hz), theta (4–9 Hz), alpha (9–13 Hz), beta (13–28 Hz), and gamma (28–80 Hz) oscillation power was analyzed during the first 4 h following treatment together with the total power contained in the signal. Statistical analyses revealed that treatment only with the high dose resulted in significant (*$p < 0.05$ vs. vehicle, #$p < 0.05$ vs. low) decrease of delta and total powers and increase of beta and gamma powers. Symbols (x for vehicle, Δ for low, and + for high dose) show values for individual animals. All animals are shown, outliers are not indicated separately, the upper and lower hinges on the boxplots show the 25th and the 75th percentiles, respectively, horizontal bar in the boxplot shows median value, whiskers extend to the minimum and the maximum values. **(B)** Systemic PF-6142 treatment significantly and dose dependently increases the time spent in wakefulness in freely moving rats. The fraction of time rats spent awake (Wake), in slow-wave sleep (SWS), or in REM sleep during the first 4 h following treatment were analyzed. Statistical analyses revealed that treatment with the high dose (5.6 mg/kg, SC) resulted in a significant (*$p < 0.05$ vs. vehicle, $^{\#}p < 0.05$ vs. low) decrease of the time spent in SWS and REM sleep, and an increase of the time spent awake. Boxplots are set up as described in **(A)**.

caused a robust decrease in percent correct on the SDR task from 70.63 ± 1.75% to 28.75 ± 2.45% [$F(\text{treatment})_{1,\ 6} = 62.089$; $p < 0.001$]. Pretreatment with PF-6142 (0.0015–0.15 mg/kg, s.c.) significantly attenuated the ketamine-induced deficits in the task at all doses tested [$F(\text{pretreatment})_{3,\ 18} = 5.733$; $p = 0.006$], improving performance by more than 10% correct (range 42.5–52.1%). Pretreatment followed by placebo (sterile saline) instead of ketamine indicated that PF-6142 had no effect on its own on working memory performance under normal conditions.

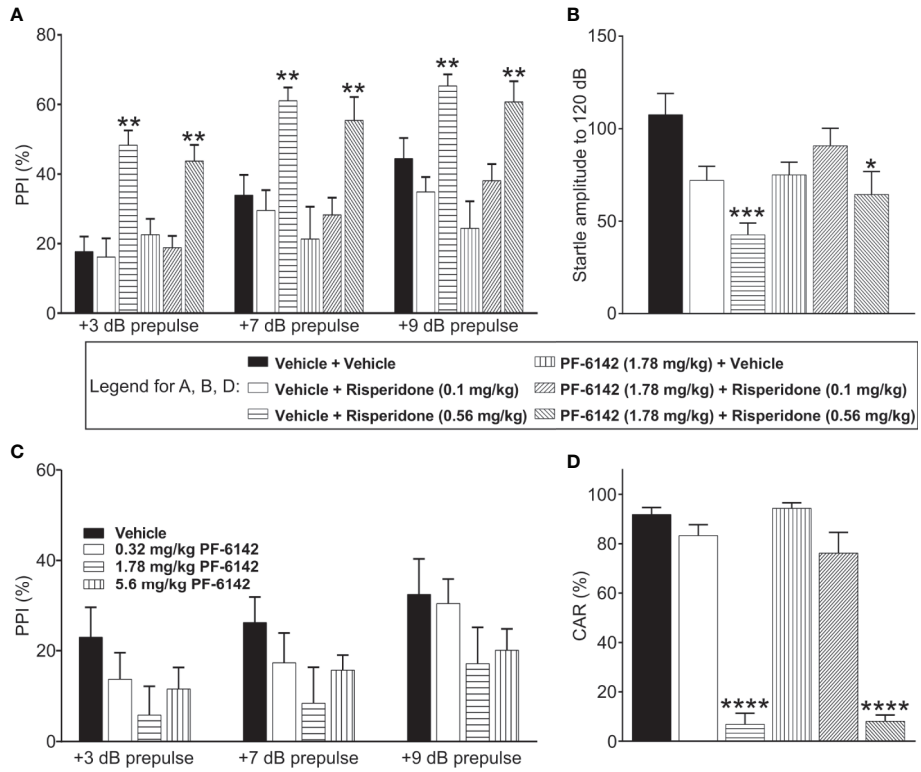

FIGURE 4 | PF-6142 Does not alter the antipsychotic-like activity of risperidone in rodents. **(A)** As expected, risperidone produced increases % PPI in adult male C57BL/6J mice consistent with an antipsychotic-like profile in this assay. Administration of PF-6142 alone or in combination with risperidone does not alter PPI responses (n=8 per treatment group). **(B)** Statistically significant reductions in startle responses were observed with the highest dose of risperidone (0.56 mg/kg) which was not altered in the presence of PF-6142. (**C)** PF-6142 alone produced modest non-significant reductions in % PPI in adult C57BL/6J mice. (**D)** Risperidone produces significant reductions in avoidance responding consistent with antipsychotic-like activity in adult male rats (n=8–9 per treatment) in the conditioned avoidance responding assay. There is no effect of PF-6142 alone or in combination with risperidone in avoidance responding. One-way ANOVA with Tukey's post-test adjusted *p < 0.05, **p < 0.01 vs. vehicle + Risperidone (0.56 mg/kg) ***p < 0.001 vs. vehicle + vehicle. Note:2-way RM ANOVA (A). One-way ANOVA with Dunnett's post-hoc test ****p < 0.0001 vs. vehicle + vehicle **(D)**.

In Vivo Anesthetized Electrophysiological Recordings

PF-6142 Significantly Reverses N-Methyl-D-aspartate (NMDA) Receptor Blockade-Induced Changes in Paired Pulse Facilitation (PPF)

In agreement with previous studies (Kiss et al., 2011), administration of NMDA receptor antagonist MK-801 (0.1 mg/kg, IV) resulted in significant increase of both the P1 (t_4 = −10.49, p = 0.0005) and P2 (t_4 = −8.05, p = 0.001) response amplitudes (**Figure 6A**). However, magnitude of the increase of P1 amplitude was proportionately much greater than that of P2 (44% vs. 10%, respectively). The resulting effect was a significant decrease of the corresponding PPF (PPF = P1 amplitude/P2 amplitude; t_4 = 2.99, p = 0.04). MK-801 (0.1 mg/kg, IV) decreased PPF and subsequent cumulative PF-6142 administration (0.1–1 mg/kg, IV) rescued this effect. The reversal of MK-801 was an all-or-none effect. Once the effective dose of PF-6142 was reached, the onset of reversal was rapid and almost maximal for each animal with minimal further effect observed after additional cumulative dosing. In two out of the five animals in this study a reversal effect was observed after a cumulative IV dose of 0.3 mg/kg of PF-6142 while the remaining three animals required the maximal cumulative dose of 1.0 mg/kg tested to reverse MK-801. On the population level, animals exhibited significantly higher PPF values following the administration of PF-6142 (1.0 mg/kg, IV) than under MK-801 challenge (t_4 = −5.28, p = 0.006). The ED50 for PF-6142 reversal of MK-801-induced effects on PPF was 0.35 mg/kg (95% confidence levels = 0.21–0.57 mg/kg) as calculated using the Spearman-Karber method.

PF-6142 Significantly Reverses NMDA Receptor Blockade-Induced Medial PFC (mPFC) Low Frequency Delta Activity Increase

The effects of MK-801 and subsequent cumulative IV dosing of PF-6142 on mPFC low frequency delta activity are shown in **Figure 6B**. MK-801 (0.1 mg/kg, IV) resulted in a significant increase (t_4 = −4.36, p = 0.01) in low frequency (0–1.8 Hz) irregular delta activity. Subsequent cumulative dosing of PF-6142 (0.1–1 mg/kg, IV) dose-dependently reversed MK-801-induced increases in low frequency delta that paralleled its reversal of MK-801-induced changes in PPF. At the highest dose animals

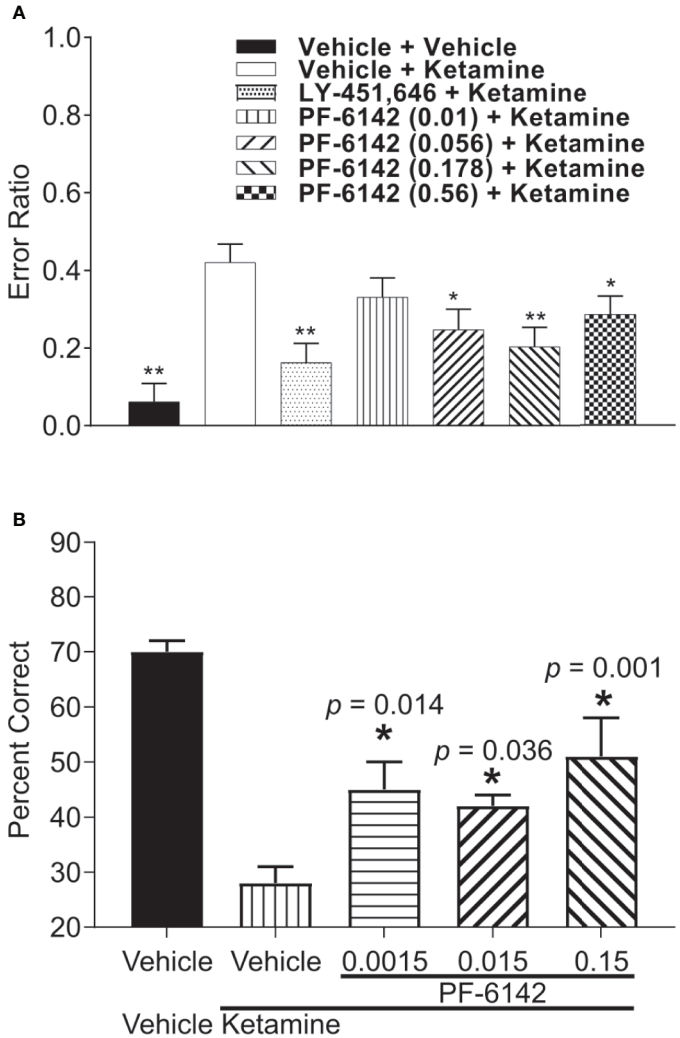

FIGURE 5 | PF-6142 effects on ketamine-induced working memory deficits. **(A)** Pre-treatment with PF-6142 dose-dependently prevents ketamine-induced deficits in the rat radial arm maze assay. Data are presented as mean errors + SEM. Kruskal-Wallis test with Dunn's multiple comparison post-test *p < 0.05, **p < 0.01 vs. vehicle + ketamine. N=10–21. **(B)** Treatment with PF-6142 prevents ketamine-induced deficits in the non-human primate spatial delayed response task. One-way ANOVA with Dunnett's post-test adjusted *p < 0.05 vs. vehicle + Ketamine and N = 8 NHP.

showed significantly decreased low frequency power compared to MK-801 challenge (t_4 = 4.49, p = 0.01).

Antagonism of D1Rs Blocks Effects of PF-6142 Both on PPF and mPFC Low Frequency Delta Activity

To test selectivity and specificity of PF-6142 in generating PPF and low delta activity effects, SCH-23390 was used to pretreat animals before MK-801 and subsequent PF-6142 administration (**Figures 6C, D**).

SCH-23390 (0.32 mg/kg, IV) had no effect on baseline P1 (t_4 = 1. 47, p = 0.21) and P2 (t_4 = −0.17, p = 0.86) response amplitudes (**Figure 6C**). Furthermore, subsequent administration of MK-801 (0.1 mg/kg, IV) still resulted in significant and selective increase in P1 response amplitude (t_4 = −4.90, p = 0.008) in all five of the animals in this study causing significant decrease of PPF (t_4 = 4.88, p = 0.008) similarly to previous results without D1R antagonism. SCH-23390 (0.32 mg/kg, IV), had no impact on control PPF (t_4 = −2.53, p = 0.06), and did not prevent MK-801 from decreasing PPF (two-sample t-test assuming equal variances: t_8 = 1.00, p = 0.34). SCH-23390 did, however, block the potential of PF-6142 (1–3 mg/kg, IV) to reverse the MK-801 effects: in four out of five animals cumulative IV administration of PF-6142 (1.0–3.0 mg/kg) had no effect on MK-801-induced changes in PPF, and therefore no significant effect of PF-6142 was detected (at the highest dose of 3 mg/kg, IV t_4 = −0.14, p = 0.89). These effects were largely due to the effect on P1 while P2 was unaffected.

The effects of SCH-23390, MK-801, and subsequent cumulative IV dosing of PF-6142 on mPFC low frequency delta activity are shown in **Figure 6D**. SCH-23390 (0.32 mg/

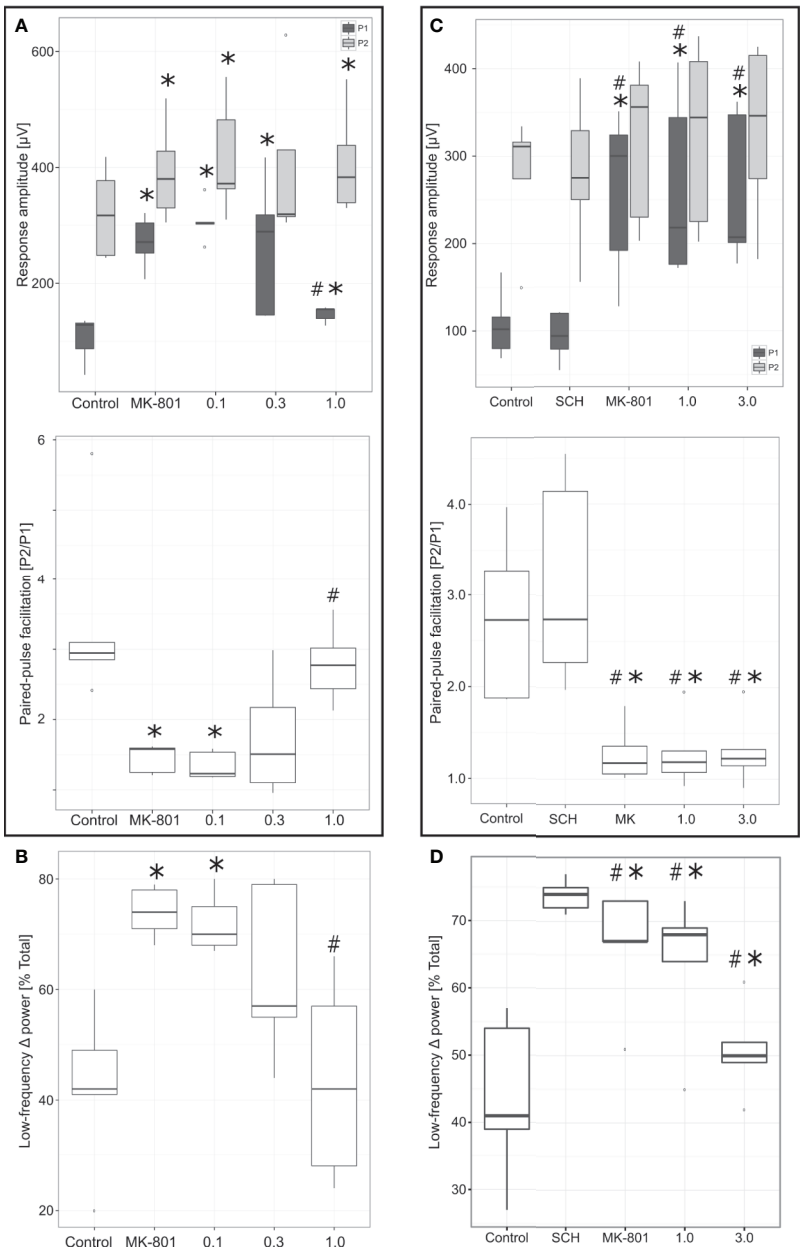

FIGURE 6 | Effects of PF-6142 on NMDA antagonist disrupted paired-pulse facilitation (PPF) and delta oscillations. **(A)** PF-6142 significantly reverses NMDA blockade-induced changes in PPF. PPF (calculated as P2/P1) values as a function of drug treatment. Note that PF-6142 reverses MK-801-evoked decrease of PPF with an ED_{50} of 0.35 mg/kg (*: $p<0.05$ vs. Control, #: $p<0.001$ vs. MK-801; $n=5$). The upper and lower hinges on the boxplots show the 25th and the 75th percentiles, respectively, horizontal bar in the boxplot shows median value, whiskers extend to the minimum and the maximum values, "o" indicates data points outside of the 1.5*inter-quartile range of the hinges. **(B)** PF-6142 significantly reverses NMDA blockade-induced mPFC low frequency delta activity increase. Power contained in the low frequency delta (0–1.8 Hz) band expressed as a percentage of the total delta (0–4 Hz) power. Note that PF-6142 completely reverses MK-801 induced increase of low frequency delta oscillation (*: $p<0.02$ vs. Control; #: $p<0.02$ vs. MK-801; $n=5$). **(C)** Antagonism of D1Rs blocks effects of PF-6142 on PPF. Figure shows PPF values as a function of drug treatment. Note that administration of SCH-23390 alone had no effect on either the P1 or P2 components or PPF, while this pretreatment completely blocked PF-6142 effects even at high doses. **(D)** Antagonism of D1Rs blocks effects of PF-6142 on mPFC low frequency delta activity. Power contained in the low frequency delta (0–1.8 Hz) band expressed as a percentage of the total delta (0–4 Hz) power. Note that as with PPF SCH-23390 completely blocks effects of PF-6142 on reversing MK-801-induced changes (*$p<0.05$ vs. Control, $^{\#}p<0.03$ vs. SCH-23390; $n=5$).

kg, IV) alone had no significant effect on mPFC low frequency delta activity ($t_4 = -1.53$, $p = 0.19$). Subsequent MK-801 (0.1 mg/kg) administration resulted in a significant increase ($t_4 = -6.15$, $p = 0.003$) in low frequency irregular delta activity, statistically similar to the case when treatment with SCH-23390 did not precede MK-801 treatment (two-sample t-test assuming equal variances: $t_8 = 0.09$, $p = 0.93$). Similar to PPF, cumulative IV dosing of PF-6142 in animals pretreated with SCH-23390 had no effect on MK-801-induced increases in low frequency delta activity (at the highest dose of 3 mg/kg, IV $t_4 = 2.08$, $p = 0.11$).

Receptor Occupancy Estimate for PF-6142

Maximal exposure was observed to occur between 0.5 and 2 h following dosing. Exposures increased in a generally dose proportional manner across most of the doses used in the pharmacology studies, and inter animal variability was typically low. Exposures were obtained in brain tissues for rat and mouse, and plasma protein binding and brain tissue binding were measured. From these data (not presented), we observe that PF-6142 is fully brain penetrant, with unbound concentrations approaching unity between the brain and plasma compartments.

A selection of representative exposure data is presented in **Table 2** to help contextualize the results of these *in-vivo* pharmacology studies with PF-6142 with published data reported on other D1R agonists. To account for any species differences in the affinity of PF-6142 for binding to D1R in each of the test species, radiolabel displacement assays were conducted in tissue from each one (data not shown) and measured values used in the receptor occupancy calculation.

DISCUSSION

Prefrontal cortical (PFC) functional alterations have been associated with the symptoms of multiple neuropsychiatric and neurodegenerative diseases, such as schizophrenia and Parkinson's disease (Howes and Kapur, 2009). In the PFC, DA D1Rs play a key role in cognitive control circuits that support working memory and executive function; thus, potentiation of these receptors offers a potential therapeutic pathway to counteract cognitive symptoms. Accordingly, a number of selective D1R agonists have been explored to date, all of which contain the catecholamine structural motif of DA itself, however, the catechol structural element imparts generally unfavorable aspects to their *in vivo* PKs (Zhang et al., 2009).

Recently, a novel chemotype of D1R agonists was described (Davoren et al., 2018; Gray et al., 2018; Soutschek et al., 2020b) that does not contain a catechol group and has generally good PKs and brain penetration. These compounds are reported to bind to the orthosteric DA site on D1Rs and activate cyclic adenosine monophosphate (cAMP). They also show evidence of biased G protein–coupled receptor (GPCR) signaling with respect to β-arrestin, with functional consequences on receptor internalization *in vitro* and on repeat-dose *in vivo* behavioral pharmacology. Recently, direct iontophoretic application of another compound from this new chemical series to aged monkeys performing a delay-dependent spatial working memory task yielded electrophysiologic evidence of D1R mediated excitatory actions on dlPFC task-related firing (Wang et al., 2019).

In this paper, a prototypical member of the novel non-catechol D1R agonist series, PF-6142, is characterized in various preclinical models. A specific goal of these studies was to assess if prior observations of selective D1R agonist pharmacology from *in vivo* models using catechol-based compounds (Roberts et al., 2010) would translate to these new compounds given their novel structure, signaling properties, high D1/5 selectivity, and different *in vivo* PKs. Assays were selected to cover different aspects of D1R-relevant circuitry with a general focus on cognitive and motor systems.

PF-6142 has moderate affinity for the human D1 and D5 receptors. Current literature is ambiguous regarding the differential expression, functional impact, and developmental changes of these receptor subtypes (Ciliax et al., 2000), thus effects observed in this paper can be attributed to an action *via* both D1 and/or D5 receptors. However, PFC dependent activity is likely due to the activation of D1 receptors that have higher cortical density including in PFC pyramidal cells in rats (Araki et al., 2007) and in NHP (Smiley et al., 1994; Montague et al., 2001; Lidow et al., 2003). The predominance of D1 was experimentally observed in experiments with D1R knockout mice where the effects of PF-6142 was absence.

D1R agonist-like activity was demonstrated by measuring aCh levels in the PFC of rats and mice using microdialysis. Like other D1R agonists, PF-6142 caused a robust increase in ACh level in the PFC which could be attenuated by administration of SCH-23390, a highly selective D1R antagonist in mice or by D1R knockout. Importantly, unlike currently available D1 agonists (Damsma et al., 1990; Imperato et al., 1994), the ACh release-promoting effect of PF-6142 was maintained following subchronic administration supporting previous observations that compounds from this non-catechol chemotype produce lasting functional effects without the rapid tolerance (Gray et al., 2018) that has been observed with catechol based D1 agonists (Kebabian et al., 1992).

Acute treatment of freely moving rats with PF-6142 resulted in a significant and dose-dependent increase of wakefulness and associated low-amplitude, high frequency electroencephalographic brain oscillations, primarily in the beta and gamma range. This stimulant activity is in line with increased selective activation of D1Rs (Herrera Solís et al., 2016) and is compatible with previous observations showing wake-promoting and EEG desynchronizing action of D1R agonist in normal (Ongini et al., 1985) and in a narcoleptic rodent model. Interestingly, it has also been shown recently that the D1R agonist SKF-38393 successfully alleviated excessive daytime sleepiness and restored REM sleep to baseline values in a macaque monkey model of Parkinson's disease (Hyacinthe et al., 2014).

Similarly, to other D1R agonists, PF-6142 significantly and dose dependently increased locomotor activity in mice (Dracheva et al., 1999). Specificity of the response to D1R agonism was validated pharmacologically by administering the

D1R antagonist SCH-23390 and by using a D1R knock-out mouse model. In both cases the hyperlocomotor response induced by PF-6142 administration was significantly attenuated. Importantly, the lack of effect in knockout mice suggests a D1R subtype-dependent action, although it does not fully discard the potential role of D5 receptors. Testing the effect of PF-6142 in D5R knockout mice is required to better understand the contribution of each receptor subtype.

Importantly, PF-6142 does not interfere or compromise the efficacy of risperidone, an antipsychotic drug, in the PPI assay and the CAR assay, two preclinical models used to demonstrate antipsychotic efficacy. All clinical antipsychotics agents show efficacy in these two preclinical models. Therefore, it is central to discard any potential interference with the standard care. These null results support the notion that D1R agonist administration will not interfere with the positive symptom efficacy of current antipsychotics medication that is likely used by patients.

Accumulating data suggesting that D1 receptors play a critical role in orchestrating function within the PFC and striatum for neuroadaptive processes which influence higher level functioning. Neuroimaging studies have shown increased D1R expression in PFC early in the course of illness in drug naïve schizophrenic patients and increased [^{11}C]NNC 112 binding in the DLPFC was predictive of poor performance on a working memory task (Abi-Dargham et al., 2002).

This data has led to the premise that D1 receptor agonist therapy may ameliorate working memory impairment by modulating the insufficient DA tone in patients with schizophrenia.

To assess the potential of PF-6142 to improve working memory, two preclinical deficit models were used taking advantage of NMDA antagonism for inducing cognitive impairment. NMDA receptor (NMDAR) dysfunction can directly impact synaptic plasticity and modify circuit output. Previous studies have shown (Kiss et al., 2011) that systemic administration of the non-competitive NMDAR antagonist MK-801 disrupts short-term synaptic plasticity between hippocampal CA1 and the PFC and increases low frequency electrical activity in the PFC of anesthetized rats. Importantly, our results demonstrated that administration of PF-6142 significantly reversed these effects similarly to LY451395, an AMPAkine shown to reverse NMDA-antagonist-induced deficits in preclinical models of cognition in NHP (Roberts et al., 2010).

At the functional level, in the RAM assay, a rodent spatial working memory task, the partial D1R agonist PF-6142 reversed ketamine-induced deficits, another way to compromise NMDAR functioning, in a dose dependent manner.

Similarly, pretreatment with PF-6142 prior to an acute ketamine challenge prevented ketamine-induced impairment in the SDR task model of primate spatial working memory. At the doses PF-6142 was tested in both of these paradigms, PF-6142 appears not to show the expected U-type dose response pattern that was found previously for the partial agonist SKF38393 in this model, in contrast to the inverted-U-type response found for the full agonists SKF-81297 and A77636 (Zahrt et al., 1997; Roberts et al., 2010). However, assessment of wider dose range is required to confirm this observation.

The results of these studies highlight significant differences from previous observations and suggest wider efficacy window underscoring the therapeutic potential of this novel class of D1 agonist. However, it is reasonable to conclude the optimal dose of a D1R agonists for improving cognitive function in a disease state may vary according to individual differences and neuropsychiatric conditions and also suggest that dopaminergic treatments of psychiatric disorders should consider baseline DA levels in order to avoid side effects of over-or underdosing on cognition (Floresco, 2013).

These findings indicate this novel class of D1R agonists shows efficacy for improving functionality under conditions in which NMDAR transmission is impaired, as hypothesized in schizophrenia. We note the low doses of PF-6142 that were associated with reversal of ketamine-induced working memory deficit in rat and NHP, a finding consistent with data obtained using catechol D1 agonists. Independent of chemical class, the positive effects in these models occurred at low doses and consequently a very low estimated receptor occupancy (<5%; see **Table 2**). Given prior data on D1R full agonists in this model, additional study is warranted to fully understand the exposure-response relationship.

In summary, the collected data support the hypothesis that PF-6142 a novel, non-catechol-based compound has functional pharmacology that is generally consistent with the expected profile for a D1R agonist acting *via* increased cAMP signaling. The new tool has favorable PK properties compared to previously available D1R agonists that might enable further research on the D1R system, particularly chronic studies or paradigms which look to assess the impact of continuous D1R receptor occupancy over a sustained period. Taken together, results of these studies replicate published pharmacology and also extend what is known about the performance of D1R agonists in other models and provide data that encourages further development of D1R agonists as potential therapies for cognitive impairment in schizophrenia and other psychiatric illness.

DEDICATIONS

The authors would like to dedicate this work to one of our co-authors, DJ, who passed away unexpectedly during the final stages of writing this manuscript. He was an extraordinary colleague whose commitment and expertise in drug discovery was critical to this work. DJ dedicated his life's work to making a difference for patients, and he will be missed greatly.

DATA AVAILABILITY STATEMENT

The datasets presented in this article are not readily available because they contain proprietary information owned by Pfizer

Inc. Requests to access the datasets should be directed to DG (david.gray@cerevel.com).

ETHICS STATEMENT

The animal study was reviewed and approved by the Institutional Animal Care and Use Committee at Pfizer Inc. and Yale Animal Care and Use Committee.

AUTHOR CONTRIBUTIONS

RK, DG, TK, DJ, PS, SS, MH, GW, and SC contributed conception and design of the study. BH, WH, PS, RG, KK, KD, SS, JD, and AA conducted experiments. BH, WH, TK, DV, and KD performed data analysis. TK, RK, KD, DG, SS, MH, and JD wrote or significantly contributed to the writing of the manuscript. All authors contributed to the article and approved the submitted version.

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REVIEW
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Dopamine Receptor Subtypes, Physiology and Pharmacology: New Ligands and Concepts in Schizophrenia

Jean Claude Martel[1] *and Silvia Gatti McArthur*[2]*

[1] Independent Researcher, Amos, QC, Canada, [2] McArthur and Associates, Basel, Switzerland

Dopamine receptors are widely distributed within the brain where they play critical modulator roles on motor functions, motivation and drive, as well as cognition. The identification of five genes coding for different dopamine receptor subtypes, pharmacologically grouped as D1- (D1 and D5) or D2-like (D2S, D2L, D3, and D4) has allowed the demonstration of differential receptor function in specific neurocircuits. Recent observation on dopamine receptor signaling point at dopamine—glutamate-NMDA neurobiology as the most relevant in schizophrenia and for the development of new therapies. Progress in the chemistry of D1- and D2-like receptor ligands (agonists, antagonists, and partial agonists) has provided more selective compounds possibly able to target the dopamine receptors homo and heterodimers and address different schizophrenia symptoms. Moreover, an extensive evaluation of the functional effect of these agents on dopamine receptor coupling and intracellular signaling highlights important differences that could also result in highly differentiated clinical pharmacology. The review summarizes the recent advances in the field, addressing the relevance of emerging new targets in schizophrenia in particular in relation to the dopamine – glutamate NMDA systems interactions.

Keywords: schizophrenia, dopamine receptor, NMDA, antipsychotic, psychosis, D1, D2, D3

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University of Ioannina, Greece

***Correspondence:**
Silvia Gatti McArthur
silvia.gatti_mcarthur@rmcaa.org

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INTRODUCTION

The dopaminergic system undergoes a delayed maturation in the brain, suggesting important stabilizing and integrating functions on neural circuits (Grace, 2016; Ohira, 2020). Schizophrenia (SCZ) is associated with dopamine (DA) neurotransmission alterations during puberty and adult life causing deficits in motivation, cognition and sensory functions (Simpson and Kellendonk, 2017; Abi-Dargham, 2018; Grace and Gomes, 2019; Sonnenschein and Grace, 2020). DA release measures in SCZ clinical studies and in preclinical models have clearly documented a fronto-cortical DA hypoactivity and a striatal (mainly dorsal) DA hyperactivity, associated with the occurrence of different SCZ symptoms (Terrillion et al., 2017; McCutcheon et al., 2019; Rao et al., 2019; Li et al., 2020). A summary of the most recent experimental evidence linking SCZ to DA alterations can be found in **Table 1** (McCutcheon et al., 2020). Recent studies are however questioning the causal role of DA in SCZ in favor of a more "NMDA hypofunction hypothesis" of the disease. The limited SCZ genetic links to dopamine receptors (DR) and

TABLE 1 | Summary of most recent evidence of dopaminergic alterations in schizophrenia.

Method	Results	References
Functional Imaging	Impaired PFCx control. Dorsal striatum alterations	(McCutcheon et al., 2019)
PET studies	Increased DA synthesis - release. Reduction during symptoms remission/D2 occupancy of antipsychotics./Hypo DA in PFCx. Antipsychotic treatment response./Effect of stress and DA in the reward circuit/DA alterations and white matter reduction./Pre- and postsynaptic alterations./SCZ subtypes./High risk SCZ patients.	(Abi-Dargham, 2018; Mitelman et al., 2018; Tseng et al., 2018; Avram et al., 2019; D'Ambrosio et al., 2019; Kim et al., 2019; Rao et al., 2019; Sekiguchi et al., 2019; Weidenauer et al., 2020; Girgis et al., 2020; Brugger et al., 2020; Wulff et al., 2020; Frankle and Narendran, 2020)
Post mortem	DAT levels./Presynaptic dysregulation.	(Tseng et al., 2017; Purves-Tyson et al., 2017)
Genetic/ epigenetic	DA sensitization in SCZ./NMDA DR epigenetic./Cumulative DA genetic and response inhibition./DR genetic variants and heterodimerization.	(Oishi et al., 2020; Enge et al., 2020; Faron-Gorecka et al., 2020; Jackson, 2020)
Transcriptional	SCZ risk genes control on D2 pathway expression.	(Torretta et al., 2020)
Protein level	Impact of DA on posttranslational control	(Kos et al., 2018)
Developmental	Netrin1/DCC on DA neuronal dev./MAM model.	(Grace and Gomes, 2019; Sonnenschein and Grace, 2020; Vosberg et al., 2020)
Biomarker	Anti-NMDA antibodies reduces D1 trafficking/Neuromelanin imaging.	(Grea et al., 2019; Wengler et al., 2020)
Therapy	Review on antipsychotics/Clinical effect of TAAR1 agonist.	(Koblan et al., 2020; Willner et al., 2020)
Cognitive	DA breakdown and working memory/D2 and cognition, area volumes -IQ./D2-like receptors and executive function.	(Bolton and Constantine-Paton, 2018; Veselinovic et al., 2018; Chang et al., 2020)
Animal models	Blonanserine in SCZ-like symptoms rodent models/DA alterations in rodents with NMDA hypofunction/model relevant for prodromal SCZ.	(Petty et al., 2019; Nakao et al., 2019; Takeuchi et al., 2019)
Translational	Extensive review from bentch to bed-side.	(Abi-Dargham, 2020)
Pharmacology	Lumateperone D1 and D2-like antipsychotic profile./Cariprazine new data.	(Vyas et al., 2020; Periclou et al., 2020)
Morphology	Rodent dorsal striatum synaptosome and Disc1	(Sialana et al., 2018)

the main glutamatergic alterations observed in SCZ imaging studies are among the most compelling reasons for this debate (Coyle et al., 2010; McCutcheon et al., 2020) (see also supplementary material **Table 1** for genetic links). This clearly does not question the well documented therapeutic benefit of DR antagonists as antipsychotics, but challenges two decades of efforts to develop new and improved SCZ therapies. This review aims at providing a summary of the most recent advances in DR control in SCZ with focus on DR—glutamate NMDA interactions across the genetic, intracellula,r and synaptic aspects of the disease. (Rampino et al., 2018).

SECTION 1: DOPAMINE RECEPTORS

DA Neurophysiology

DA is a neurotransmitter produced in neuronal terminals by successive hydroxylation and decarboxylation of tyrosine and loaded into synaptic vesicles by the monoamine transporter 2 (VMAT2/SCL18A2). When glutamate is coreleased with DA, VGLUT2-mediated glutamate uptake causes vesicular acidification and increases DA packing (El Mestikawy et al., 2011). Released DA is targeted for reuptake by two solute carriers, DAT1/SLC6A3 and DAT/SLCA2, with a prevalence of the effect of DAT1. The degradation of DA is under the control of a methylation enzyme, COMT (highly expressed in prefrontal cortex) and presynaptic monoamine oxidases. The by-product of this oxidation, H_2O_2 is funneled into the mitochondrial transport chain to support further DA release (Chen and Jonas, 2020). DA release occurs in a rather diffuse manner and ultrastructural studies show DA neuron axonal arborization and intricate projections covering large areas. DA transmission is tightly controlled at presynaptic level, while only varicosity elements define the postsynaptic sites with a variety of inputs (cholinergic, glutamatergic) in close proximity. DA neurons are specialized to receive high volumes of afferent signals and transform this information into a modulatory tone through a large projection area. It is estimated that one DA neuron provides input to several thousand neurons in the striatum and vice-versa, any given individual striatal neuron is influenced by DA released from more than one hundred DA projections. The DA neuronal system is often described in terms of DA release (tonic or phasic) and several models have tried to explain how multiple functions can be effectively impacted by different temporal DA release patterns (Eshel et al., 2015; Berke, 2018; Lohani et al., 2019; Mohebi et al., 2019). DA neurons are intrinsic pacemakers, with a slow (2–4 Hz) rhythmic activity associated with a tonic feed-forward control on DA receptor activation. The ionic channels/voltage sensitive mechanisms controlling DA tonic firing activity can differ even in within each DA nucleus. DA neurons can also fire in rapid bursts in response to relevant (salient) stimuli. This transient increase in firing rate induces a temporally precise rise in DA concentrations that can be synchronized in within local circuits. The lack of canonical synaptic release sites and the low probability of release for DA containing vesicles allow a scaling of neurotransmitter release as a function firing frequencies (Lebowitz and Khoshbouei, 2020). DA neurons in normal conditions always contain a "reserve pool" of DA vesicles that are rather insensitive to stimulation and more than half of DA synaptic release sites are functionally silent when stimulated. The DA system is therefore also sensitive to a local presynaptic modulation from other neurotransmitters (like acetylcholine or endocannabinoids) (Xu et al., 2018). DAT exerts a main presynaptic master control on DA release as recently demonstrated (Condon et al., 2019; Walters et al., 2020). DA release is in fact directly modulated at the presynaptic terminals by

a Rho-dependent internalization of DAT. This prolongs DA availability after burst stimulation, causing a prolonged postburst increase (>20 min) (Lohani et al., 2018). Differences in presynaptic Ca^{2+} channels and Ca^{2+} buffering further contribute to DA release synaptic heterogeneity (Chuhma et al., 2017). Large postexperience DA stimulation phases are important during learning procedures and in motivational drive, reward processes (Lak et al., 2020; Song and Lee, 2020). Most likely both D1 and D2 receptors subtypes are differentially engaged when in presence of DA burst firing at least in cortical and striatal regions (Hunger et al., 2020). Experimental evidence points at presynaptic alterations in DA nerve terminals in the striatal region and in prefrontal cortex in SCZ (Chuhma et al., 2017; McCutcheon et al., 2020; Weidenauer et al., 2020). Independent groups have reported alterations in the DAT level or function in SCZ patients (Artiges et al., 2017; Tseng et al., 2017; Lucarelli et al., 2019; Sekiguchi et al., 2019), but some of the results are still contradictory (Fusar-Poli and Meyer-Lindenberg, 2013). The described SCZ increase in DA synthesis/release in the rat dorsal striatum can be reproduced in preclinical models with alterations which resemble SCZ early symptoms (Petty et al., 2019). These general features are confirmed in a mouse model of NMDA receptor hypofunction in GABAergic neurons during development (Nakao et al., 2019), in mouse models studying SCZ genetic links to CACNA1C (Terrillion et al., 2017) and in Neuregulin 2 KO mice (Yan et al., 2018). Recent data managed to shed further light on the synaptic proteins involved in DA release, and how these are linked to SCZ by genetic studies. For instance both the somato-dendritic and axonal release of DA are controlled by RIM protein isoforms in the active zone and by the Rab3 counterpart *via* D2L receptors (Robinson et al., 2019). Glutamatergic effects on the DA release machinery are most likely indirect and sustained by GABAergic interneurons at least in cortical regions (Molinaro et al., 2015). In fact, antipsychotic agents do not completely manage DA synthesis/release alterations, even in presence of efficacy on psychotic symptoms (Wheeler et al., 2015; Weinstein, 2019).

DR Subtypes

DR are integral membrane receptors coupled to G proteins (Beaulieu and Gainetdinov, 2011; Thal et al., 2018). The dopaminergic system signals through "D1-like" D1 and D5 receptor subtypes and "D2-like": D2Short (S), D2Long (L), D3 and D4 receptor subtypes (Xin et al., 2019). There is some difference in the affinity of DA for D1-like receptors and D2-like receptors, mostly reported on the basis of receptor-ligand binding studies in recombinant systems (**Supplementary Material: Table 1**). D2-like receptors have a 10- to 100-fold greater affinity for DA than the D1-like family, suggesting that the balance of D2-like vs. D1-like receptor signaling can change depending on extracellular DA concentrations. A general view supports the specific engagement of D1 receptors in cortical regions when in presence of burst firing (Dreyer et al., 2010; Nair et al., 2014) while DA tonic activity affects only postsynaptic D2-like receptor signaling (Caravaggio et al., 2020). Differences in DR affinity may not be however the only relevant factor when discussing DR engagement in physiological conditions. The timescale of DR engagement (minutes) and the relative DR abundance in complex circuits need to also be taken into account (Hunger et al., 2020). The role of DR in different neuronal populations in striatum can be an example of this complexity. D1 and D2 receptors are generally segregated in striatal GABAergic medium spiny neurons (MSNs). D1-MSNs respond mostly to DA burst signals (Yapo et al., 2017), while optogenetic studies show that the effect of DA burst firing on D2 is not occluded by the presence of a background DA tone. D2-MSNs can therefore respond to a broader range of stimuli (Marcott et al., 2014). Cholinergic interneurons in the same region also receive an important DA/glutamate corelease input during burst firing. These cholinergic neurons express the receptor D5 (D1-like) responsible for an excitatory response after a bursts of DA release and D2-like receptors which trigger an hyperpolarization (a pause in the cholinergic signaling sequence) when activated. These events are in temporal sequence with the NMDA activation after glutamate/DA corelease creating a specific pattern of activity in these interneurons (Wieland et al., 2014). In the nucleus accumbens (nAcc) finally D1 and D2-like receptors work in cooperativity (heterodimers) in the same neuronal population and still a local complex coding of response to DA release fluctuations can support motivation and decisional processes (Hamid et al., 2016).

The original classification of DRs subtypes signaling mechanisms on the basis of cAMP stimulation and/or inhibition is no longer so useful given the substantial complexity of the heterocomplexes formed by DR. The DR - cAMP cascade is in any case directly linked to mRNA translation enhancement *via* PKA and serine-residues phosphorylation of ribosomal protein S6. So transcriptional - translational control can be considered a specific part of the DRs activation cascade. Only D1 and D2/D3 will be further discussed in this review as DR most involved in SCZ related alterations. D5 research did not produce convincing evidence so far of robust SCZ association (Hwang et al., 2012) and a link to stress and GABA transmission is the only new element of relevance for D4 in SCZ psychosis (Tan et al., 2019).

D1 Receptors

When discussing D1 in the context of SCZ, the most important aspects are certainly related to the prefrontal cortex (PFCx) regions and the cognitive deficits observed during the disease (Arnsten et al., 2017). D1 activates a postsynaptic Gs/Golf protein complex with a final increase in intracellular cAMP levels. PDE1b is the most relevant enzyme for the cAMP degradation upon D1 activation (Yamamoto et al., 2013; Yano et al., 2018). Two cAMP sensors link D1 activation to the ERK cascade: PKA and NCS-RAP/GEF2. Both proteins are important to trigger neuroplasticity effects (Jiang et al., 2017). Prolonged agonist activation of the D1 receptor leads to phosphorylation of the intracellular domains by G protein coupled receptor (GPCR) serine and threonine kinases (GRKs) and other kinases like GSK3b. They trigger the translocation and coupling of β-arrestins and D1 receptor endocytosis (Wang et al., 2017). The scaffolding function of β-arrestins enables the gathering of various other signaling components (cAMP independent). D1/

D3 heterocomplexes transactivation can also switch D1 signal toward a cAMP independent cascade (Guitart et al., 2019). D1 has been the focus of past SCZ research because of its functional role in the potentiation of postsynaptic NMDA currents *via* a receptor complex with NR1a/NR2a including PSD95 (Zhang et al., 2009; Desai et al., 2017). D1 activation triggers NR1-CaMKII coupling and enhancement of CaMKII activity; mGlu5 phosphorylation by MAPK and potentiation of the effect of Pin1 - Homer1 (Nai et al., 2010). A multicompartment model of this control in striatal medium spiny neurons (MSN) involves STEP tyrosine phosphatase (Beutler et al., 2011; Gutierrez-Arenas et al., 2014). The D1-dependent engagement of Fyn kinase leads to an enhancement of NMDA NR2b subunit channel activity also of specific relevance in MSN in striatum (Hu et al., 2010) NMDA – D1 interplay *via* Fyn kinase could be also more broadly relevant across glutamatergic synapsis in cortical regions given the long term effect on the function of ELF2 (David et al., 2020). A more downstream control on the same path can be made *via* PKA activation and by PDE10 inhibitors and similar considerations can be applied to D2 intracellular cascade in MSN (Nishi et al., 2011; Harada et al., 2020). D1 may be present in heterologous glutamatergic pre-synapsis possibly in heterocomplexes (D3)? in prefrontal cortex and hippocampus with an effect on glutamate release (Hikima et al., 2016).

D2/D3 Receptors

D2-like receptors (D2/D3) are the main targets of antipsychotics (Zhang et al., 2020). The D2 receptor is present in two isoforms D2S and D2L which differ because of a 29 AA insertion in the third intracellular loop on D2L (Zuk et al., 2020). Both receptors can inhibit intracellular cAMP *via* Gi. The inhibitory effect of D2 (and D3) on membrane excitability is generally due to the coupling to GIRK channels *via* Go (Kv 1.1, 1.2, or 1.6 - possibly Kv3) (Huang et al., 2013; Bonifazi et al., 2019). Both D2S/L receptors can initiate a cAMP-independent pathway by promoting the association of a signaling complex containing AKT1, PP2A, and β-arrestins leading to the activation of both ERK1/2 and GSK3b signals (Chen et al., 2016). The D2 receptor establishes a complex with DISC-1 that facilitates GSK3 mediated signaling and inhibits D2 agonist mediated receptor internalization, further enhancing the final D2 mediated effects (Su et al., 2014). Antipsychotics seem to be able to uncouple this complex (Zheng et al., 2019). The D2S is dominant in the cell bodies and projection axons of the dopaminergic cells in mesencephalon, while the D2L is a mainly postsynaptic receptor strongly expressed by neurons in the striatum and nAcc, brain structures targeted by DA terminals. In cell types of relevance for SCZ like MSN or cortical pyramidal neurons, D2L is able to trigger PKA activation possibly because of receptor transactivation (Castellani et al., 2017). DARPP32, RCS, and ARPP16 are the most important PKA targets of the D2 effects (Walaas et al., 2011). D2L activation can also recruit c-Src to transactivate the PDGF receptor and downstream Ras/Raf/MEK/ERK signaling cascade. This pathway represents a main stimulus for dendritic formation in striato-pallidal MSN (Shioda et al., 2017). D2S auto-receptors (on dendrites and soma) are known to inhibits cell firing, activate DA reuptake and inhibit DA synthesis. The work of Purves-Tyson confirms that D2S, VMAT2, and DAT mRNAs are significantly decreased in schizophrenia, with no change in *DRD3* mRNAs, and DAT protein between groups (Purves-Tyson et al., 2017). Other studies have verified that these alterations are sensitive to stress (Sallis et al., 2020) and present in drug-naïve SCZ patients not previously treated with antipsychotics (Tseng et al., 2018). In the same presynaptic compartment D2S can inhibit the trace amine receptor TAAR1 with a final potentiating effect on the DA release in striatum (Leo et al., 2014; Su et al., 2014). The distribution of TAAR1 is predominantly intracellular thus being uniquely positioned to regulate aminergic activity (possibly including DAT function) (Asif-Malik et al., 2017). The recent positive clinical results obtained with the TAAR1 agonist SEP-363856 tested as antipsychotic provide a confirmation of the relevance of the observed alterations in presynaptic DA release in SCZ (Pei et al., 2016; Koblan et al., 2020).

The D3 receptor is efficiently coupled to Gi/o at pre- and postsynaptic sites and in cell bodies. Some D3 intracellular pathways are similar to those observed for D2 (Guitart et al., 2019). The D3 receptor can however be sequestered in an inactive state at the membrane level rather than internalized (Zhang et al., 2012; Zhang et al., 2016; Zheng et al., 2016). D3 can work in complex with D1 receptor and thanks to this, D3 agonists can stimulate cAMP production and even GABA release. This D1/D3 interaction also facilitates non cAMP related intracellular signaling as demonstrated with biased ligands (Guitart et al., 2019) (see section 3). At postsynaptic level in MSN, D3 modulates Ca^{2+} channels *via* PLC and PP2B. At extra-synaptic location (cell bodies) D3 receptors have been reported to selectively modulate Ca^{2+} influx through low-voltage activated (Ca_V3, T-type) Ca^{2+} channels, in a β-arrestin-dependent mechanism. In other cases, non-canonical DR mediated events like the D3 interaction with the ghrelin receptor need to be invoked (in hippocampus) to explain a final effect *via* Galphaq-PLC-IP3-Ca^{2+} (Kern et al., 2015). The D3 receptor is able to interact with nicotinic receptors (for instance alpha 4 containing nicotinic receptors) in particular in VTA (Bontempi et al., 2017) and represents a main point of cross talk with the cholinergic system (Matera et al., 2019). D3 turnover is controlled by the EGFR tyrosine kinase signaling cascade (Zhang et al., 2020). EGFR phosphorylates GRK2 which then phosphorylates the intracellular domain of the D3 receptor to trigger D3 intracellular receptor degradation (Sun et al., 2018). PICK1 instead seems to be able to control surface D3 levels. PICK1 is present in dopaminergic neurons in close proximity with D3 (also D2 and DAT) at cytosolic level and an increase in PICK1 lowers the surface density of D3 (Zheng et al., 2016). D3 effects can be increased in presence of NMDA receptor hypofunction. Upon NMDA activation CaMKII alpha is recruited to D3 by rising Ca^{2+} to increase the CaMKII alpha-mediated phosphorylation of D3, thereby transiently inhibiting D3 efficacy (Liu et al., 2009). This CAMKII control on DA/NMDA interplay is potentially very relevant in SCZ and core to

the therapeutic interventions required to limit D3 overactivation. See **Figure 1** for DR and signal transduction at synaptic level.

DR Dimerization and Complexes

As for many GPCRs, all DR subtypes form homo and heterodimers *in vivo* with effects on native receptors signaling. DR dimerization involves transmembrane domains 5 and 6. This interaction can be a transient process, stabilized in presence of agonists like dopamine or quinpirole (Kasai et al., 2018) and it is of potential pathophysiological significance for SCZ. The balance of D2 homodimers to monomers has been also associated to amphetamine sensitization in animals, a further element related to SCZ (Weidenauer et al., 2020). This is why the generation of bivalent DR ligands has been attempted by several groups (Carli et al., 2018). The most common DR heterodimers/tetramers observed *in vivo* are D1/D2, D1/D3, D1/H3 and D2/A2A (Borroto-Escuela and Fuxe, 2019). They all affect the MAPK response of these receptor systems, D1/D3 also modify recruitment of β-Arrestin-1 and heterodimer internalization. mGlu5/D2, D2/mu opioid receptor, D2/neurotensin 1 receptor, and D2/5-HT1a heterodimers have been also described, but not necessarily in the context of SCZ (Lukasiewicz et al., 2016; Qian et al., 2018a; Qian et al., 2018b). They can all be potentially relevant for the effects of antipsychotic agents and for the generation of new ligands with unique pharmacological properties (Hubner et al., 2016). A different type of interaction has been described for D1 and NMDA receptors. In this case the presence of a membrane cluster in hippocampal neurons has been convincingly demonstrated during the past decade (Ladepeche et al., 2013). D1 activation is associated with increased NMDA trafficking to the synaptic surface and vice-versa. The proposed model shows D1 receptors dynamically retained in clusters in the vicinity of glutamate synapses where they interact with NMDAR. DR activation disrupts this interaction and favors the lateral redistribution of both receptors. D1Rs moves to extra-synaptic areas, whereas NMDA receptor reaches the glutamatergic postsynaptic density. Most importantly anti-NMDA antibodies from SCZ patients disrupt NMDA trafficking and reduce D1 trafficking as well. A region contained in the intracellular C-terminus of the D1 receptor is involved in this interaction with the NMDA receptor (Grea et al., 2019). More complex structures are also reported in the cortex involving D1, H3 and NMDA receptors (Rodriguez-Ruiz et al., 2017).

DR Turnover

Palmitoylation at the C-terminus of the DR protein has been documented for D1, D2, and D3 receptors as reversible switch for DR signaling *via* the cAMP path (Ebersole et al., 2015; Arango-Lievano et al., 2016). The most important

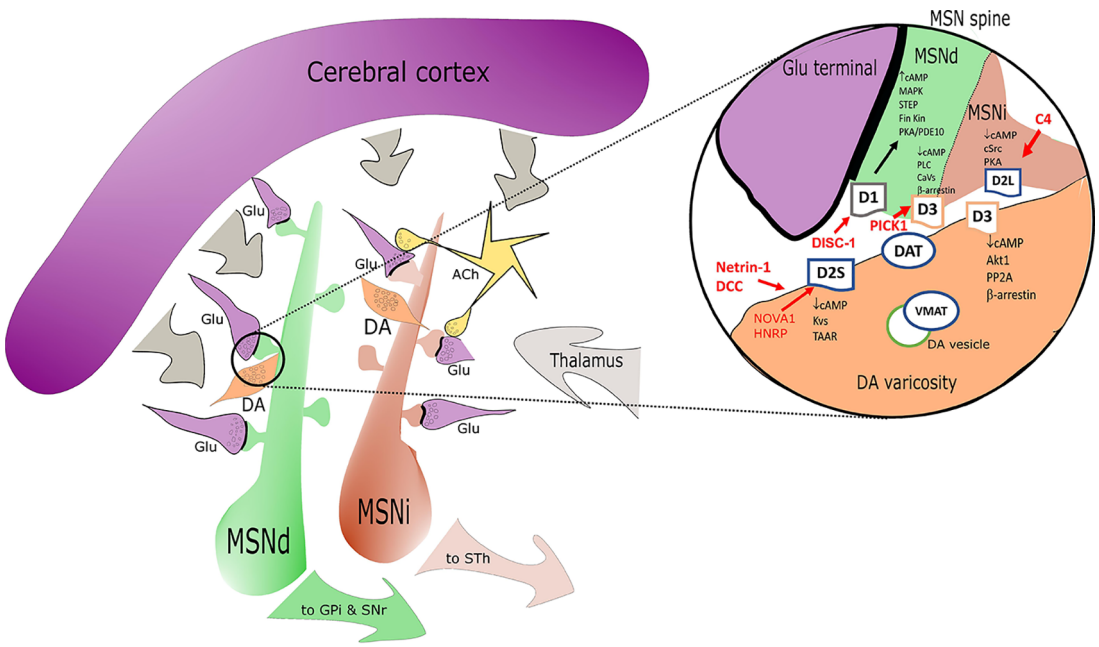

FIGURE 1 | Simplified sketch of the dopamine receptors (DR) connectome in the basal ganglia/striatum with a zoom (right circle) on signal transduction at presynaptic level in medium spiny neurons (MSN) dendritic boutons. Highlights on the elements associated with SCZ alterations are depicted in red. D1 positive medium spiny neurons of the direct pathway (MSNd) are in green, inhibitory D2 positive MSN of the indirect pathway (MSNi) are in red. Glutamatergic cortical input - presynaptic terminals are in magenta. DA "en passant" boutons are indicated in orange and in close proximity of glutamatergic postsynaptic spines. Cholinergic interneurons are in yellow. In the magnification on the right note the distribution of DR: D2s and D3 are presynaptic in DA terminals; D1/D3 postsynaptic in MSNd and D2L postsynaptic in MSNi. Other projections are in gray. Abbreviations: ACh, acetylcholine; DA, dopamine; Glu, glutamate; MSNd/i, direct/ indirect path projecting MSN neurons; GPi, internal segment of globus pallidus; SNr, substantia nigra, reticular part; STh, subthalamic nucleus; other common abbreviation and protein names as cited in text.

posttranscriptional modification of D2 and D3 receptors is the N-linked glycosylation that classically affects both correct cell surface expression and signaling/internalization (caveolin - chlatrin mediated) (Min et al., 2015). D1 and D2 are localized to different endocytic vesicles after internalization. D1 is recycled back to the cell surface in a process controlled by the VPS35 complex (Wang et al., 2016), while prolonged agonist stimulation causes D2 trafficking into lysosomes and subsequent receptor degradation by a Rab5 GTPase controlled pathway (Shioda et al., 2017; Shioda, 2017). A specific presynaptic control on D2S membrane density is exerted by the L1 close homolog adhesion factor (also a risk gene for SCZ) (Kotarska et al., 2020). Presynaptic D2S receptor density is directly or indirectly affected by ALK and possible transactivation mechanisms (He and Lasek, 2020). The overall complexity of the control of D2 receptor internalization (vs D3) is possibly justified by the major biological role of D2 surface density adjustments, required in different circuits depending on DA content. A specific example is the D2 vs D3 relative control by Dysbindin 1 (Leggio et al., 2019). Dysbindin (SCZ risk gene associated with cognitive symptoms) is mainly expressed in hippocampus and dorsolateral (DL) PFCx. It is a component of the multi-subunit complex BLOC-1 where it interacts directly with MUTED (also probably associated with SCZ). Both dysbindin and MUTED siRNAs increase cell surface D2 receptors and block DA-induced D2 internalization in human and rat cells. Dysbindin variants are known to modify the cognitive response to antipsychotics. This effect is most likely related to the parallel Dys1/D3 signal reduction that favors a D2 component in cortical regions (Leggio et al., 2019).

Other types of control on DR density are exerted at source at the transcriptional level. A recent analysis of proteasome alterations in SCZ points at spliceosome nuclear protein and calmodulin related pathways. The control on the splice variants of the D2 receptor is exerted by NOVA1 and HNRP (Min et al., 2015), and D2 mRNA 3′UTR binding of microRNAs mir-9 and mir 328 inhibits messenger translation (Shi et al., 2014). Development mechanisms are directly impacting on DR expression. In particular DISC-1 can translocate with KLF16 into the nucleus and recruit SIN3A corepressor to the D1 locus (Suh et al., 2019). The DISC-1 related complex is a main hub that could bring more specific information on SCZ developmental aspects in terms of consecutive development related alterations in glutamatergic (NMDA/AMPA) and dopaminergic responses (D1 +D2+D3) in key SCZ regions like dorso-lateral PFCx and the striatum (Onishi et al., 2018; Jacobi et al., 2019). The expression control can also be exerted more dynamically on the D1 intracellular signal transducers by nuclear receptors like Nr4A1 (Nurr77) (Cirnaru et al., 2019). Another nuclear factor involved in shaping dopaminergic terminals is Nurr1, highly relevant for the D2 receptor network and its circadian cycling (Chung et al., 2014; Torretta et al., 2020). See **Table 1** supplementary material for a summary. Until puberty, the DA system maturation is controlled by the netrin-receptor DCC mediated organization of DA neurons in the meso-cortical limbic system and the projections to PFCx (Vosberg et al., 2020). Axon navigation is directed by extracellular axon guidance cues, which induce molecular changes in the axonal growth cones in response to extracellular levels of DA (via D1 in complex). The DCC gene keeps being a confirmed SCZ genetic link across several studies (Vosberg et al., 2020) with a particular effect on the anatomical connectivity of the nigra/VTA dopaminergic pathways and the final distribution and relative density of DR. In animal models, SCZ-like symptoms seem to correlate with netrin 1 - DCC related alterations in size, complexity and density of DA spines (medial PFCx layer V pyramidal neurons). Other genetic SCZ links (for example RGS12) concur on DA synthesis and release (Gross et al., 2018; Kos et al., 2018). A common upstream element affecting the expression of D2, COMT and structural proteins at presynaptic DA level is the zinc finger element ZFN804A (Girgenti et al., 2012), coded by another SCZ risk gene (Zhou et al., 2020).

SECTION 2: DR ALTERATIONS IN SCHIZOPHRENIA

The current understanding of the role of DR in SCZ is in full expansion, thanks to developmental brain studies and the advancements of imaging techniques. DR expression is segregated across neuronal populations and associated with temporal and coupling differences in activation properties. This distribution is respected in SCZ, while a variety of DR β-arrestin mediated intracellular signaling show clear alterations in SCZ disease models. Some developmental and connectivity aspects of DR distribution are maintained across species and useful for the definition of SCZ as a developmental disease across circuits (Sonnenschein and Grace, 2020).

Prefrontal Cortex Neurocircuit(s) Affected by SCZ and DR

Connectivity measures across different SCZ studies are not always easy to compare, but some key elements are constant across patient groups, detection modalities and data interpretation: the involvement of striatal-thalamic and PFCx connections in SCZ (Zhao et al., 2020). Imaging, functional and circadian studies are also in general agreement on the presence of main alterations in the PFCx of SCZ patients, in particular dorso-lateral and cingulate regions (Seney et al., 2019). PFCx circuits are central to cognitive functions and linked to the different aspects of cognitive deficits and positive symptoms as observed in SCZ. Dorso-lateral PFCx weaker processing of sensory information from thalamus is in fact associated with hallucination experiences which are common in > 50% of the SCZ patients (Daskalakis et al., 2020). The molecular studies point at parvalbumin positive (PV+) GABAergic interneurons and cortical pyramidal cells networks as both altered in SCZ PFCx and across species in SCZ models (Chung et al., 2018; Petralia et al., 2020; Wang et al., 2020; Weidenauer et al., 2020). Dopaminergic ascending terminals reaching these neurons are also hypofunctional (Rao et al., 2019). Dopamine release enables the PFCx to compute and generate spatio-temporally diverse and specialized outputs, but these are not a linear function of the DA

release input. Thus, it is quite complex to establish the functional correlates for cortical functions. Rapid, transient changes in DA transmission in PFCx are observed in response to task events, such as cues and rewards whereas prolonged responses are relevant to emotional states and motivation (Lohani et al., 2019). DA neurons in the region are mainly coming from the VTA and the terminal density in PFCx is much lower (in terms of DAT content) when compared to the striatal regions.

D1 receptors are enriched in pyramidal cells in both layers 5 (thin-tufted layer) and 6 projecting in turn to contralateral cortex, striatum, and claustrum. D1 receptors are also present in interneurons and enriched in a specific population of VIP+ calretinin positive interneurons (Anastasiades et al., 2019; Saffari et al., 2019). D1 receptors strongly enhance action potential firing in this subset of cortico-cortical neurons and VIP+ interneurons and the modulation *via* D1 receptors can influence both excitatory and disinhibitory microcircuits in the PFCx (Anastasiades et al., 2019). This PV+ interneuron circuits are a the main point of interaction between mGlu5/NMDA and D1 (D2-like) receptors, both involved in the control of the glutamatergic input from pyramidal cells (Nicoletti et al., 2019). D1 is important for the correct migration of the dopaminergic terminals which increase throughout adolescence across species. Developmental studies in netrin-1 receptor DCC deficient mice demonstrate a role for DA in adolescent brain axon growth. DCC controls in fact the extent of this protracted growth by determining where and when DA acts. Pyramidal neuron morphology studies and cognitive performances show that the lack of DCC causes dopaminergic deficit across PFCx and morphological changes in pyramidal neurons (Reynolds et al., 2018). This process can be influenced by stress. The DA deficit in PFCx regions following this hypothesis may be then of developmental origin and caused by morphological alterations affecting DA terminals, pyramidal cells and interneurons.

D2/3 receptors are also differentially expressed in PFCx and their activation contribute to specific cognitive processes (Robinson and Sohal, 2017; Bailey et al., 2020; Papenberg et al., 2020). D2 are enriched within subcortically projecting L5 pyramidal neurons thick-tufted pyramidal cells, with projections to thalamus and pons, but not contralateral cortex (Yu et al., 2019). These neurons exhibit a prominent hyperpolarization-activated cationic current. In this population, pharmacological activation of D2 elicits a profound after depolarization that only occurs when NMDA receptors are coactivated. D2 signal in this case is triggering a Gs- cAMP/PKA pathway in a non-canonic manner (Robinson and Sohal, 2017). D2 are also expressed in PV+ interneurons, a property acquired during adolescent brain maturation (Urs et al., 2016). The D2 network controls the connection to the hippocampal system (Tomasella et al., 2018; Khlghatyan et al., 2019). Species related differences in this circuitry could be large, so human data are needed for the correct interpretation of the results (Gonzalez-Burgos et al., 2019). The cortical D2 mediated effects of the most common antipsychotics (antagonists and partial agonists) have been extensively evaluated. This is mostly because these agents cannot rescue the cognitive impairment associated with schizophrenia, with possibly few exceptions (amisulpride or 5-HT1A partial agonists) (Park et al., 2019; Huang et al., 2020).

D3 are expressed by a distinct population of prefrontal neurons and they also represent the main auto-receptor controlling DA release in prefrontal cortex. D3 expression defines an additional class of L5 pyramidal cells that largely lack D1 or D2 coexpression. L5 D3-expressing neurons are similar to D1-expressing cells in their synaptic connectivity, with projections to contralateral cortex. D3-expressing neurons could be distinguished from D1- or D2-expressing neurons by dendritic morphology, intrinsic electro-physiological properties and by the manner in which DA regulates neuronal function. In these neurons in fact D3 selectively regulates the dynamics of voltage-gated calcium channels localized to the site of action potential initiation in the axon initial segment, with a marked suppression in the generation of high-frequency action potential bursts. D3 regulates $Ca_V3.2$ channels through a non-canonical, arrestin-dependent pathway. The D3 plays therefore a unique role in the regulation of pyramidal cell excitability (Clarkson et al., 2017). The D3 receptor function has received attention because it could be a discriminant of the clinical effect of different antipsychotics (Girgis et al., 2020) and because of the potential to address SCZ negative symptoms. In fact, D3 are associated to a cortical circuit important for all the different SCZ symptoms. The D3 controlled PFCx projections to hippocampus are interesting in this sense (Provenzano et al., 2020). The recent paper from Meier et al. shows the effect of a preferential D3 partial agonist Cariprazine on gamma oscillations in hippocampal slides further supporting the general assumption that gamma waves could predict psychosis and *in vitro* NMDA hypofunction, and that D3 functional reduction can stabilize the alterations of the signal caused by NMDA hypofunction (Meier et al., 2020). Treatment response to antipsychotics may be predicted looking at the effect on hippocampal- cortical connections and again these changes could be in part D3 related (Guma et al., 2019; Blessing et al., 2020). The observed hippocampal alterations in some SCZ patients (psychotic) also support the presence of hippocampal immaturity at least in a subgroup of SCZ patients (Alvarez et al., 2020; Cachia et al., 2020). There is therefore a renewed interest for the hippocampal models in SCZ, because it is possible to study developmental changes which are closer to those observed in man and because it is easier to obtain NMDA receptor hypofunction (Alvarez et al., 2020). In a mouse model of postnatal NMDA hypofunction (NR1a KO) the effect seems to be selectively associated with PV+ interneurons (in cortex and hippocampus among other areas). In this animal model both cortical hypo- and striatal hyperdopaminergic phenotypes can be observed (Nakao et al., 2019). The reason(s) behind these extensive dopaminergic changes across areas are still not fully understood, but SCZ genetic data related to ancillary proteins for the NMDA receptor function also support this hypothesis. Very recent work has also given renewed attention to circuit(s) involving PFCx areas like DL or the orbitofrontal (and cerebellum) in relation to some aspects of negative symptoms in SCZ (Walton et al., 2018; Brady et al., 2019). It is possibly too early to include a conclusive map of DR expression in within

these pathways. The DISC-1 developmental mouse model could however help to analyze these circuit(s), considering the main impairment observed in sociability measures (Sultana and Lee, 2020). The PV+ interneurons can also be a starting point to address the network in terms of developmental changes. Recent DISC-1 studies report a reduction of spontaneous inhibitory transmission onto L2/3 PV+ interneurons in medial PFCx and a decreased feed forward inhibition onto L2/3 pyramidal neurons (Delevich et al., 2020).

Striatal Circuits Alteration(s) in SCZ and DR

The main role of the striatum is the integration of cortical and thalamic glutamatergic projections (Hunnicutt et al., 2016; McCutcheon et al., 2019). The striatum is at the center of a DA-sensitive basal ganglia circuit associated with psychosis, SCZ related motor dysfunctions and reward deficits. A summary of all the direct and indirect evidences of striatal DA alterations in SCZ was recently published (McCutcheon et al., 2020). All data confirm the presence of presynaptic DA sensitization and elevated DA synthesis and release capacity (Brugger et al., 2020; Weidenauer et al., 2020). Higher striatal DA synthesis and higher DA release correlated with worsening of psychotic symptoms in SCZ patients and were also supported by neuromelanin observation (Weinstein et al., 2017). Excess striatal DA in SCZ is not related to changes in DA innervation (Wengler et al., 2020). There have been extensive efforts to describe the neuroanatomy of striatum, and the cellular distribution of DR (Soares-Cunha et al., 2016; Clarkson et al., 2017). Substantia nigra DA projections mainly reach the dorsal striatum (Uchigashima et al., 2016) while ventral tegmental area (VTA) projections from the mesencephalon reach the ventral striatum (nAcc). Striatal neurons that receive DA inputs are mainly GABAergic medium spiny neurons (MSN). MSN neurons are the recipients of both DA and glutamatergic (from PFCx and thalamus) projections, they represent therefore a core neuronal element for both DA and NMDA hypothesis in SCZ. The MSN projecting to the internal segment of globus pallidus/nigra pars reticulata express D1 receptors, while those projecting to the external segment of globus pallidus are essentially expressing the D2 receptors. The two types of neurons are finely intermingled across the whole striatum (Ren et al., 2017). There is also a not so small population of MSN that express both D1 and D2 receptors. They are usually described as enkephalin receptor positive neurons, they express specifically the subunit GluA3 of the AMPA receptor and project broadly to nuclei containing DA neurons cell bodies, to the nAcc and the ento-peduncular nucleus among others (Perreault et al., 2011). The cross talk of interneurons at this level is a main filter on the cortical input. Clearly, different DR contribute to the final effect, depending on receptor distribution across different types of interneurons (Burke et al., 2018). For example the D1 activity in MSN is inhibited by the cholinergic tonus (M4 mediated) (Nair et al., 2019). In SCZ increased spine density have been observed in dorsal striatum MSN. Converging evidences suggest a critical role of the dopaminergic system in adapting synaptic plasticity of glutamatergic inputs (synaptic spines). Early in development, the DA system has fundamental roles in forebrain differentiation and circuit formation (Brignani and Pasterkamp, 2017), but DA tone also has clear effects on glutamatergic spine density at adult stage. It is however not clear how SCZ specific NMDA alterations could impact on the system. The recent and seminal work of the group of Prof. Groc, using single molecule-based imaging shows that NMDA antibodies present in some SCZ patients with psychotic symptoms are specifically changing the surface dynamics and nanoscale organization of synaptic NMDA and its anchoring partner the EphrinB2 receptor in synaptic spines in hippocampal neurons, ultimately preventing LTP potentiation (Jezequel et al., 2017; Jezequel et al., 2018). As expected this causes a small reduction of the D1 surface expression in the same cellular system (Grea et al., 2019). The associated intracellular DA signaling effects however could be more deeply modified because of this lack of NMDA/D1 interaction. It would be equally important to study these NMDA-antibody related changes in the context of the striatal circuits in particular on MSN D1 mediated signal and during development. The D1 receptor in dorsal striatum has been also involved in the sensorimotor gating alterations observed in SCZ but these mechanisms needs to be verified in man and with selective agents given the main differences in anatomical connectivity (Aguilar et al., 2018).

Striatal D2/D3 Receptors and SCZ

There are main differences in the DA input across the different striatal regions. This is particularly true for the D2 receptor function across dorsal striatum and nAcc. Increased DA D2 sensitivity in the nAcc is related to differences in coupling to Go vs. Gi (Marcott et al., 2018). The striatal D2 related control on reward is a key aspect of the effects of antipsychotics. Psychotic symptoms have been in fact linked to salience changes in the reward system circuit and blocking D2 controls psychotic symptoms including a normalization on reward disturbances (Han et al., 2020). A direct relationship between D2 receptor blockade, normalization of reward processing and symptom improvement was recently further supported by a small study in antipsychotic-naive first-episode SCZ patients (Wulff et al., 2020). Cognitive flexibility (reversal learning) is another aspect of D1/D2 related deficits that is linked to DA striatal functional regional differences (Sala-Bayo et al., 2020). The cellular basis of the role of striatal D1 vs. D2 in reward and learning have been further clarified by the work of Iino et al., 2020, showing in rodents the presence of a D2 controlled spine plasticity in MSN, that can be reversed with a D2 antagonist (Iino et al., 2020).

D2 antagonism is still recognized as a main stay of SCZ therapy and the D2 receptor is considered to be directly or indirectly responsible for the efficacy of the majority of typical and atypical antipsychotics. This is coherent with the general observation of a main role of DA control of cortico-striatal synchronization of D2-MSN neurons (via D2-GPRIN AKT) (Karadurmus et al., 2019). The tetra complex A2A-D2 receptors (plus AC5) is really central to multiple effects of both adenosine and DR ligands in the striatal region (Ferre et al., 2018; Bonifazi et al., 2019). mGlu5 receptor can be also included in a complex interaction with D2-A2A in GABAergic neuronal

terminals providing a multiple way to increase GABA release (Borroto-Escuela et al., 2016; Sahlholm et al., 2018). It is becoming therefore apparent that D2 receptor function is heterogeneous and possibly strictly dependent on the neuronal type expressing the receptor in different cortical and sub-cortical regions. Considering the role of D2 receptor in the control of emotional, cognitive and sensory functions alterations in SCZ it is therefore important to revisit the molecular aspects of this receptor and possibly even the pharmacology of the different antipsychotics (Quintana and Beaulieu, 2019). For instance the D1/D2 complex (possibly) present in some MSN exhibits the remarkable property of a coupling to a Gq- PLC mediated increase in intracellular calcium release and CAMKII phosphorylation (Perreault et al., 2011). This complex may represent an interesting new pharmacological target in SCZ. The D2S receptor is involved whenever SCZ treatment resistance is discussed or phenomena of presynaptic D2 receptor supra-sensitivity induced by antipsychotics (Amato et al., 2019).

Motivational deficits in SCZ are most likely associated with cortico-striatal circuits involving the VTA, and the ventral striatum (Aberg et al., 2020; Kontaris et al., 2020). Clinical observation keep suggesting some involvement of ventral striatum in the control of motivation, emotions and social behavior as relevant for negative symptoms in SCZ with regular debates on the matter (Fareri et al., 2017; Stepien et al., 2018; Waltz et al., 2018). Interestingly, D3 receptor expression is enriched in midbrain ventral striatum (including nAcc) (Slifstein et al., 2020) where the receptor is present on pre- and postsynaptic locations and can also work in cooperation with the receptor D1 (in MSN - AKT signal) (Castrellon et al., 2019; Guitart et al., 2019). The D3 receptor has been linked to control of DA firing in VTA, emotion and reward control in animal models (Takeuchi et al., 2019), but the lack of selective D3 ligands has so far hampered specific research on the subject (Correll and Schooler, 2020). Cholinergic interneurons in the ventral striatum, particularly those in the insula major of Calleja are highly enriched in D3 receptor, making these cells extremely sensitive to DA from VTA projections. Also in this case a D1/D3 complex is probably present. In this region as well as in cerebellum or other extra-striatal circuits, the D3 receptor has been linked to thermoregulation and sleep/wakefulness, which are potentially relevant for the control of some aspects of SCZ (Luo et al., 2018). Calleja islands are also a site related to adult neurogenesis in ventral striatum across species: these neurons are D3, Erb4 and neuroregulin1 positive.

SECTION 3. DR LIGANDS AND SCZ THERAPIES. THE NEW WAVE OF LIGANDS WITH POTENTIAL RELEVANCE FOR THERAPY OR BRAIN IMAGING

The discovery that DA effective drugs for treating SCZ is redeemable to the elegant work of Carlsson and Lindqvist in the early 60's and to the identification, a decade later, of the antipsychotics/DA receptor. Atypical antipsychotics developed in the 70's and 80's, included serotoninergic complementary mechanisms, as observed with clozapine, the prototypical atypical antipsychotic, to improve treatment compliance (Aringhieri et al., 2018). Historical perspectives on SCZ drugs generally highlight the DA receptor D_2 antagonism as main mechanism of action (Madras, 2013), but the pharmacology of antipsychotics is much more complex and requires a specific discussion on DR selectivity and serotonin receptor poly-pharmacology (Butini et al., 2016; Aringhieri et al., 2018; Moritz et al., 2018; Bueschbell et al., 2019). Important discoveries were made in the DA field during the past decade, in particular in relation to the pharmacology of DR ligands. DR heterodimers have been described in different brain regions and used to explain the complex biological effects associated with DR activation (Borroto-Escuela et al., 2018). Exciting data from crystallographic studies have supported a wave of drug discovery projects looking for new antipsychotics (Chien et al., 2010; Wang et al., 2017; Wang et al., 2018). DR signaling versatility is further magnified by context dependent dissecting signatures or "bias" (Urs et al., 2017) extending the potential for optimized pharmacological interventions. It is possible for instance to separate β-arrestin mediated signals using biased D1 agonists (Urs et al., 2011; Gray et al., 2018). Several recent contributions are available on this matter (Vyas et al., 2020). The potential therapeutic applications of biased D2 ligands to new SCZ therapies, has fuelled new interest on D2S vs. D2L or cAMP independent intracellular pathways, looking for agents with less motor side effects. D2 β-arrestin-biased ligands are now available (Park et al., 2016) and they may provide some pharmacological advantages, at least on the basis of the results in preclinical models (Urs et al., 2017). These agents are not per se D2 selective since they also interact with the D3 receptor and might require the presence of an heteromeric complex with the receptor A2a for the final effect. There is therefore a need for a different look at DR ligands pharmacology *in vitro*. We should possibly reconsider aspects like receptor internalization or intracellular recycling also for the main active metabolites or when comparing antagonists and partial agonists (De Vries et al., 2019). See **Table 2 Supplementary Material** for chemical series of DR ligands and representative compounds described in section 3.

DR Ligand Receptor Interactions

The most interesting finding in the field of DR is certainly the crystal structure of D2, D3. and D4 receptors and how this was used to identify new series or new mechanisms of ligand receptor interaction. Homology models are also extremely helpful for D1 and D5 with some main limitation for specific domains with reduced identity (Bueschbell et al., 2019). The DA binding site is contained in a membrane pocket formed by the TM3/5/6/7 with similarities across biogenic amines GPCRs. Molecular docking studies for the D1 receptor were able to demonstrate the presence of allosteric sites that were further targeted to obtain highly selective positive allosteric modulators with high potency, weak agonist properties and able to increase DA response (cAMP) (Bruns et al., 2018). The mode of interaction of biased agonists is different since they fail to trigger D1 receptor desensitization *in vitro*. The current model

supposes a docking in within the DA site, but with differences in interactions with TM3/5 and extracellular loop 2 (Gray et al., 2018). The rapid advance of the pharmacology of D1 receptors bringing new drugs to the clinic is a clear demonstration of the therapeutic impact of research on DR-ligand interactions (Hall et al., 2019). For D2/D3 biased ligands the drug design is complicated by the needed poly-pharmacology vs. 5-HT1A or 5-HT2A receptors which contribute to the clinical efficacy and also is intrinsic to some pharmacophore (Ma et al., 2019). The ligands cocrystallized in the different D2/D3 studies are haloperidol, risperidone, nemonapride and eticlopride, non-selective but potent antagonists (Fan et al., 2020). Thus no main difference was expected. In reality the results show differences in D2 inactive conformation that suggest different receptor inactive states (Lane et al., 2020). In addition the agonist binding pocket in the D2 allows an extension that has been used to study D2 > D3 and D4 selectivity (with agonist ligands) and to determine the possibility to obtain biased agonists for D2 (Fan et al., 2020). The re-assessment of the D2 interaction profile of different classes of D2 antagonists is also on the way (Zieba et al., 2019). The case of D3 is complementing this picture given the variety of new ligands currently available. Subtype-selective compounds have been sought for more than two decades with difficulties achieving sufficient selectivity and central exposure. Clinical PET data have recently provided encouraging results with cariprazine and F17464 (Slifstein et al., 2020). More recent D3 over D2 new ligands have been obtained exploiting the presence of a secondary allosteric D3 pocket to generate bitopic ligands with long molecular bridges. This strategy has allowed a powerful expansion in chemical possibilities even while maintaining the capacity to generate agents with biased activities (Rossi et al., 2017; Bonifazi et al., 2019). The concept of bitopic ligands is associated with the presence of two separated regions of the receptor with different vectors relevant for the affinity and the allosteric pocket interaction (usually driving D3/D2 selectivity considerations). Shorter D3 ligands will necessarily reside instead only in within the orthosteric pocket. Some interesting caged ligands for the D2/D3 orthosteric pocket could possibly help further pharmacological studies on this subject in native systems (Gienger et al., 2020). There is a second interesting aspect in the pharmacology of D3 bitopic ligands. It has allowed to show the presence of an alternative mechanism of D3 receptor internalization independent of β-arrestin and used by group II GPCR (Xu et al., 2019). Considering the excess D2 homodimers detected in schizophrenia (Wang et al., 2010), the effects of DA antagonists on these entities has been specifically explored using bivalent ligands (Pulido et al., 2018; Wouters et al., 2019). A molecular model of the homodimer has been also generated for D2 to provide docking information relative to bivalent ligands with different pharmacological properties (for example orthosteric and allosteric agents) (Kaczor et al., 2016). Other DR heterodimers were also considered as selective targets for this type of ligands (Carli et al., 2018), mainly because the differential expression of these dimeric receptor entities may allow a more precise approach to specific brain structures and pathways (Cortes et al., 2016; Foster and Conn, 2017).

DR-Ligand Interaction Dynamics and Efficacy Studies

There are classic aspects of receptor pharmacology like constitutive activity or equilibria across receptor conformations which are quite difficult to address with DR, in particular when considering heterocomplexes. It should be however possible to better distinguish antagonists from partial agonists and systematically discuss on and off rates vs. affinity measures when presenting new DR ligands. Species specific differences are also seldom acknowledged. This systematic pharmacological work is required to make sense of the complex *in vivo* pharmacology of DR ligands (in particular D2/D3) also for antipsychotics already on the market. The case of D2 and D3 receptors is indeed quite interesting in this sense because of the complexity of the structure/activity database required to select new candidates and validate efficacy in comparison to reference antipsychotics. Several groups have generated a variety of synthetic ligands concurring to build similar molecular models including dynamic aspects of DR receptor activation over time. In recombinant systems at least, we witness some amazing activity switches between agonist and "antagonist" properties across different series that require further dynamic considerations (Tan et al., 2020). Destabilization of D3 inactive state(s) and flexibility of the ligands are among the elements that the most recent model available is proposing (Ferraro et al., 2020). Molecular recognition steps, changes in hydration of the ligand binding pocket and ligand dependent receptor configuration changes are also important considerations for D2 and D3 in particular when docking flexible ligands and establishing comparisons (Pal et al., 2019). Native system pharmacology studies are due to confirm the relevance of the observed *in vitro* differences. It would be indeed interesting to obtain a database of consistent functional information for all the ligands generated to further advance in the direction of new therapeutics. A re-evaluation of known DR ligands in the clinic on the basis of the latest available molecular model would be useful to help DR drug developers to build a more integrated view on the efforts, the tools and the information available and needed to move forward.

CONCLUSION

This article reviews current knowledge on DR subtypes in SCZ, anatomical distribution, and new pharmacological tools that can help dissect out subtype-specific functions. The aspects of DR research described hereby are strictly related to SCZ or risk genes associated with it. What appears is that the current molecular understanding of Glutamate NMDA - DA interactions in SCZ has improved, but it is still insufficient in particular in brain areas like the ventral striatum and in relation to negative symptoms. A better understanding of the circuit(s) will possibly further reduce boundaries between cognitive and negative SCZ symptoms domains (Robison et al., 2020). The DA - NMDA research is

also bringing the neurodevelopmental aspects of the SCZ disease to the core of current efforts and hopefully this will improve our understanding of SCZ disease onset and the relevance of DR research in SCZ animal models. It is therefore essential to integrate all the most recent DR findings and further discuss the NMDA Glutamate – DA dysregulation hypothesis for SCZ with a focus on the key interactors between the two systems (Kesby et al., 2018; Potkin et al., 2020). This may also help drug discovery to address the complexity of DR heterocomplexes in native systems using multiple intracellular markers and benefiting from the available more selective DR tools.

AUTHOR CONTRIBUTIONS

SG and JM contributed to the text, tables, and JM contributed the figure in the review manuscript.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2020.01003/full#supplementary-material

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SYSTEMATIC REVIEW
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Antipsychotic Treatment Failure: A Systematic Review on Risk Factors and Interventions for Treatment Adherence in Psychosis

Kawtar El Abdellati[1,2]*, *Livia De Picker*[1,2] *and Manuel Morrens*[1,2]

[1] *Collaborative Antwerp Psychiatric Research Institute, University of Antwerp, Antwerp, Belgium,* [2] *Scientific Institute for Neuropsychiatric and Psychopharmacological Studies (SINAPS), University Psychiatric Centre Duffel, Duffel, Belgium*

Objective: Antipsychotic medication non-adherence has detrimental effects on patients' clinical outcome. It is unclear which risk factors affect adherence most and which interventions are effective at improving adherence to antipsychotic medication. The aim of this systematic review is to summarize evidence exploring risk factors of non-adherence to antipsychotic treatment and effectiveness of intervention to improve adherence in patients with psychotic spectrum disorders.

Methods: We conducted a systematic search in PubMed from 1994 to 2019 using a structured search strategy. Studies were quality assessed, and studies reporting on possible risk factors and intervention strategies were synthesized.

Results: We reviewed 26 studies on factors related to antipsychotic medication adherence and 17 studies on interventions to improve adherence in patients with psychosis spectrum disorders. Risk factors of non-adherence included younger age, poor illness insight, cannabis abuse, and the presence of severe positive symptoms. Antipsychotic medication adherence was associated with positive attitude toward medication of both patients and their family, family involvement, and illness insight. Somewhat consistent evidence was found for interventions involving family and technology-based interventions and strategies combining depot medication with psychoeducation. However, given the wide range of heterogeneous interventions and methodological limitations, findings must be interpreted with caution.

Conclusion: Despite much effort invested in the research area of antipsychotic medication adherence, the heterogeneity in study design and outcome, adding to confounding effects and possible biases, and methodological restraints complicate comparability of the results. Future research in this field should therefore be conducted on patient-tailored interventions, considering risk factors affecting the patient and implementing well-validated, standardized assessment methods. Accordingly, this systematic review seeks to facilitate endeavors improving adherence to antipsychotic treatment by identifying modifiable and non-modifiable risk factors, outlining effective intervention strategies, and proposing recommendations to enhance adherence strategies.

Keywords: adherence, non-adherence, compliance, antipsychotic, psychosis, schizophrenia, therapeutic drug monitoring

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Ole A. Andreassen,
University of Oslo, Norway

***Correspondence:**
Kawtar El Abdellati
kawtar.elabdellati@uantwerpen.be

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INTRODUCTION

Treatment resistance	Kane et al. (1988): (1) a minimum of three treatment periods in the preceding 5 years with antipsychotics (from at least two difference chemical classes) at dosages ≥1,000 mg/day chlorpromazine for a period of 6 weeks, each without significant symptomatic relief and (2) no period of good functioning within the preceding 5 years. Kane et al. (2019): failure to respond on any two antipsychotic medications, each at an adequate dose (i.e., equivalent to ≥600 mg/day chlorpromazine) and treatment duration + objective symptom measurements should be used to assess treatment response and medication adherence.
Pseudo-resistance	Lack of response to antipsychotic treatment not attributed to pharmacological inefficiency of the compound but depending on modifiable and non-modifiable factors such as non-adherence (de Bartolomeis et al., 2018)
Non-adherence	Only some or none of the prescribed medication is taken (Kane et al., 2019)

Psychotic disorders are severe mental disorders that are characterized by episodic or long-term dysfunctions of perceptual, cognitive, and emotional processes that cause severe impairments with regard to social and occupational functioning (Howes et al., 2012). A proportion of patients exhibit little clinical response despite treatment with multiple different antipsychotic drugs (Howes et al., 2017), implicating that therapeutic assistance is often challenging with results that are incomplete and unsatisfactory. This therapeutic failure may be partially or completely due to various factors, including not only treatment resistance, regimen appropriateness, and drug tolerability (Lindenmayer et al., 2009) but also adherence to prescribed treatment (Garcia et al., 2016; Howes et al., 2017). Approximately 30% of patients with schizophrenia and related disorders obtain little benefit from standard antipsychotic treatment and are considered to have a treatment-resistant illness profile (Conley and Buchanan, 1997; Meltzer, 1997; National Collaborating Centre for Mental Health, 2009; Lally et al., 2016; Wimberley et al., 2016; Demjaha et al., 2017). Pioneering work by Kane et al. (1988) initiated a chain of works on treatment resistance in schizophrenia, and accordingly, the topic has been discussed at length [see Howes et al. (2017), Kane et al. (2019)]. Notwithstanding, defining treatment resistance and deriving pragmatic recommendations for clinical practice remains problematic. Current guidelines broadly agree in terms of their definition of treatment, with key criteria that include no significant improvement in psychotic symptomatology after treatment with at least two different non-clozapine antipsychotics at adequate dose and duration of time. However, recommendations and clinical outcomes used to evaluate the level of treatment response vary among the guidelines, which is further complicated by the already heterogeneous psychotic patient population (Kane et al., 2019; Barnes et al., 2020), such that substantially inconsistent results can be found across the studies involving these patients (Suzuki et al., 2012).

Another issue in determining treatment response is the concept of pseudo-resistance (Howes et al., 2017), which postulate that certain components can make it appear as if a patient is non-responsive while in reality treatment response can be altered, i.e., through improvement of adherence behavior (de Bartolomeis et al., 2018). Indeed, at least a third of the patients thought to have a treatment-resistant profile have shown to have subtherapeutic plasma antipsychotic levels due to pharmacokinetic factors or to poor adherence (McCutcheon et al., 2015, 2018). Additionally, antipsychotic treatment non-adherence has been identified as one of the main causes for antipsychotic treatment failure (Goff et al., 2010). Although medication non-adherence is a common problem throughout medicine, several factors make it especially challenging in treating patients with psychotic disorders: direct impact of symptoms on cognitive functions (El-Missiry et al., 2015; MacKenzie et al., 2018), lack of illness insight, stigma, comorbid substance abuse, and social isolation (Haddad et al., 2014). Astoundingly, while the number of patients taking antipsychotics has increased over the years, little progress has been made with regard to improving medication adherence in these patients, possibly because the choice of measurement of adherence is a long-standing methodological problem. Measures of medication adherence can be classified in (1) objective indicators of medication intake, such as pills counts, electronic monitoring, and serum or plasma

Abbreviations: AIMS, abnormal involuntary movement scale; AP, antipsychotics; ARS, Adherence Rating Scale; AT, adherence therapy; AP, antipsychotic medication; BIS, Birchwood Insight Scale; BPRS, Brief Psychiatric Rating Scale; CAE, customized adherence enhancement; CAT, cognitive adaptation training; CBT, cognitive behavioral therapy; CDR, concentration to dose ratio; CDSS, Calgary Depression Scale for Schizophrenia; CFI, Camberwell Family Interview; CGI, Clinical Global Impression scale; CRS, Clinician Rating Scale; CVLT, California Verbal Learning Test DAI, drug attitude inventory; DB, double-blind; DKEFS, Delis–Kaplan Executive Function System; EE, expressed emotion; EPS, extrapyramidal symptoms; ES, effect size; ESRS, Extrapyramidal Symptom Rating Scale; FEP, first-episode psychosis; FU, follow-up; GAF, Global Assessment of Functioning; IDS, Inventory of Depressive Symptomatology; LAI, long-acting injectable antipsychotics; LUNSERS, Liverpool University Side Effects Rating Scale; MADRS, Montgomery–Asberg Depression Rating Scale; MAQ, Morisky Green Adherence Questionnaire; MARS, Medication adherence Rating Scale; MeM, Med-eMonitor; MFG, multifamily group therapy; MPR, Medication Possession Ratio; NART, National Adult Reading Test; PANSS, Positive and Negative Syndrome Scale; PE, psychoeducation; PETiT, Personal Evaluation of Transitions in Treatment; PSST, Psychosocial Skills Training; QLS, Quality of Life Scale; QoL, quality of life; RoB, risk of bias; ROMI; Rating of Medication Influences; SAI-C, Schedule for the Assessment of Insight-Compliance; SB, single-blind; SE, side effects; SZ, schizophrenia; SZA, schizoaffective disorder; SPH, schizophreniform disorder; TRQ, Tablet Routines Questionnaire; TAU, treatment-as-usual; WASI, Wechsler Abbreviated Scale of Intelligence; WIS, Wechsler Intelligence Scale; WMS, Wechsler Memory Scale.

levels of antipsychotics and (2) subjective measures of medication use via patient report or interviewer ratings. Adherence is an observable, measurable behavior and is often reported as a dichotomous variable (adherence vs. non-adherence), while it can vary along a continuum in which absolute adherence and non-adherence are the two ends. However, the absence of consensus on cutoff points prevents comparability of the literature (Sendt et al., 2015). Although continuous observation of actual medication intake is the true gold standard of adherence estimation, such conspicuous monitoring would prompt better adherence than would occur in unobserved environments. Nonetheless, measuring adherence behavior does not reveal underlying reasons for non-adherence (Sajatovic et al., 2010).

Adherence difficulties complicate the clinical management for prescribers as well. Psychiatrists may have trouble distinguishing between poor adherence and poor treatment response, especially since partial non-adherence occurs as frequently as complete medication cessation (Svestka and Bitter, 2007). A 15-year Belgian population-based study reported that a vast majority of antipsychotic-treated patients took their prescribed medication for a brief period of time (81.8% of the prescribed antipsychotics were administered for a maximum of 3 months), indicating that a considerable part of the patients with psychosis are inadequately or even untreated (Morrens et al., 2015). By underestimating non-adherence, prescribers may prematurely discontinue treatment, add concomitant medications, or increase dosages. Treatment failure in covert non-adherent individuals may lead to the faulty assumption of treatment resistance (Velligan et al., 2013). Clearly, vigorous efforts should be made to determine medication adherence and exclude so-called pseudo-resistant individuals (Howes et al., 2017) in order to improve clinician's decision-making process and prevent further iatrogenic harm (Lopez et al., 2017). In this regard, one could wonder if the routine blood level monitoring for antipsychotics may thus contribute to its superior effectiveness in previously non-responsive patients (Patteet et al., 2012). Moreover, non-adherence has been significantly associated with poorer clinical outcome, including greater risk of hospitalization, longer duration of hospitalization (Higashi et al., 2013; Olivares et al., 2013), and greater risk of suicide (Leucht and Heres, 2006; Llorca, 2008; Forsman et al., 2019). In addition, partial and total medication non-adherence are strongly associated with psychotic relapse as non-adherent patients with schizophrenia having a 5-fold increase in risk of relapse (Robinson et al., 1999; Caseiro et al., 2012). This systematic review will therefore summarize key factors predicting non-adherence in psychotic spectrum disorders (PSDs) in order to better identify at-risk patients. In addition, we evaluate the existing evidence on the efficacy of interventions to improve medication adherence in PSD and their effect on other patient outcomes. To our knowledge, this is the first systematic review combining and linking risk factors and interventions of (non)adherence in psychosis.

METHODS

In August 2019, an electronic search was conducted in the PubMed database for English-language publications from January 1994 to August 2019, using the following MeSH terms: medication adherence, medication compliance, antipsychotics, antipsychotic agents, psychosis, and psychotic disorder. Additionally, we used the following PubMed filters: study type (clinical trials, meta-analysis, observational study, randomized controlled trial, systematic reviews) and study subject (human). Subsequently, reference lists from studies included in our systematic review were manually searched for additional relevant publications. Year 1994 was selected as the start date for the search because of the publication of the Diagnostic and Statistical Manual of Mental Disorders, fourth edition (DSM-IV) in that year.

All abstracts were screened for the following predefined inclusion criteria: clinical trials, observational studies, randomized controlled trials, systematic reviews, and meta-analyses in which the study population consisted of patients with psychosis and schizophrenia spectrum disorders (corresponding to schizophrenia, schizophreniform disorder, schizoaffective disorder, delusional disorder, brief psychotic disorder, other specified schizophrenia spectrum and other psychotic disorder. and unspecified schizophrenia spectrum and other psychotic disorder as described in DSM-V) being treated with antipsychotic agents and in whom factors or interventions associated with treatment adherence were assessed. All studies must include direct and/or indirect measures of medication adherence behavior. Exclusion criteria were other primary diagnosis and narrative or qualitative reviews. To facilitate interpretation of the studies published to date, we considered the distinction between adherence behavior and attitude and excluded studies with an adherence assessment based on adherence attitudes solely.

Quality and risk of bias of the articles related to the objective of our review were assessed using the Critical Appraisal Skills Programme (CASP) Appraisal Checklist (Critical Appraisal Skills Programme, 2019) and the Cochrane risk of bias for randomized studies (Higgins et al., 2011).

Data Extraction

Two independent reviewers, KEA and LJDP, extracted predefined data and checked the data extraction sheet. Discordant results were resolved through discussion. We developed a standardized data extraction sheet regarding interventions with following data: intervention type, methodology, diagnosis, age, ethnicity, type of antipsychotic, duration, number of included cases, adherence outcome and effects, other outcome measures and effect, definition of (non)adherence, classification of adherence, quantification of adherence, and limitations of the study. A data extraction sheet regarding risk factors and predictors with following data was also created: type of factor, diagnosis, stage of illness, age, ethnicity, type of antipsychotic, methodology, duration of study, number of cases, outcome measures and effect, definition of (non)adherence, and classification and quantification of adherence.

RESULTS

The search of the PubMed database resulted in an initial 71 records (cf. PRISMA flowchart in **Figure 1**). For three records,

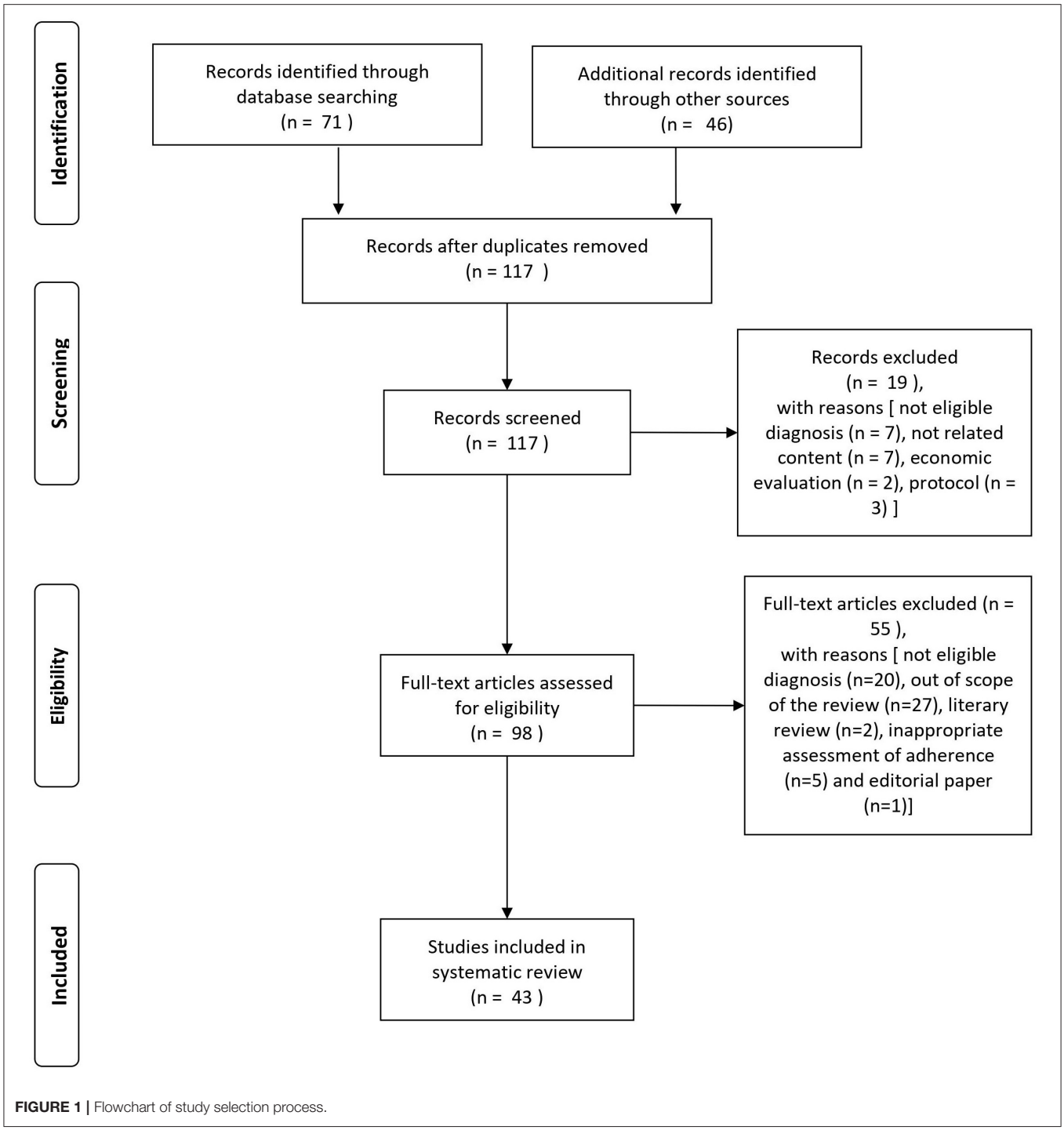

FIGURE 1 | Flowchart of study selection process.

we contacted the study authors in order to obtain more information on the characteristics of the study population or for clarification of the results. One of these could provide the necessary information (Beebe et al., 2017). An additional 46 eligible articles were identified by hand search of reference lists. Nineteen articles were excluded at screening with the following reasons: not eligible diagnosis ($n = 7$), not related content ($n = 7$), economic evaluation ($n = 2$), and protocol ($n = 3$). After full-text assessment, additional 55 articles were excluded [not eligible diagnosis ($n = 20$), out of scope of the review ($n = 27$), literary review ($n = 2$), inappropriate assessment of adherence ($n = 5$), and editorial paper ($n = 1$)].

A total of 43 studies was found eligible for the systematic review: 17 studies provided information on intervention strategies to improve antipsychotic medication adherence, and 26 studies were on factors influencing adherence outcome.

For a schematic representation of the study selection process, see **Figure 1**.

Study Specific Characteristics

Most studies included an adult population, with the exception of one study with an age range of 14–19 years (Molteni et al., 2014). Several studies on the factors associated with medication adherence enrolled participants at early stage of illness (first episode of psychosis, recent onset of psychosis) (Coldham et al., 2002; Mutsatsa et al., 2003; Kahn et al., 2008; Quach et al., 2009; Weiden et al., 2012; Molteni et al., 2014; Winton-Brown et al., 2017). Not all studies reported ethnic background. In general, medication was either taken orally, by depot injection, or in combination. Some studies did not detail specific medication information, reporting them only as antipsychotic or neuroleptic medication.

Risk Factors and Predictors of Adherence

The main factors that might influence treatment non-adherence were associated with patients themselves, their drug treatment, and family involvement.

Patient-Related Risk Factors and Predictors

Twenty individual studies and three systematic reviews investigated patient-related predictors of non-adherence. The details on each individual study are summarized in **Table 1**.Sociodemographic features, clinical symptoms, adverse effects, cognitive functioning, illness insight, alcohol and illicit substance use, and patient attitudes are the main factors that have been studied in the context of antipsychotic medication adherence (see **Table 2**). For an overview of risk factors and predictors related to antipsychotic medication adherence and non-adherence, see **Table 3**.

Sociodemographic Risk Factors

Evidence from 13 studies assessing the relation between sociodemographic risk factors or predictors and adherence are summarized below.

One randomized controlled study (RCT) with 599 patients with schizophrenia and schizoaffective disorders (Lindenmayer et al., 2009), two cross-sectional studies (Meier et al., 2010; Jonsdottir et al., 2013), and two longitudinal cohorts (Acosta et al., 2009; Yang et al., 2012) investigated baseline demographics as potential risk factors but found none to be good predictors of non-adherence. Results were mixed concerning age as a predictor. Both younger age and, to a lesser extent, also younger age at illness onset have been identified as a strong predictor of non-adherence, although other studies have failed to replicate this finding. Findings were mixed regarding adherence rates in ethnic minorities compared to Caucasian patients (Aldebot and de Mamani, 2009; Winton-Brown et al., 2017). Furthermore, adherence behavior is not related to patients' marital status (Acosta et al., 2009; Higashi et al., 2013; Jonsdottir et al., 2013; Bayle et al., 2015; Sendt et al., 2015), gender (Janssen et al., 2006; Morken et al., 2007; Klingberg et al., 2008; Acosta et al., 2009; Aldebot and de Mamani, 2009; Higashi et al., 2013; Jonsdottir et al., 2013; Bayle et al., 2015; Sendt et al., 2015), occupation (Klingberg et al., 2008; Higashi et al., 2013; Bayle et al., 2015; Sendt et al., 2015), and level of education (Klingberg et al., 2008; Acosta et al., 2009; Aldebot and de Mamani, 2009; Higashi et al., 2013; Jonsdottir et al., 2013; Sendt et al., 2015), with the exception of one longitudinal study that found a small association with non-adherence (OR, 0.59; 95% CI, 0.41–0.86, $p < 0.01$) (Janssen et al., 2006).

Clinical Risk Factors

Twenty studies investigated the relation between symptom severity and antipsychotic adherence behavior. While a significant association between increasing severity of illness and decreasing antipsychotic adherence was reported in four individual studies (Morken et al., 2007; McCabe et al., 2012; Yang et al., 2012; Bayle et al., 2015), no association of symptom severity was reported in three others (Klingberg et al., 2008; Aldebot and de Mamani, 2009; Meier et al., 2010; Jonsdottir et al., 2013). However, generalization of the results is complicated by the fact that symptoms were assessed using different scales, i.e., Positive and Negative Syndrome Scale (PANSS), Clinical Global impression (CGI) scale, and Brief Psychiatric Rating Scale (BPRS).

Positive symptoms have been linked to non-adherence in a longitudinal cohort of patients with first-episode psychosis (FEP) (Coldham et al., 2002) and in stable patients (Borras et al., 2007). However, no significant association with adherence was observed in another cohort of stable patients (Klingberg et al., 2008). In addition, high intensity of excitement (Yang et al., 2012), hostility (Lindenmayer et al., 2009), and a high PANSS paranoid subscore (Janssen et al., 2006) were also identified as risk factors of non-adherence, while higher scores on disorganization syndromes (Mutsatsa et al., 2003; Acosta et al., 2009) were weak predictors of non-adherence. Evidence for other factors such as a higher negative subscore on the PANSS (Mutsatsa et al., 2003; Janssen et al., 2006; Klingberg et al., 2008) was weak, while poor impulse control and preoccupation have been associated with non-adherence (Yang et al., 2012). A significant association has also been found between both depressive symptoms as measured by the MADRS (total score, $p = 0.01$; reported sadness, $p = 0.04$; pessimistic thoughts, $p = 0.01$) and the PANSS (depressive factor HR = 1.2; 95% CI, 1.06–1.35; $p = 0.003$), in an RCT (Lindenmayer et al., 2009). In contrast, no association for depressive symptoms as measured by the CDSS and IDS was found in a longitudinal (Yang et al., 2012) and cross-sectional cohort (Jonsdottir et al., 2013), respectively. Although the design and included sample size of the prospective cohort generates limited evidence, the CDSS can differentiate depressive symptoms more accurately from other symptoms (Lako et al., 2012) compared to the MADRS. No association was found for manic symptoms as measured by the YMRS and adherence (Jonsdottir et al., 2013).

Furthermore, illness characteristics (Lindenmayer et al., 2009), including specific diagnosis (Janssen et al., 2006; Klingberg et al., 2008; Baloush-Kleinman et al., 2011; Bayle et al., 2015), and duration of illness (Janssen et al., 2006; Acosta et al., 2009; Baloush-Kleinman et al., 2011; Sendt et al., 2015) were poor predictors for adherence behavior. Other factors, such as

TABLE 1 | Summary of the characteristics of the individual studies on potential risk factors of adherence and non-adherence.

Study type	Study	Sample characteristics	Cases	Duration of study	Adherence measure	Adherence rate	RoB
RCT	1. Olivares et al. (2013)	Chronic; stable; SZ + SZA;	599 (10 vs. 20 vs. 40 mg/day, pooled)	8 w (4w and 8 w assessment); DB	Pill counts, response rate, plasma levels for half of patient population	Adherent: 65.5%; non-adherent: 34.5%	Low
	2. Morken et al. (2007)	Recent-onset; stable; SZ + SPH;	30 intervention vs. 20 TAU	24 m (assessments every 2 months); SB	Clinician-rated 4-point scale (based on patient interviews and other measures), family/caregiver reports; plasma levels of AP	Non-adherent: 20%	Low
	3. Weiden et al. (2012)	FEP, acute; PSD	26 intervention vs. 11 TAU	104 w; open-label; SB	Time to initial non-adherence	Non-adherent: 81%	Moderate
	4. Kahn et al. (2008)	FEP; state NR; PSD	Haloperidol (n = 103) vs. SGA [amisulpride (n = 104), olanzapine (n = 105), quetiapine (n = 104), ziprasidone (n = 82)]	12 m; open-label; unblinded	One-item 7-points rating scale	Non-adherent: haloperidol, 72%; amisulpride, 40%; olanzapine, 33%; quetiapine, 53%; ziprasidone, 45%	Low
CT—open label, naturalistic, flexible-dose	5. Guo et al. (2011)	Early-stage; stable; SZ + SPH	1,133	12 m	Treatment discontinuation rate, including non-adherence or changing initial AP	Non-adherent: chlorpromazine, 41.4%; sulpiride, 39.5%; clozapine, 36.7%; risperidone, 40.2%; olanzapine, 39.6%; quetiapine, 46.9%; aripiprazole, 40.2%	Low
CT—observational, longitudinal	6. Winton-Brown et al. (2017)	FEP; state NR; PSD	136	18 m; retrospective	Self-report, breaks in treatment	Non-adherent: 40.2%	Low
	7. Coldham et al. (2002)	FEP; state NR; PSD	186	3 y (3-monthly assessment 1st year, half-yearly in 2nd year and then annually); prospective	3-point scale	Adherent: 40.9%; inadequately adherent: 19.9%; non-adherent: 39.3%	Low
	8. Mohamed et al. (2009)	Chronic; stable; SZ	1,432	18 m (3-monthly assessment); prospective	Patient, clinician, and family reports; pill counts	Adherent: ±75%	Low
	9. Quach et al. (2009)	FEP; state NR; PSD	547	2 y (annual assessment); prospective	Observer-rated (based on structured interviews with the patient, information from the primary case manager, the psychiatrist, and by systematic examination of the case notes and prescription cards)	Non-adherent: 35–39%	Moderate
	10. Baloush-Kleinman et al. (2011)	Early stage; state NR; SZ + SZA	112	6 m (assessments at admission, discharge, 3 and 6 m FU); prospective	Visual analog scale for assessing treatment adherence (Smith et al., 1992), and rated by patients, relatives, and treating clinician.	Non-adherent: 29.7%	Low
	11. Janssen et al. (2006)	Mixed (10.1% FEP); state NR; PSD	670	Assessment weekly during the inpatient stay (mean stay 43 days), and at discharge; prospective	Likert-type scale within a structured interview, adapted from Amador et al. (1993).	Adherent: 47.0%	Moderate

(Continued)

TABLE 1 | Continued

Study type	Study	Sample characteristics	Cases	Duration of study	Adherence measure	Adherence rate	RoB
	12. Acosta et al. (2009)	Mixed (% FEP NR); stable; SZ	74	3 m; prospective	MEMS device, depot visits; estimation by psychiatrist, patients, and family/caregiver reports	Non-adherent: 42.3%	Low
	13. Yang et al. (2012)	Chronic; stable; SZ	65	8 w (assessments at baseline, w 4 and w 8); prospective	MEMS; Pill count; clinician-rated 7-point adherence scale (based on patient interview), patient self-report scale (0–100%)	Non-adherent: 41.2% (MEMS), 7.8% (pill counting), 7.8 %(clinician rating scale), 25.5%'(self-report)	Low
CT—cross-sectional	14. Klingberg et al. (2008)	Mixed (30.6% FEP); stable; SZ + SZA	108	NA	CRS; AP plasma levels	Non-adherent: 0.9%	Low
	15. Mutsatsa et al. (2003)	FEP; acute; SZ + SPH	101	NA	CRS	Non-adherent: 44%	Low
	16. Bayle et al. (2015)	Mixed (% FEP NR); stable; PSD	1,887	NA	MAQ	Non-adherent: 53.2%; partially adherent: 29.5%,; adherent: 17.3%	Low
	17. Molteni et al. (2014)	Early onset (14–19 years); stable; PSD	67	NA	4-point Likert-type questionnaire	Non-adherent: 8.96%; partially adherent: 25.73%; adherent: 65.67%	Low
	18. Day et al. (2005)	Mixed (%FEP NR); acute; SZ + SZA	228	NA	Morisky, DAI	NR	Low
	19. Meier et al. (2010)	Chronic; stable; SZ	409	NA	MAQ, CRS	NR	Low
	20. Borras et al. (2007)	Chronic, stable; PSD	103	NA	Self-report, blood drug monitoring	Non-adherent: 15.5%	Moderate
	21. Aldebot and de Mamani (2009)	Mixed (% FEP NR); stable; SZ + SZA;	40	NA	Modified subscales of the COPE inventory; MARS	NR	Low
	22. McCabe et al. (2012)	Chronic; stable; PSD	507	NA	Clinician-rated: 3-point Buchanan criteria (based on routine clinical contact); for 29% of sample: information from social contacts used to complement clinician rating; objective measures for 49% of sample: depot records, supervised medication taking or drug testing used to inform rating	Poor adherence (<25%): 4.1%; good adherence (>75%): 75.7%	Low
	23. (Jonsdottir et al., 2013)	Illness stage NR; stable; PSD	154	NA	Self-report (Likert 0–100%) + serum concentration (AP in 94.8% of patients)	Full adherence (100% self-report, serum concentration within reference level): 55.2%; no adherence (<12% adherence self-report, no detectable levels): 11.0%; partial adherence (12–95% self-report, detectable serum levels not within reference levels): 51.3%	Low

TABLE 2 | Evidence table on risk-factors of antipsychotic medication adherence and non-adherence.

Study type	Study	Outcome measures	Sociodemographic factors	Clinical factors	Treatment-related factors	Family involvement and therapeutic relations
RCT	1. Lindenmayer et al. (2009)	PANSS; MADRS; GAF; CGI-S; QLS; Simpson–Angus Scale; BARS and AIMS	Demographics (gender, age, ethnicity), illness characteristics, baseline weight (n.s.)	MADRS scores [baseline total mean (SD), adherent 13.90 (8.80) vs. non-adherent 15.85 (8.50), $p = 0.010$]; worsening PANSS depressive factor (HR = 1.2, 95% CI 1.06–1.35, $p = 0.003$); hostility (HR = 1.14, 95% CI 1.02–1.26, $p = 0.020$); change in PANSS total score and history of substance abuse (n.s.)	adverse events (n.s.) and weight change (n.s.)	
	2. Morken et al. (2007)	Expressed emotion assessment based on CFI	male sex (OR = 6.11, 95% CI 1.2–29.74, $p = 0.025$)	Symptom severity (BPRS) (OR = 1.13, 95% 1.01–1.27, $p = 0.034$)		Patients living with family with high expressed emotion (OR = 36.43, 95% CI 2.18–608.01, $p = 0.012$); lower expressed emotion: 1st year (OR = 19.59, 95% CI 1.64–234.22, $p = 0.019$); both years (OR = 6.04, 95% CI 1.07–34.13, $p = 0.042$)
	3. Weiden et al. (2012)				Route of administration (n.s.)	
	4. Kahn et al. (2008)				FGA vs. SGA (n.s.)	
CT—open label, naturalistic, flexible dose	5. Guo et al. (2011)				FGA vs. SGA (n.s.)	
CT—observational, longitudinal	6. Winton-Brown et al. (2017)	GAF, PANSS, CDSS, insight rating scale (David et al., 1992), relapse	Non-Caucasian (OR = 3, 95% CI 1.3–7.2, $p = 0.01$)	Use of illicit substances (OR = 0.3, 95% CI 0.1–0.5, $p < 0.001$)	Presence of EPS (OR = 8.1, 95% CI 1–65.3, $p = 0.050$)	Carer involvement (OR = 2.2, 95% CI 1–4.9, $p = 0.048$);
	7. Coldham et al. (2002)	QLS; ESRS; Bares Akathisia Scale; Premorbid Adjustment Scale	Young age ($F = 4.5$, $p = 0.010$); young age of onset ($F = 6.7$, $p = 0.002$); younger age (OR = 1.13, 95% CI 1.02–1.24, p = 0.015)	Relapse in first year ($F = 4.16$, $p = 0.020$); positive symptoms at 1 year ($F = 7.88$, $p = 0.001$); QoL at baseline ($F = 3.45$, $p = 0.030$); QoL at 1 y ($F = 4.47$, $p = 0.010$); poor premorbid functioning (OR = 0.07, 95% CI 0.00–0.24, $p = 0.006$); alcohol at baseline ($F = 3.31$, $p = 0.020$); alcohol at 1 y ($F = 6.21$, $p = 0.003$); cannabis at baseline ($F = 3.17$, p = 0.040); cannabis at 1 y ($F = 3.17$, $p = 0.001$); cannabis use (OR = 0.46, 95% CI 0.25–0.84, $p = 0.012$); alcohol abuse n.s.; insight at baseline ($F = 4.08$, $p = 0.020$); insight at 1 y ($F = 4.26$, $p = 0.02$)		lack of family involvement (OR = 0.19, 95%CI 0.05–0.75, $p = 0.017$)
	8. Mohamed et al. (2009)	GAF; ITAQ; DAI		Baseline illness insight ($t = 2.48$, $p < 0.050$); change in insight scores from baseline to follow-up up (ITAQ: 0.078, $p < 0.001$; DAI: 0.235, $p < 0.001$); positive attitudes toward medication ($r = 0.154$, $p < 0.001$)		

(Continued)

TABLE 2 | Continued

Study type	Study	Outcome measures	Sociodemographic factors	Clinical factors	Treatment-related factors	Family involvement and therapeutic relations
	9. Quach et al. (2009)	GAF; SUMD; ROMI	Young age (OR = 1.79, 95% CI 1.16–2.75, $p = 0.008$)	Comorbid addiction (OR = 2.03, 95% CI 1.17–3.52); high global functioning (GAF) (OR = 1.73, 95% CI 1.07–2.81, $p = 0.0300$); unawareness of the effect of medication (OR = 2.34, 95% CI 1.44–3.82, $p = 0.0010$); negative attitude toward medication (OR = 2.13, 95% CI 1.43–3.17, $p = 0.0001$)		No upbringing by both parents (OR = 1.64 95% CI 1.11–2.42, $p = 0.010$); no key supporting relative (OR = 1.54, 95% CI 1.05–2.25, $p = 0.030$)
	10. Baloush-Kleinman et al. (2011)	CGI, SAPS; SANS; Cognitive Appraisal of Health Scale; Scale to Assess Unawareness of Mental Disorder; MacArthur Competence Assessment Tool; ESRS; Liverpool University Neuroleptic Side Effect Rating Scale; patient-rated Trust in Physician Scale; DAI; Visual Analog Scale (perception of family involvement)	Mode of admission, diagnosis of schizoaffective disorder, duration of illness (all n.s.)	Higher levels of insight into illness ($t = 0.13$, $p = 0.009$), awareness of the need for treatment ($t = 3.82$, $p < 0.001$), awareness of the social consequences of illness (n.s.)	Side-effects in adherent group ($t = 2$, $p = 0.036$); medication class (n.s.)	Perceptions of doctor–patient trust in the therapeutic alliance ($t = 3$, $p = 0.012$), perceived family involvement and attitudes toward medication in the family ($t = 5$, $p < 0.001$)
	11. Janssen et al. (2006)	GAF; DOTES; PANSS	Number of previous psychiatric hospitalizations ($p < 0.010$); involuntary admission (OR = 0.60, 95% CI 0.41–0.89, $p < 0.050$); no school graduation (OR = 0.59, 95% CI 0.41–0.86, $p < 0.010$); gender, primary diagnosis, first or multiple episode admission, duration of illness (all n.s.)	History of aggressive behavior (OR = 0.57, 95% CI 0.38–0.85), PANSS negative subscore above 25 (admission) (OR = 0.61, 95% CI 0.43–0.85, $p < 0.01$), PANSS paranoid/belligerence subscore above 9 (admission) (OR = 0.69, 95% CI 0.48–0.99, $p < 0.01$); substance disorder (OR = 0.52, 95% CI 0.32–0.85, $p < 0.01$)	Neurological side effects (n.s.); SGA monopharmacy at discharge > FGA mono or FGA + SGA ($p < 0.005$, $\chi^2 = 17.6$); FGA monotherapy switch to SGA vs. continue to take FGA ($p < 0.001$, $\chi^2 = 12.6$); mean dosage of initial antipsychotic treatment (n.s.); route of admin at admission (n.s.); depot vs. oral AP at discharge ($p < 0.05$, $\chi^2 = 6.3$)	
	12. Acosta et al. (2009)	Amador Insight scale, PANSS	Age, sex, marital status, education level, living alone or with someone, length of illness, number of prior hospitalizations, time since last hospitalization (all n.s.)	PANSS conceptual disorganization (OR = 1.74, CI 0.96–3.17, $p = 0.068$); present and past substance use or abuse (n.s.); poor insight (OR = 1.22, 95% CI 1.01–1.48, $p = 0.040$)	Medication class and dosage (n.s.)	

(Continued)

TABLE 2 | Continued

Study type	Study	Outcome measures	Sociodemographic factors	Clinical factors	Treatment-related factors	Family involvement and therapeutic relations
	13. Yang et al. (2012)	CDSS; CGI; PANSS; LUNSERS; DAI; SWN; Revised Insight Scale for Psychosis; WIS	All n.s.	CDSS (n.s.); CGI-S at baseline ($r = -0.301$, $p < 0.050$); CGI-S at 4 w ($r = -0.403$, $p < 0.010$); CGI-S at 8 w ($r = -0.426$, $p < 0.010$); PANSS score excitement [mean (SD), adherent 1.23 (0.43) vs. non-adherent 1.63 (0.83), p = 0.032], poor impulse control [mean (SD), adherent 1.23 (0.43) vs. non-adherent 1.58 (0.77), $p = 0.049$], and preoccupation [mean (SD), adherent 1.27 (0.58) vs. non-adherent 1.74(0.93), $p = 0.035$]; neurocognitive functions and insight (n.s.); attitudes toward medication ($r = 0.49$, $p < 0.010$)	Side effects (n.s.); polypharmacy ($r = 0.358$, $p < 0.050$);	Lower perceived support from significant other (only significant in parts of analysis; mean (SD), adherent 3.49 (1.54) vs. non-adherent 4.59 (1.62), $p = 0.017$);
CT—cross-sectional	14. Klingberg et al. (2008)	PANSS, GAF, SCL-GSI; UKU; EPS; AIMS	All n.s.	PANSS, GAF, SCL-GSI, global functioning and neurocognitive function (all n.s); lack of insight (OR = 0.41, 95% CI 0.183–0.915, $p = 0.030$); positive attitude toward medication ($r = 0.382$; $p < 0.001$)	Medication class and dosage (n.s.)	Frequency social contact, patient has a close friend, contact to relatives >10 h per week, influence family criticism, resignation and overprotection (all n.s.)
	15. Mutsatsa et al. (2003)	LUNSERS; ROMI; SAI; SWN; PANSS		Negative symptoms ($t = -1.98$, $p = 0.050$); disorganization ($t = -2.01$, $p = 0.050$); alcohol or non-alcohol substance misuse (n.s.); poor insight ($t = 5.71$, $p < 0.001$); negative attitudes toward medication ($t = 3.01$, $p = 0.003$)	Akathisia, parkinsonism, non-neurological side effects and subjective well-being (all n.s.)	
	16. Bayle et al. (2015)	CGI; PANSS	Age <40 years (OR = 1.566, 95% CI 1.313–1.869, $p < 0.001$); diagnosis of schizophrenia ($p = 0.008$, χ^2 test, adherent 43.7% vs. non-adherent 56.3%); sex, marital status, and living arrangements or occupation (all n.s.)	CGI-S ≥4 (OR = 1.986, 95% CI 1.518–2.598, $p < 0.0001$); lower insight (PANSS-G12) (OR = 1.459, 95% CI 1.225–1.738, $p < 0.001$)		
	17. Molteni et al. (2014)	SE using DAI-30		Positive subjective experience with medication (DAI-30) (OR = 1.10, $p = 0.002$)		
	18. Day et al. (2005)	PANSS; LUNSERS; attitude (DAI, Van Putten, Morisky); BIS; relationship with staff; admission experience		Attitude toward medication ($r = 0.26$, $p = 0.001$)		PEESSS ($r = 0.73$, $p < 0.001$); PEESSC ($r = 0.79$, $p < 0.001$); PEESSI ($r = 0.16$, $p < 0.001$)

(Continued)

TABLE 2 | Continued

Study type	Study	Outcome measures	Sociodemographic factors	Clinical factors	Treatment-related factors	Family involvement and therapeutic relations
	19. Meier et al. (2010)	Illness history (CSSRI); BPRS; GAF; MHS; LUNSERS; DAI	Age, marital status, and living arrangements or occupation gender (all n.s.)	Symptom scales (all n.s.); positive attitude to psychotropic medication (for clinician-rated adherence; $T = 3.46$; $p < 0.001$)	Side effects (n.s.); medication class (n.s.)	
	20. Borras et al. (2007)	PANSS; CGI; "Multidimensional Measurement of Religiousness/Spirituality for Use in Health Research," the "Religious Coping Index," and a questionnaire on spiritual and religious adjustment to life events		PANSS positive symptoms (OR = 0.91, 95% CI 0.84–0.98, $p < 0.001$); substance abuse (OR = 4.0, 95% CI 1.5–10.6, $p < 0.001$)		Positively influenced by spiritual beliefs (31%); negatively influenced by spiritual beliefs (26%);
	21. Aldebot and de Mamani (2009)	BPRS; denial coping from COPE inventory	Gender, ethnicity, years of education (n.s.)	BPRS (n.s.); acceptance (n.s.); denial coping ($t = -2.83$, $p = 0.008$)		
	22. McCabe et al. (2012)	PANSS; therapeutic alliance (Helping Alliance Scale)		PANSS total score (OR = 0.984, 95% CI 0.971–0.996, $p = 0.014$)		Therapeutic relationship (clinician-rated OR = 1.51, 95% CI 1.01–2.25, $p = 0.042$; patient-rated OR = 1.35, 95% CI 0.95–1.90, n.s.)
	23. (Jonsdottir et al., 2013)	PANSS; IDS; YMRS; BIS; UKU; NART; WIS; WASI; Bergen n-back test; DKEFS; WMS; CVLT	Age, gender, marital status, education (all n.s.); BMI full adherence > partial adherence ($p = 0.012$)	PANSS n.s.; IDS n.s.; YMRS n.s.; insight: BIS no adherence < full adherence ($p = 0.013$); neurocognition: WAIS n.s.; NART n.s.; WASI no adherence > full adherence $p < 0.05$; WMS and CVLT no adherence > full and partial adherence $p < 0.05$;executive functioning: DKEFS no adherence > full adherence $p < 0.05$; lifetime diagnosis of addiction or abuse of illicit drugs and alcohol partial adherence > full adherence ($p = 0.000$)	SE: UKU poor adherence significant for diarrhea, nausea, and orthostatism (p-value NR)	

TABLE 3 | Overview of risk-factors and predictors of antipsychotic medication adherence and non-adherence.

Predictors		Sociodemographics	Clinical factors	Substance use and abuse	Insight and attitude	Treatment related	Family involvement and therapeutic relations
Commonly involved	In adherence				Illness insight (8; 10)		Family involvement and support (14; 10; 6)
	In non-adherence	Younger age (7; 9; 16)			Lack of insight (7; 15; 14; 12; 9; 16); positive attitude (18; 19; 13; 9; 17; 14)		
Possibly involved	In adherence				Change toward more positive attitudes (8)		Positive attitude of family members toward medication (10)
	In non-adherence	Age at illness onset (7; 14)	Paranoia (11); hostility (1); excitement (13); poor impulse control and preoccupation (13); poor premorbid functioning (7)	Cannabis (7); comorbid substance dependence syndrome (11; 9)	Negative attitude (15; 9; 13)		Lack of family involvement (7)
Insufficient evidence	In adherence		Subjective well-being (15), neurocognitive functioning (13;14)	Absence of cannabis use (20)	Positive change in insight (8), lower score on "lack of insight" (14)		Therapeutic environment (10; 22); admission experience with regard to psychiatric care (18)
	In non-adherence	Ethnic minorities (6;21)	Positive symptoms (7; 20; 14), negative symptoms (15; 11; 14), poor QoL and high relapse rate (7), disorganization syndromes (15; 12), illness severity (2; 14; 21; 19; 22; 13;16), depressive symptoms (1; 13); denial coping (21), comorbid harm or dependence syndrome (9); mode of admission (10; 11); number of previous admissions (11; 12); global functioning (9; 14;19)	Substance use (15; 20; 1; 6); alcohol (7; 15)	Lower positive attitude (15; 9)	Administration route (11; 14; 3); EPS (15; 11; 6), weight change (1), non-neurological SE (15), adverse events (1; 19; 10; 13)	Living with family with high EE (2)
Low evidence	In adherence	Gender (11; 2; 14; 12; 16); occupation (14; 16), marital status (12; 16); level of education (11; 14; 12); duration of illness (11; 12; 10); illness characteristics (1)	Treatment efficacy (1)			Medication class (11; 4; 14; 19; 10; 5); mean AP dosage (11; 14; 12; 13)	
	In non-adherence		Problem-solving ability (14)	History of substance abuse (12; 1)			

denial coping (Aldebot and de Mamani, 2009), comorbid harm or dependence syndrome (Quach et al., 2009), poorer impulse control, poorer quality of life, and higher relapse rate (Coldham et al., 2002) were also weakly associated with non-adherence. Surprisingly, higher subjective well-being (Mutsatsa et al., 2003) and treatment efficacy (PANSS total score, $p = 0.38$) (Lindenmayer et al., 2009) do not predict adherence. With the exception of poor premorbid functioning in FEP (Coldham et al., 2002), general functioning, including current score on the Global Assessment of Functioning (GAF) scale and self-rated problem-solving ability (Klingberg et al., 2008), was not predictive of non-adherence. Additionally, neurocognitive function domains, including IQ, as assessed by the Wechsler Intelligence Scale, executive functioning (verbal fluency and trail making test) (Yang et al., 2012), working memory, and attention (Klingberg et al., 2008) did not predict adherence, and one study even found an inverse relationship between neurocognitive functioning and adherence (Jonsdottir et al., 2013).

Findings regarding previous psychiatric hospitalizations were mixed. Mode of admission ($n = 112$) (Baloush-Kleinman et al., 2011), number of prior admissions, and time since last hospitalization ($n = 74$) (Acosta et al., 2009) were reported to be not significant as predictors in two longitudinal trials. Another study with a larger sample size ($n = 670$) found that (Janssen et al., 2006) while first or multiple episode admission were not different in predicting non-adherence, the number of previous admissions and involuntary admission were significantly predictive of non-adherence.

Substance Use

Although a dual diagnosis of substance dependence syndrome as comorbidity to psychosis has been associated with poor adherence (Janssen et al., 2006; Quach et al., 2009; Jonsdottir et al., 2013), evidence is lacking for both alcohol abuse and illicit substance abuse as reliable individual predictors of medication non-adherence.

In FEP, a longitudinal cohort demonstrated significantly higher levels of alcohol use in the non-adherent group (Coldham et al., 2002). No significant association between non-adherence and alcohol was found elsewhere (Mutsatsa et al., 2003).

Misuse of illicit substances has been significantly associated with poor adherence in two studies (Winton-Brown et al., 2017) but refuted elsewhere (Mutsatsa et al., 2003; Acosta et al., 2009; Lindenmayer et al., 2009). Unsurprisingly, among different substances, cannabis—the most used illicit drug among patients with psychosis—was the strongest predictor of non-adherence to antipsychotic medication (Coldham et al., 2002), and absence of cannabis use was predictive of adherence (Borras et al., 2007).

Illness Insight and Medication Attitudes

Some of the most consistent results were found for the relationship between low illness insight and adherence. It is proposed that because patients with psychosis lack insight into their disease, this affects adherence to their medication regimes. Indeed, lack of insight, including unawareness of the effect of medication and negative medication beliefs, were significantly associated with medication non-adherence in all but one study (Yang et al., 2012). This finding was consistent over the different illness stages: in FEP (Coldham et al., 2002; Mutsatsa et al., 2003; Quach et al., 2009), in patients with a recent acute psychotic episode (Bayle et al., 2015), and in clinically stable patients (Klingberg et al., 2008; Acosta et al., 2009; Mohamed et al., 2009). Positive change in insight scores also predicted adherence in clinically stable patients (Mohamed et al., 2009). In the same line, illness insight (Mohamed et al., 2009; Baloush-Kleinman et al., 2011), including better awareness of the need for treatment and social consequences of illness (Baloush-Kleinman et al., 2011), is a consistent predictor of good adherence.

Unsurprisingly, an overall positive attitude toward antipsychotic medication is highly associated with adherence (Day et al., 2005; Quach et al., 2009; Meier et al., 2010; Yang et al., 2012), a finding that has been replicated in adolescents with psychosis (Molteni et al., 2014), and in clinically stable patients with schizophrenia and schizoaffective disorders (Klingberg et al., 2008). In addition, a change toward more positive attitudes (Mohamed et al., 2009) was correlated with greater medication adherence. Inconsistent findings were reported for lack of positive attitude and medication adherence in FEP. Although lower positive attitude has been found to be unrelated to adherence in one study (Mutsatsa et al., 2003), lack of positive attitude was identified as a predictor of antipsychotics non-adherence in another (Quach et al., 2009). In this line, negative attitude toward antipsychotic medication may be a relevant predictor of poor adherence to antipsychotic medication (Yang et al., 2012), particularly among patients with FEP (Mutsatsa et al., 2003; Quach et al., 2009). In addition, a study reported on the direct impact of spiritual beliefs adherence and found that 26% were negatively and 31% positively influenced by their spiritual beliefs (Borras et al., 2007).

Treatment-Related Factors

Factors related to antipsychotic treatment, such as type, dosage, and route of medication administration are difficult to evaluate reliably outside of RCTs due to the confounding effect of clinical characteristics occurring in naturalistic studies.

One prospective cohort of 670 subjects (Janssen et al., 2006) found that patients using second-generation antipsychotics (SGA) monopharmacy had better adherence at discharge than patients using first-generation antipsychotics (FGA) either as monotherapy or in combination. In addition, those on FGA monotherapy who switched to an SGA (55 %) also had a significantly higher good adherence rate at discharge than those who had continued to take FGA medication, which, according to the authors, may be explained by the prescribers' preference for SGAs in patients with better adherence. Interestingly, the finding that antipsychotic medication class was associated with adherence rates has not been replicated in an open RCT of haloperidol vs. SGAs in patients with FEP (Kahn et al., 2008) nor in open-label (SGA vs. FGA) (Guo et al., 2011), cross-sectional (Klingberg et al., 2008; Meier et al., 2010), and longitudinal setting (Baloush-Kleinman et al., 2011) with stable schizophrenia patients.

On the same note, giving patients control over the choice of route of antipsychotic medication administration did not

lead to better adherence in an RCT of intramuscular vs. oral antipsychotics in FEP (Weiden et al., 2012). In addition, administration route of medication in stable subjects did not significantly impact adherence (Janssen et al., 2006). If anything, patients prescribed with antipsychotic depot formulations at discharge even had a significantly higher non-adherence rate (34.7% of $n = 149$) compared to those on oral medication (48.4% of $n = 521$; $p < 0.05$). Yet, in such a naturalistic setting, obviously considerable selection bias would exist with clinicians being more likely to prescribe depot formulations in patients considered *a priori* to be at risk for non-adherence. Finally, the mean dosage of antipsychotic treatment did not influence adherence behavior in the reviewed studies. One small-sized cohort did report a correlation of non-adherence with polypharmacy of antipsychotic drugs ($r = 0.358$, $p < 0.05$) (Yang et al., 2012).

Although low tolerability of antipsychotic medication is often viewed as an important reason for non-adherence, medication side effects do not seem to carry strong predictive effects. Two individual cohorts found no association between antipsychotic-induced side effects and medication adherence behavior (Meier et al., 2010; Yang et al., 2012), while one identified side effects as an impediment to adherence. Weight change has been demonstrated to be a poor predictor of non-adherence (Lindenmayer et al., 2009), and extrapyramidal side effects (EPS), such as akathisia and parkinsonism, significantly predicted medication non-adherence in some but not all studies (Mutsatsa et al., 2003; Winton-Brown et al., 2017). Unexpectedly, adherence did not differ between patients with EPS compared to those without (47.8% adherent vs. 41.3% non-adherent) in a study of inpatients of different illness stages (Janssen et al., 2006). Moreover, no non-neurological side effects were reported to be significant (Mutsatsa et al., 2003). Overall tolerability, measured by the maximum severity of adverse effects, was a poor predictor of non-adherence (Lindenmayer et al., 2009).

Family Involvement and Therapeutic Relations

The relative contribution of social and family involvement and therapeutic relations to medication adherence is suggested to be highly relevant. Indeed, higher level of family and career involvement and support (Baloush-Kleinman et al., 2011; Winton-Brown et al., 2017) and positive attitudes of family members toward medication (Baloush-Kleinman et al., 2011) are good predictors of medication adherence. One study failed to find an association between medication adherence and "expressed emotions" (i.e., degree of criticism, resignation, and overprotection expressed by relatives) (Klingberg et al., 2008). This may be explained by the inclusion of patients of different illness stages, as one systematic review emphasized that social support and family involvement are particularly beneficial for adherence in younger study populations (Sendt et al., 2015). In addition, another study suggested living with family with high expressed emotions was associated with higher adherence rates (Morken et al., 2007). Moreover, lack of family involvement and social support was also found to be predictive of poor adherence to antipsychotic treatment (Coldham et al., 2002; Yang et al., 2012). One longitudinal study reported that patients who were not upbrought by both parents or had no key relative that came to entry interview were at greater risk of medication non-adherence (Quach et al., 2009). The quality of the therapeutic relationship, as rated by both patients and clinicians (Baloush-Kleinman et al., 2011; McCabe et al., 2012), can indirectly influence adherence by mediating better attitudes to medication (Sendt et al., 2015) or to the psychiatric care in general (Day et al., 2005).

Interventions to Improve Antipsychotic Medication Adherence

We identified 17 distinct studies involving individuals with psychotic spectrum disorder undergoing an intervention to improve antipsychotic medication adherence. Four main intervention groups were identified: behavioral interventions, family interventions, LAI + interventions, and technology interventions (see **Table 4**). Objective adherence measures included pill counts, prescription refill rates, or blood plasma concentration levels. Subjective clinician-rated or self-reported measures quantifying medication adherence were also eligible (e.g., Medication Adherence Questionnaire).

Behavioral Interventions

Adherence Therapy

Adherence therapy (AT) is a 12-session patient-centered therapy that mainly involves a combination of techniques derived from motivational interviewing, cognitive behavioral therapy, and psychoeducation to promote treatment adherence (Kemp et al., 1996). All five included individual studies here employed the modified, brief (six to eight sessions) course designed by Gray et al. (2006). Mixed findings were demonstrated concerning the efficacy of AT in terms of improving adherence. Antipsychotic medication adherence was measured with different tools in all five studies, with four using only subjective measures (Gray et al., 2006; Anderson et al., 2010; Chien et al., 2015, 2016) and one combining subjective and objective tools (Schulz et al., 2013).

No significant differences in adherence behavior between the intervention and control group was found in three single-blind RCTs (Gray et al., 2006; Anderson et al., 2010; Schulz et al., 2013), irrespective of outcome measure used. AT was not found to be more effective than health education in improving participant's adherence to medication and quality of life (measured by different self-rating scales) after the intervention or at 1-year follow-up (total $n = 409$) (Gray et al., 2006). AT did also not significantly affect patients' adherence and treatment attitudes in a study using both subjective and objective (serum concentrations of antipsychotic medication) measures for adherence. Yet, despite the lack of improvement in adherence in this study, the symptom severity scores improved significantly more in the AT group compared to treatment-as-usual (TAU) (Schulz et al., 2013). We cannot exclude the possibility that selection bias (of patients with positive medication attitudes), ceiling effects (high mean baseline CDR levels), and a lack of power may have obscured any effect of the intervention in Anderson et al. (2010) and Schulz et al. (2013).

Only two out of five studies, both of them conducted by the same research group, found AT to be effective in improving medication adherence at small-to-large effect sizes (effect size, 0.72 and 0.30) (Chien et al., 2015, 2016). Both of these studies

TABLE 4 | Evidence table on interventions to improve medication adherence.

Intervention type	Study	Study type	Sample characteristics	Cases	Duration of study	Adherence measure	Effect on adherence	Effect other outcome measures	RoB
Behavioral—adherence therapy	Anderson et al. (2010)	RCT, SB	Mixed (%FEP NR); stable; SZ + SZA	12 intervention vs. 14 TAU	8 w	PETiT	$t = 1.20$, n.s.		Low
	Chien et al. (2015)	RCT, SB	Mixed (%FEP NR); stable; PSD	57 intervention vs. 57 TAU	4 m; FU at 6 m	ARS	$F = 7.45$, $p = 0.007$; ES = 0.72	PANSS score ($F = 7.32$, $p = 0.008$); positive symptoms score ($F = 7.28$, $p = 0.008$); negative symptoms score ($F = 7.81$, $p = 0.006$); ES = 0.70–0.75; number of rehospitalizations ($F = 5.01$, $p = 0.030$), ES = 0.48; insight into illness and/or treatment ($F = 6.58$, $p = 0.021$), ES = 0.51; functioning ($F = 6.89$, $p = 0.014$), ES = 0.68	Low
	Chien et al. (2016)	RCT, SB	Mixed (%FEP NR); stable; PSD	67 intervention vs. 67 TAU	12 w; 18 m FU (2w, 6m, 18m)	ARS	Non-adherent: 85 vs. 90% ($F = 9.10$, $p = 0.005$), effect size = 0.30	Insight ($F = 10.98$, $p = 0.001$), ES = 0.40; functioning ($F = 8.90$, $p = 0.005$), ES = 0.29; symptom severity (PANSS) ($F = 10.10$, $p = 0.003$), ES = 0.32, hospital rate duration ($F = 8.80$, $p = 0.005$), ES = 0.28; hospital rate frequency ($F = 3.47$, $p = 0.092$)	Low
	Gray et al. (2006)	RCT, SB	Chronic; state NR; SZ	204 intervention vs. 205 HE (control)	52 w (8 weekly sessions within first 5 m)	MAQ, SAI-C	MAQ: n.s.; SAI-C: -n.s.	n.s. QoL and BPRS	Low
	Schulz et al. (2013)	RCT, SB	Mixed (%FEP NR); acute; SZ	80 intervention vs. 57 TAU	12 w	CDR, MARS	CDR: $F = 2.29$, n.s.; MARS: difference 0	PANSS ($F = 6.19$, $p < 0.05$); beliefs about treatment (DAI) n.s.; GAF n.s.	Low
Behavioral—CBT	Bechdolf et al. (2010)	RCT, SB	Mixed (% FEP NR); acute; PSD	16 CBT vs. 27 PE	8 w, results FU at 24 m	4-point rating scale	$F = 1.31$, $p = 0.26$	Rehospitalization rate 37.5% vs. 59.3%, ($\chi^2 = 2.50$, n.s.); symptom severity n.s.	Low

(Continued)

TABLE 4 | Continued

Intervention type	Study	Study type	Sample characteristics	Cases	Duration of study	Adherence measure	Effect on adherence	Effect other outcome measures	RoB
Behavioral—cognitive adaptation training	Velligan et al. (2008)	RCT, SB	Chronic; stable; SZ + SZA	34 CAT vs. 32 PharmCAT vs. 29 TAU	9 + 6 m FU (3 and 6 m)	Unannounced in-home pill counts; prescription refill rates	Pill count adherence: CAT vs. TAU ES = 1.09; Pharm-CAT vs. TAU ES = 1.05; prescription refill rates: main effect of group ($F = 3.93$, $p < 0.020$), CAT vs. TAU ($F = -2.85$, $p < 0.006$), Pharm-CAT vs. TAU n.s.; CAT vs. TAU ES = 0.51 and Pharm-CAT vs. TAU ES = 0.33	Symptom severity n.s.; relapse rate CAT vs. TAU ($\chi^2 = 8.29$, $p < 0.004$); Pharm-CAT vs. TAU ($\chi^2 = 8.20$, $p < 0.005$); relapse in 15 m >65% CAT and Pharm-CAT vs. 19% TAU; functional outcome CAT vs. TAU 6 m treatment ES = 1.47 and 6 m FU ES = 0.50, Pharm-CAT vs. TAU at 3 m ES = 0.42, at 6 m treatment ES = 0.44, at 6 m FU ES = 0.22	Low
	Velligan et al. (2013)	RCT, SB	Chronic; stable; SZ + SZA	46 MeM vs. 46 PharmCAT vs. 45 TAU	9 m	Electronic monitor, pill counts	e-monitoring: treatment group effect $F = 47.29$, $p < 0.0001$; effects for time $F = 0.06$, n.s.; time × group effect $F = 0.44$, n.s.; PharmCAT vs. TAU ES = 1.03 and MeM vs. TAU ES = 0.98. Pill counts: significant main effect of group $F = 7.83$, $p < 0.0001$ and n.s. effects of time $F = <1$, n.s.; time × group interaction $F = 2.34$, $p = 0.06$; adherence rate PharmCAT 91% vs. MeM 86%, $t = 2.05$, $p = 0.04$; PHARMCAT 91% vs. TAU 80%, $t = 3.95$, $p = 0.0001$; MeM 86% vs. TAU 80%, $t = 1.82$, n.s.	Symptom severity and functioning (all n.s.)	Low
Family therapy	Kopelowicz et al. (2012)	RCT, SB	Mixed (%FEP NR); stable; SZ + SZA	64 MFG-adherence vs. 53 MFG-standard vs. 57 TAU	12 m (FU at 18 m and 24 m)	Treatment Compliance Interview	Group effect ($F = 6.41$, $p = 0.003$); Time effect ($F = 3.5$, $p = 0.009$); Group × time effect n.s.	Group differences in time to first hospitalization ($\chi^2 = 13.3$, $p = 0.001$); at FU MFG-A vs. MFG-S ($\chi^2 = 6.3$, $p = 0.01$) and MFG-A vs. TAU ($\chi^2 = 8.7$, $p = 0.003$); hospitalization rate: MFG-A (39%) vs. MFG-S (66%) ($\chi^2 = 8.2$, $p = 0.004$), MFG-A vs TAU (70.2%) ($\chi^2 = 11.3$, $p < 0.001$); MFG-S vs. TAU ($\chi^2 = 0.2$, n.s.)	Low

(Continued)

TABLE 4 | Continued

Intervention type	Study	Study type	Sample characteristics	Cases	Duration of study	Adherence measure	Effect on adherence	Effect other outcome measures	RoB
	Valencia et al. (2010)	RCT, SB	Mixed (%FEP NR); stable; SZ	47 intervention vs. 36 TAU	12 m	Prescription renewals, patient's and key relative's monthly report to the treating psychiatrist	Medication adherence 91.5 vs. 77.8% ($p < 0.050$); visit adherence 82.5 vs. 70% ($p < 0.050$)	Global functioning ES = 1.30 vs. TAU ES 0.30 (effect for time, group and time × group all $p < 0.010$); relapse rate 12.8 vs. 33.3%, $p < 0.05$; rehospitalization 2.1 vs. 14%, $p < 0.050$	Low
LAI	Noordraven et al. (2017)	Open label RCT	Chronic; stable; PSD	84 intervention vs. 85 TAU	12 m (+6 m FU)	MPR, longest uninterrupted period during which depot medication was received, time to first discontinuation of depot medication, total number of days without depot medication, and time between prescription date and the date the depot was actually received	MPR 14.9% (95% CI 8.9–20.9), $p < 0.0001$; good adherence (MPR ≥80 %) = 33.1% (95% CI 20.2–45.4), p = 0.031; 6 m FU MPR 6.5% (95% CI 2.0–10.9), $p = 0.047$; 6 m FU good adherence: 22.1% (95% CI 4.2–39.8%), $p = 0.010$	Attitudes, clinical symptoms, psychosocial functioning, substance use, QoL, side effects (all n.s.)	Moderate
	Lee et al. (2010)	CT—prospective, controlled, unrandomized	Mixed (% FEP NR); stable; SZ + SZA	21 intervention vs. 25 TAU	12 m (+FU at 2 y)	Visits for injection/planned visits for injection; treatment discontinuation; injection discontinuation	1 y FU intervention: 94.6%, TAU: 75.9%, ($t = 3.5$, $p < 0.010$); 2 y FU intervention: 92.1%,TAU: 74.2%, ($t = 2.7$, $p < 0.010$); treatment discontinuation: intervention 14% vs. TAU 28% ($\chi^2 = 6.0$, $p = 0.010$); injection discontinuation: intervention 23% vs. TAU 68% ($\chi^2 = 13.0$, $p < 0.010$)	1 y relapse rate intervention vs. TAU $p < 0.010$; 2 y relapse rate intervention vs. TAU $\chi^2 = 4.2$, $p = 0.040$; symptom severity n.s.; side effects n.s	Moderate
	Sajatovic et al. (2013)	CT—prospective, uncontrolled trial	Mixed (% FEP NR); state NR; SZ + SZA	30	6 m	TRQ, MAQ, injection frequency	TRQ (incl. oral medication, mean) −38.9 (95% CI, −75.7–−2.0), $p = 0.028$; MAQ, mean (SD): 1.4 (1.6), $p = 0.001$; injection frequency, mean (SD): only at week 13: 83 (35), and week 25: 76 (35)	Improvements in psychiatric symptoms ($p < 0.001$; BPRS ($t = 2.51$, $p = 0.029$), PANSS ($p = 0.005$), CGI ($p < 0.001$), and functioning ($p < 0.001$), akathisia (40%); BMI and total cholesterol n.s.; changes in hospitalizations n.s.	Low

(Continued)

TABLE 4 | Continued

Intervention type	Study	Study type	Sample characteristics	Cases	Duration of study	Adherence measure	Effect on adherence	Effect other outcome measures	RoB
Technology	Frangou et al. (2005)	RCT, open	Chronic; stable; SZ	36 pill counting vs. 36 @HOME vs. 36 TAU	8 w	MAQ-based questionnaire; pill counting; e-monitoring (incl. electronic dispenser)	TAU, mean (SD; range)%: 77.3 (22.1; 18–95)%; pill counting, mean (SD; range)% = 78.5% (14; 50–95); e-monitoring, mean (SD; range)%: mean of 92.3% (4.8; 82–100); effect of group ($F = 8.9$, $p = 0.0001$); TAU vs. pill counting (n.s.); e-monitoring group vs. TAU ($p = 0.001$); e-monitoring vs. pill counting group ($p = 0.007$)	Group differences in the PANSS total score ($F = 5.7$, $p = 0.004$); control vs. pill-counting group ($p = 0.008$) and e-monitoring ($p = 0.04$); pill-counting vs. e-monitoring ($p = 0.8$); end-point medical ($p = 0.01$) and emergency ($p = 0.0001$) visits in the @HOME patient, group difference ($F = 3.6$, $p = 0.002$)	Moderate
	Montes et al. (2012)	RCT; open	Chronic; stable; SZ	100 intervention vs. 154 TAU	6 m (3 and 6 m)	MAQ	MAQ [mean (95% CI)] 3 m: mean total score change intervention-−1.0 (−1.02–−0.98) vs. TAU −0.7 (−0.72–−0.68) $p = 0.02$; 6 m: mean total score change intervention-−1.1 (−1.12–−1.08) vs. TAU 0.8 (0.81, 0.78), $p = 0.04$	Symptom improvement [mean (95% CI)] 3 m: improvement in negative [intervention 3.3 (3.10–3.50) vs. TAU 3.5 (3.36–3.64), $p = 0.020$], cognitive [intervention 3.3 (3.12–3.48) vs. TAU 3.6 (3.46–3.74), $p = 0.010$] and global [intervention 3.2 (3.02–3.38) vs. TAU 3.5 (3.36–3.64), $p = 0.012$) symptoms; 6 m negative (n.s.), cognitive (n.s.) and global (n.s.) symptoms; attitude [mean (95% CI)] 3 m: intervention 2.0 (1.94, 2.06), vs. TAU 0.4 (0.35, 0.45), $p = 0.0003$; 6 m: intervention 2.3 (2.24, 2.36), vs. TAU 0.9 (0.85, 0.95), $p = 0.002$; insight n.s.; QoL intervention 6.6 (6.38–6.82) vs. TAU 3.1 (2.91–3.29), $p < 0.03$; 6 m: n.s.	Moderate

(Continued)

TABLE 4 | Continued

Intervention type	Study	Study type	Sample characteristics	Cases	Duration of study	Adherence measure	Effect on adherence	Effect other outcome measures	RoB
	Velligan et al. (2013)	RCT, SB	Chronic; stable; SZ + SZA	46 MeM vs. 46 PharmCAT vs. 45 TAU	9 m	Electronic monitor, pill counts	e-monitoring: treatment group effect $F = 47.29$, $p < 0.0001$; effects for time $F = 0.06$, n.s.; time × group effect $F = 0.44$, n.s.; PharmCAT vs. TAU ES = 1.03 and MeM vs. TAU ES = 0.98. Pill counts: significant main effect of group $F = 7.83$, $p < 0.0001$ and n.s. effects of time $F = <1$, n.s.; time × group interaction $F = 2.34$, $p = 0.06$; adherence rate PharmCAT 91% vs. MeM 86%, $t = 2.05$, $p = 0.04$; PHARMCAT 91% vs. TAU 80%, $t = 3.95$, $p = 0.0001$; MeM 86% vs. TAU 80%, $t = 1.82$, n.s.	All n.s. ($p > 0.090$; symptom severity and functioning)	Low
	Moncrieff et al. (2016)	RCT, open	Mixed (% FEP NR); state NR; PSD	31 intervention vs. 29 TAU	3m (FU 2–3 w; 2–3 m)	MAQ	OR = −0.44, 95% CI, −0.76–−0.11	Positive attitudes to antipsychotic medication (DAI, 1.65; 95% CI, −0.09–3.40); PANSS, side effects and dosage (all n.s.)	Moderate
	Beebe et al. (2017)	RCT, SB	Mixed (% FEP NR); stable; SZ + SZA	53 intervention vs. 52 TAU	6 m	Pill counts; serum medication levels	Pill counts adherence: 66% vs. 50%, (χ^2, n.s.); serum AP levels within therapeutic range: 54.7% vs. 32.7% ($\chi^2 = 5.2$, $p = 0.023$)		Low

[$n = 114$ (Chien et al., 2015) and $n = 134$ (Chien et al., 2016)] featured a slightly modified treatment with a larger proportion of motivational interviewing techniques. Along with a significantly greater improvement over time in medication adherence of the AT group, there was also a significantly greater improvement of symptom severity, illness insight, global functioning, and rate of hospitalization at 6-month follow-up. Importantly, the study that found the larger effect size only included previously non-adherent patients and had a very low (7%) refusal rate as well as a high family support; which may all have inflated the results.

CBT

Only one RCT has studied (Bechdolf et al., 2005) group cognitive behavioral therapy (CBT) vs. psychoeducation (PE) group training for medication adherence. The group CBT treatment consisted of coping strategy enhancement, problem solving, and relapse prevention in patients with psychosis. The intervention was focused on the treatment of symptoms, relapse prevention and associated problems, and enhancing medication adherence and included 16 sessions in 8 weeks. The eight PE training sessions were covered in the same time window.

Adherence was measured posttreatment and at the 24-month follow-up, using a 4-point rating scale based on multiple sources, including patients, relatives, and clinical staff. Although no significant differences were reported on adherence levels between the two interventions at any assessment point, both interventions led to relevant clinical improvement, in terms of rehospitalization, symptom severity, and medication use, at the end of treatment and at follow-up. Readmission was not significantly related to non-adherence. Baseline medication adherence was high in both groups, with a mean score of 3.9 $\pm$ 0.3 and 3.77 $\pm$ 0.5 for the CBT and PE group, respectively, possibly leaving no room for further improvement. Moreover, the author reported that the follow-up sample might have been unrepresentative due to the high lost-to-follow-up rate.

Cognitive Adaptation Training

Two studies by the same research group investigated cognitive adaptation training (CAT) for medication adherence in schizophrenia. In one study (Velligan et al., 2008), patients were randomized to receive either CAT, Pharm-CAT, which is a subset of techniques from the CAT program, or TAU. CAT is a series of compensatory strategies and environmental supports designed to improve multiple domains of adaptive functioning including medication adherence, grooming, and independent living skills in patients with schizophrenia (Velligan et al., 2008). Pharm-CAT uses environmental supports such as checklists, signs, and electronic cueing devices to improve medication adherence. In contrast to full CAT treatment, only interventions that specifically target adherence are used (Velligan et al., 2008, 2013). Treatment lasted for 9 months, and follow-up lasted to 6 months after end of treatment. Objective adherence measures in the form of unannounced in-home pill counts and prescription refill rates were used. Adherence and functional outcomes were assessed every 3 months. A superior treatment effect with large effect sizes for both CAT (ES = 1.09) and Pharm-CAT (ES = 1.03) over TAU in pill count adherence was established during intervention and at follow-up, and adherence remained close to 80%. In addition, only small-to-moderate effects were found in prescription fill rate (ES CAT = 0.51 and Pharm-CAT = 0.33). Across the treatment groups, no significant differences in symptom severity were demonstrated. However, relapse rates in the CAT and Pharm-CAT groups were significantly lower than in the TAU group, with no significant differences between the active treatment groups. Pharm-CAT was only significantly different than TAU in improving functioning in the first 6 months of treatment. The authors suggested that this slight improvement in functioning in the group receiving Pharm-CAT may be due to better medication adherence in this group as compared to patients receiving standard treatment.

In another study (Velligan et al., 2013), patients were randomized to receive either standard treatment, Pharm-CAT, or a smart pill container known as the Med-eMonitor for 9 months. Here, adherence was obtained via an electronic monitor and by monthly unannounced pill counts conducted in participants' homes. All groups received a monitoring device to assess adherence, but only in the Med-eMonitor group the monitor was set to encourage adherence. More specifically, the Med-eMonitor was capable of cueing patients to take their medication and warning them when they are taking the wrong medication, documenting adverse events complaints, and alerting clinical staff of failure to adhere to medication. Compared to TAU, medication adherence measured with e-monitoring was significantly higher in both active intervention groups (ES Pharm-CAT = 1.03 and MeM = 0.98). No differences between the Pharm-CAT and Med-eMonitor treatment groups were found. In contrast, medication adherence as measured by pill counting was higher in the Pharm-CAT group (91%) compared to the Med-eMonitor (86%, $p = 0.04$) or TAU group (80%, $p = 0.0001$). Although the active interventions significantly improved medication adherence, this did not translate to improved clinical outcomes in terms of symptom severity or global functioning.

Family Interventions

According to two single-blind RCTs, add-on family-based interventions seem to result in better medication adherence as compared to TAU alone. In one study, outpatients were randomized to either continue TAU or receive a 12-month psychosocial rehabilitation, including Psychosocial Skills Training (PSST) and family psychoeducation on top of TAU in one study (Valencia et al., 2010). Subjects' relatives who were randomized to PSST participated in 12 psychoeducational, multifamily group sessions in which they received similar information as the patients. This included providing effective support to the person with schizophrenia and coping with the disorder; information on symptoms, medication, side effects, and the importance of treatment (Kopelowicz and Liberman, 2003). Adherence assessment included both subjective measures by patient and key relative's report and objective measures in the form of prescription renewals. Medication and appointment adherence was significantly greater among patients receiving psychosocial rehabilitation than their counterparts in the TAU condition. Moreover, the addition of PSST and family

psychoeducation to antipsychotic medication significantly reduced psychiatric symptoms, relapses and rehospitalization rate, and improved global functioning (Valencia et al., 2010).

Similarly, a 12-month multifamily group (MFG) treatment, a behavioral family treatment that combines psychoeducation and skills training, as earlier described by McFarlane (2002), was employed in another RCT (Kopelowicz et al., 2012). Standard MFG therapy (MFG-S) was compared to both TAU and adherence-focused MFG (MFG-A), which focuses on attitudes, subjective norms, and perceived behavioral control. Adherence was evaluated using Treatment Compliance Interview (Weiden et al., 1995), an instrument that provides a quantified rating of the extent to which the patient did take their medication and the amount of medication they may have taken in the past month. Patient's key relatives were also interviewed using the relative version of the instrument. No significant differences in level of adherence were reported at any point between the MFG-S and control group. However, more participants in MFG-A were fully adherent than those in TAU at all assessments during the treatment but not at the 24-month follow-up. Group differences in time to first hospitalization after baseline was significant: rehospitalization was less likely for those in MFG-A than for those receiving MFG-S or standard treatment across the entire follow-up period (Kopelowicz et al., 2012).

LAI Combined With a Psychoeducation-Based or Monetary Intervention

Three studies examining interventions for medication adherence in patients prescribed a long-acting injectable (LAI) antipsychotic were included. One study (Sajatovic et al., 2013) assessed long-acting injectable antipsychotics (haloperidol) in combination with a customized adherence enhancement intervention. This intervention includes psychoeducation focused on medication, developing medication routines, and managing adherence in the context of substance abuse (Sajatovic et al., 2013). Adherence was assessed using both subjective tools [Tablets Routine Questionnaire (Scott and Pope, 2002) and Morisky scale (Morisky et al., 1986)] and objective measures (injection frequency). A significant positive change in both adherence to LAI and concomitant oral antipsychotics was illustrated through the uncontrolled 25-week intervention, as well as symptom severity and social functioning. No significant changes in hospitalizations were reported. Large dropout rate and small sample size did not permit valid statistical comparison at 6-month follow-up.

Another study (Lee et al., 2010) compared TAU vs. a psychosocial intervention for relapse prevention (PIRP) as add-on to depot antipsychotic (risperidone). The PIRP program consists of psychoeducation for long-acting injections, early detection of warning symptoms, relapse prevention, regular family education, crisis intervention, and encouragement to patients to adhere to a schedule of hospital visits over a 1-year period. Injection frequency was used as a measure for adherence. Results indicated better adherence associated with the intervention as compared to TAU at the end of treatment and 1-year follow-up ($p < 0.01$). Relapse rate at the end of the intervention ($p < 0.01$) and at 1-year follow-up ($p = 0.04$) were significantly lower in the PIRP group compared to the TAU group. Occurrence of injection discontinuation was significantly lower in the PIRP group than in the TAU group. Both groups showed significant improvement in symptom severity, with no difference between the treatment groups.

During a 12-month open-label, randomized controlled trial (Noordraven et al., 2017), patients were allocated to either receive a financial reward on top of usual treatment every time they received their prescribed depot of antipsychotic medication or to receive TAU only, in which patients were encouraged to continue their prescribed depot antipsychotic. Adherence was measured as the number of depots received over the number of prescribed depots during intervention period. Results showed that financial incentives improved LAI adherence significantly better compared to the control group by the end of treatment (33.1%; 95% CI, 20.2–45.4; $p = 0.031$). Also at 6-month follow-up, when financial incentives were discontinued, the positive effects on medication adherence decreased but remained significantly higher in the intervention group than in the control group. However, no differences between the groups were found in symptom severity, hospitalization or hospitalization duration, subjective quality of life, and psychosocial functioning.

Technology Interventions

Four domains of technological interventions were identified here: electronic monitoring (Frangou et al., 2005; Velligan et al., 2013), SMS reminders (Montes et al., 2012), a telephone intervention problem solving intervention (Beebe et al., 2017), and the Medication review tool (Moncrieff et al., 2016).

Two studies (Frangou et al., 2005; Velligan et al., 2013) evaluated the effects on adherence of electronic monitoring (e-monitoring) using smart pill containers to a number of different comparators. Results of one study, which randomized patients to receive either Pharm-CAT, Med-eMonitor, or TAU are described above (Velligan et al., 2013). Another study (Frangou et al., 2005) examined how the method of measuring medication adherence (i.e., self-report, pill counting, or e-monitoring) could influence adherence. Results indicated that adherence improved significantly in the e-monitoring group as compared to the control and the pill-counting group. Larger clinical improvement was reported for the e-monitoring group and pill-counting group.

Montes et al. (2012) demonstrated that daily SMS reminders to take medication resulted in better adherence compared with usual care. Greater improvement in clinical symptoms and quality of life at the end of intervention was observed with SMS reminders, but these differences were not preserved at 6-month follow-up. No differences in illness insight were observed between the groups at any measurement points.

Patients in another 6-month study (Beebe et al., 2017) were randomized to receive either telephone intervention problem solving (TIPS), a telephone nursing intervention that is used to provide weekly support to outpatients with PSD (Beebe and Tian, 2004; Beebe, 2005), or TAU. Although pill count adherence did not differ between the groups at the end of the study, significantly more patients in the

experimental group had serum antipsychotic levels within therapeutic range.

The Medication Review Tool (Moncrieff et al., 2016), an online form to help patients identify both benefits and issues of their current antipsychotic treatment and any desired changes, had to be taken into psychiatric consultation allowing the patients to express their views more clearly and to have their concerns addressed more systematically about medication. This method improved of adherence in the intervention group compared to controls. Moreover, attitudes toward antipsychotic treatment were also more favorable in the intervention group. No differences in symptomatology and side effects were reported.

DISCUSSION

Modifiable and Non-modifiable Risk Factors for Non-adherence

Antipsychotic medication non-adherence is one of the most important challenges that clinicians face in treating psychotic disorders. Subsequently, this review aimed at providing a comprehensive description of the most important factors associated with adherence and the endeavors to improve adherence in this highly prevalent condition. Our results indicate that predictors of medication adherence can be divided in modifiable and non-modifiable risk factors. Non-modifiable risk factors of non-adherence include sociodemographic features, such as younger age (Coldham et al., 2002; Quach et al., 2009; Bayle et al., 2015) and younger age at illness onset (Coldham et al., 2002; Klingberg et al., 2008), and can help to identify at-risk individuals for targeted adherence interventions. Modifiable risk factors, on the other hand, are of particular interest as targets for the development of specific interventions or strategies to improve adherence. Important modifiable risk factors include family and therapeutic relations, as well as some clinical symptoms that may be amenable to treatment. In particular, higher scores on PANSS positive and global psychopathology subscale items [paranoia (Janssen et al., 2006), hostility (Lindenmayer et al., 2009), excitement (Yang et al., 2012), and preoccupation (Yang et al., 2012)] may need to be tackled in order to improve medication non-adherence, although the extent to which positive and negative symptom domains are predictive of adherence behavior remains unclear. Additionally, current but not previous misuse of cannabis represents a clear risk factor for non-adherence, pointing out the importance of abstention strategies toward improving adherence behavior (Coldham et al., 2002; Janssen et al., 2006; Quach et al., 2009; Foglia et al., 2017; Winton-Brown et al., 2017). Unsurprisingly, patients' attitudes and beliefs about medication and illness represent another key modifiable risk factor (Day et al., 2005; Quach et al., 2009; Meier et al., 2010; Yang et al., 2012; Molteni et al., 2014) across all stages of the disorder. A clear positive impact on adherence may be generated by involving family members (Klingberg et al., 2008; Baloush-Kleinman et al., 2011; Winton-Brown et al., 2017) that support the patient and their treatment. Somewhat surprisingly, we found that treatment-related variables, such as administration route, dosage, type of antipsychotic, and medication side effects, do not significantly influence medication adherence. However, study designs may have confounded the results.

Evidence-Based Strategies to Strengthen Adherence

Despite these important clues, the main drivers and causes of non-adherence in psychosis remain difficult to determine due to the limited quality and heterogeneous nature of the available evidence, leading to a "black box effect," which has not been very informative for clinicians or researchers. The scarcity of evidence on interventions to improve adherence to antipsychotics stands in sharp contrast with the number of clinical trials trying to prove their effectiveness. Current evidence-based interventions to improve adherence include family therapy, technology-based interventions, and strategies combining depot medication with psychoeducation. However, these findings must be interpreted with caution, given the wide range of heterogeneous interventions, the lack of consequent

TABLE 5 | Outline potential adherence strategies.

	Low risk for non-adherence	Vulnerability for non-adherence: 1–2 risk factors	High risk for non-adherence: ≥3 risk factors
Patient profile	Patients with illness insight; positive attitude toward medication; family involvement and support; positive attitudes of family members toward medication	Young patients; patients who lack illness insight, cannabis use and substance dependence; high intensity of symptoms; poor premorbid functioning; negative attitude toward medication; lack of family involvement	
Adherence measurement method	Subjective rating scale	Subjective rating scale + unexpected pill count + prescription renewal/refill	Subjective rating scale + unexpected pill count + prescription renewal/refill + TDM or e-monitoring
Potential intervention strategies	PE	(LAI+) PE + SMS reminders If applicable: family therapy; cessation of cannabis and other substances	LAI + PE + contingency management (incl. financial incentives) If applicable: family therapy; cessation of cannabis and other substances; technology interventions

replication, and methodological restraints. Because of the large influence of patients' attitudes on adherence behavior, naturalistic or non-randomized designs are particularly problematic. There is a need for more well-controlled longitudinal RCTs, assessing both short- and long-term effects on adherence behavior as well as clinical and functional outcome measures. Additionally, rather than studying hybrid interventions consisting of multiple non-specific and partially overlapping components (e.g., CAT, CBT, AT, family therapy), we should study the effectiveness of specific elements of these interventions in tackling one or more of the abovementioned modifiable risk factors, allowing for an adherence strategy that is cost effective and tailor-made to an individual patient. **Table 5** outlines our proposed recommendations for such an integrative adherence strategy, based on patients' risk profile. Preventative strategies should be implemented for patients with low-risk profiles, as low vulnerability does not exclude future non-adherence behavior. Assessing patients' adherence behavior (i.e., self-report, family, and interviewer rating) and increasing awareness of their illness and of the benefits of their antipsychotic treatment may reinforce patients to proactively manage their disorder. Patients with a higher vulnerability for non-adherence should be monitored more closely, using both subjective and objective instruments. Where technology-assisted methods are not practical or affordable, prescription refill rates in combination with unexpected pill counts can be performed. Special attention for younger patients is advised. Aside from psychoeducational strategies, above-mentioned evidence-based interventions to improve adherence can be applied to patients at risk of antipsychotic medication non-adherence. Where applicable, patients' family should be involved and educated on this debilitating illness and benefits of a followed treatment course, and cessation of substance use should be encouraged.

Measuring Adherence

A variety of measures of adherence behaviors are available to researchers and clinicians studying populations with psychiatric disorders. However, none of these tools are exact measures of drug intake, and thus, all suffer from limitations. The so-called digital drugs, consisting of an antipsychotic embedded with a sensor to track consumption of the drug, could resolve this issue. However, evidence of better adherence with digital drug is very weak (Cosgrove et al., 2019). Although no gold standard approach to the measurement of adherence exists, some measures are clearly more sensitive and reliable in identifying mismatch between actual and prescribed use of antipsychotics. Measures of medication adherence can be classified in (1) subjective measures of medication use (patient self-report or interviewer ratings) and (2) objective indicators of medication intake, such as pills counts, electronic monitoring, and serum or plasma levels of antipsychotics (see **Table 6**). Despite the availability of sensitive instruments, no reliable prevalence data on non-adherence in psychosis are available, with reported non-adherence rates ranging from 0.9% (Klingberg et al., 2008) to 81% (Weiden et al., 2012). Shockingly, half of the included studies relied solely on subjective reports or rating scales of adherence. Several studies also used unstandardized and unvalidated subjective adherence measurement tools (Coldham et al., 2002; Bechdolf et al., 2005; Borras et al., 2007; Kahn et al., 2008; Acosta et al., 2009; Mohamed et al., 2009; Quach et al., 2009; Yang et al., 2012; Molteni et al., 2014; Winton-Brown et al., 2017). Valid and reliable therapeutic drug monitoring methods are now increasingly available for the most common antipsychotic drugs (Patteet et al., 2014), and it is hard to understand why TDM is not used more widely, both in clinical practice and in studies that have a primary or secondary focus on adherence assessment. Despite the difficulties linking adherence directly to patients' outcomes, we strongly recommend all clinical trials of treatment interventions for psychotic disorders to routinely include quantifiable and objective measures of adherence rather than only relying on intention-to-treat analysis.

In addition, given the far-reaching consequences of medication non-adherence in clinical practice, a failure to scientifically address this issue will have important implications for the treatment of patients with psychosis. A proper research agenda to define the optimal treatment of patients suffering

TABLE 6 | Advantages and disadvantages of objective and subjective measurement tools.

Measurement	Advantage	Disadvantage
Objective		
TDM	- Objective	- Dependent on patient's metabolism - Not quantitative - Does not exclude partial adherence - Cost - Availability
Pill count	- Easy to apply to all patients - Does not require training - Low cost	- Missing data - Reliability
Pharmacy refill, including MPR	- No missing data - Not obtrusive	- Accuracy - Variation in decision rules per study
Monitoring devices (smart containers)	- Reminders - Alert patients if cap is left off of bottle - Notifications of opening cap - Automatic download of data - Multiple drugs with one device	- Leaving caps off of bottle results in missing data in most devices - High cost - Training - Underestimating adherence when multiple pills are taken out at once - Overestimating adherence with multiple openings and no pills have been taken out
Subjective		
Self-report and observer-rated	- Easy - Short - Some take time into account - Some Likert-type rating scale	- Cost - Some no specific timeframe - Some dichotomous - Validity - Memory bias - Poor insight may limit accuracy

from psychotic illness must therefore include the need for a clear definition of adherence, including partial adherence and non-adherence, the need for consensus on appropriate adherence assessment methods, on how to assess individual patients' risk of non-adherence, and which interventions can be applied as part of a personalized and evidence-based treatment plan.

Limitations and Future Directions

Our review has several limitations. The existing literature is marked by lack of consensus about defining and measuring adherence in PSD, leading to a wide range of adherence rates (0.9–81%) found in the literature. Despite our rigorous inclusion and exclusion criteria, aiming at incorporating only high-quality studies, methodological flaws and heterogeneous definitions, measures, and intervention strategies complicated the quantitative comparison of effects across different studies. In terms of our methodology, while our stringent approach using MeSH terms in our search string improved the quality and specificity (Baumann, 2016) of our literature search, this may have come at the expense of losing some sensitivity to detect all relevant publications. To minimize the risk of missing some relevant studies, we made sure to manually review the reference lists of all individual studies and systematic reviews on the topic. Moreover, our systematic search has been limited to only one search engine. Despite these limitations, we have been able to classify factors associated with antipsychotic medication adherence as modifiable and non-modifiable risk factors to identify possible intervention strategies and to propose evidence-based recommendations.

CONCLUSIONS

One of the greatest problems when dealing with psychotic spectrum diseases is the effectiveness of antipsychotic treatment, which is complicated as patients often fail to adhere to their treatment, adding to the negative effect on prognosis in psychotic illness. Subsequently, this systematic review aims to facilitate endeavors to improve antipsychotic adherence behavior by identifying modifiable and non-modifiable adherence-related risk factors, synthesizing effective intervention strategies, and proposing recommendations to enhance adherence strategies. We demonstrate that non-adherence to antipsychotic medication in patients with psychotic spectrum disorders is a complex process influenced by numerous risk factors, including younger age, poor illness insight, cannabis abuse, and to some extent by present positive symptoms. Positive attitude toward medication, family involvement, and increased insight seem to positively influence adherence. Whereas, several treatment models aimed to improve adherence have been investigated, much ambiguity remains concerning effectiveness and active components. Although much efforts have been invested in investigating adherence, there is a dire need for the implementation of well-validated, standardized assessment methods. To improve long-term outcomes in psychotic patients, we strongly suggest that future treatment strategies should focus on the individual patient's characteristics and needs and the integration of evidence-based interventions into psychiatric services. Such evidence-based integrative treatment strategy is essential in addressing the impact of antipsychotic non-adherence on the patients' prognosis and cognitive and global functioning and on the society.

DATA AVAILABILITY STATEMENT

The datasets analyzed in this article are not publicly available. Requests to access the datasets should be directed to kawtar.elabdellati@uantwerpen.be.

AUTHOR CONTRIBUTIONS

All authors met ICMJE criteria and all those who fulfilled those criteria were listed as authors.

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REVIEW
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PDE10A Inhibitors—Clinical Failure or Window Into Antipsychotic Drug Action?

Frank S. Menniti[1]*, *Thomas A. Chappie*[2] *and Christopher J. Schmidt*[3]

[1] George & Anne Ryan Institute for Neuroscience, University of Rhode Island, Kingston, RI, United States, [2] Internal Medicine Medicinal Chemistry, Pfizer Worldwide Research and Development, Cambridge, MA, United States, [3] Pfizer Innovation and Research Lab Unit, Pfizer Worldwide Research and Development, Cambridge, MA, United States

PDE10A, a phosphodiesterase that inactivates both cAMP and cGMP, is a unique signaling molecule in being highly and nearly exclusively expressed in striatal medium spiny neurons. These neurons dynamically integrate cortical information with dopamine-signaled value to mediate action selection among available behavioral options. Medium spiny neurons are components of either the direct or indirect striatal output pathways. Selective activation of indirect pathway medium spiny neurons by dopamine D2 receptor antagonists is putatively a key element in the mechanism of their antipsychotic efficacy. While PDE10A is expressed in all medium spiny neurons, studies in rodents indicated that PDE10A inhibition has behavioral effects in several key assays that phenocopy dopamine D2 receptor inhibition. This finding gave rise to the hypothesis that PDE10A inhibition also preferentially activates indirect pathway medium spiny neurons, a hypothesis that is consistent with electrophysiological, neurochemical, and molecular effects of PDE10A inhibitors. These data underwrote industry-wide efforts to investigate and develop PDE10A inhibitors as novel antipsychotics. Disappointingly, PDE10A inhibitors from 3 companies failed to evidence antipsychotic activity in patients with schizophrenia to the same extent as standard-of-care D2 antagonists. Given the notable similarities between PDE10A inhibitors and D2 antagonists, gaining an understanding of why only the latter class is antipsychotic affords a unique window into the basis for this therapeutic efficacy. With this in mind, we review the data on PDE10A inhibition as a step toward back-translating the limited antipsychotic efficacy of PDE10A inhibitors, hopefully to inform new efforts to develop better therapeutics to treat psychosis and schizophrenia.

Keywords: schizophrenia, PDE10A, basal ganglia, dopamine, cyclic nucleotide phosphodiesterase, medium spiny neuron, antipsychotic action

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***Correspondence:**
Frank S. Menniti
mennitifs@gmail.com

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INTRODUCTION

Dopamine D2 receptor antagonists have been the standard of care pharmacotherapy for the treatment of psychosis in schizophrenia since the 1950's. In the intervening decades, there has been considerable research seeking to gain insight into the molecular basis for the antipsychotic mechanism of these drugs. A significant contribution to this effort has been the development

of pharmaceutical agents directed at alternative molecular targets and their clinical testing for antipsychotic efficacy (**Figure 1**). However, of the 14 mechanisms listed in **Figure 1**, only one, the muscarinic M1-selective agonist xanomeline (Shekhar et al., 2008), approached the efficacy of D2 antagonists. Certainly none of the tested mechanisms evidenced superiority to the standard of care. What has largely been missing from this effort is the back-translation of the molecular pharmacology of the tested-but-failed agents or classes of agents. Simply put, how did these agents affect the brain similarly and yet differently than D2 receptor antagonists to give insight into the nature of antipsychotic drug action? Recently, there has been an industry-wide effort to develop and test inhibitors of phosphodiesterase 10A (PDE10A) as a novel mechanism to ameliorate psychosis (Chappie et al., 2012; Jørgensen et al., 2013; Geerts et al., 2017; Jankowska et al., 2019). Several PDE10A inhibitors were tested in various settings in patients with schizophrenia. While some signs of efficacy was noted on measures of global clinical impressions in one study (Macek et al., 2019), overall these compounds failed to demonstrate convincing evidence of benefit equivalent to the standard of care D2 antagonists (DeMartinis et al., 2019; Macek et al., 2019; Walling et al., 2019). Moving forward from these disappointing results, comparing and contrasting the effects of PDE10A inhibitors with D2 antagonists provides a new opportunity for back translational research to gain insight into factors critical to the molecular basis of antipsychotic drug action. The fact that PDE10A inhibitors have an unusually precise molecular pharmacology, the enzyme is restricted to striatal medium spiny neurons and inhibitors increase cyclic nucleotide levels only in these neurons, may be particularly advantageous to such efforts. With this in mind, we review the data on PDE10A inhibition as a step toward such back-translation, hopefully to inform new efforts to develop better therapeutics to treat psychosis and schizophrenia.

Phosphodiesterase 10A (PDE10A) belongs to the phosphodiesterase superfamily of enzymes that control cAMP and cGMP signaling within cells throughout the body (Conti and Beavo, 2007). This control of cyclic nucleotide signaling is accomplished through the hydrolysis of signaling-capable cAMP or cGMP to signaling-silent AMP or GMP. PDEs are differentially and dynamically localized at the cellular and subcellular levels to control the intensity, direction, and longevity of cyclic nucleotide signaling engaged by external stimulation of G-protein coupled receptors and Ca^{2+} signaling mechanisms. Given the key regulatory role of PDEs in cellular communication, pharmacological manipulation has proven to be an attractive avenue for development of drugs to treat various human diseases (Lugnier, 2006; Menniti et al., 2006; Baillie et al., 2019). Inhibitors of PDE3 are used for the treatment of heart failure (milrinone), PDE4 inhibitors are used for treating inflammatory conditions such as COPD (roflumilast) and psoriatic arthritis (apremilast), and PDE5 inhibitors are used for erectile dysfunction (sildenafil, tadalafil, and vardenafil) and pulmonary hypertension (sildenafil). With the proven track record of identifying drugs that inhibit PDEs, there was significant excitement in the pharmaceutical world when PDE10A was identified as a potential new target in 1999 (Fujishige et al., 1999; Loughney et al., 1999; Soderling et al., 1999). PDE10A was found to be capable of hydrolyzing both cAMP and cGMP and mRNA for the enzyme was found to be highly localized to the brain and testes. Within the brain, mRNA expression is highest in the striatum and within this brain region expression is exclusive to striatal medium spiny neurons; high levels of protein expression also correspond with this restricted mRNA distribution pattern (Seeger et al., 2003; Xie et al., 2006). Thus, PDE10A is a unique signaling molecule in being highly expressed in only a single neuronal population and in having a singular molecular signaling role. This localization prompted an intensive effort to determine the role of PDE10A in regulating striatal function and to investigate the potential therapeutic utilities of PDE10A inhibitors (Kehler and Nielsen, 2011; Chappie and Verhoest, 2014; Charych and Brandon, 2014).

The striatum is a large nucleus comprised primarily of PDE10A-expressing medium spiny neurons (MSNs) that functions as the gateway for the input and processing of cortical information by the basal ganglia circuit (Albin et al., 1989; Haber, 2016). The MSNs are also recipient of a dense dopaminergic input from the substantia nigra and ventral tegmental area. In roughly half of the MSNs, the dopamine signal is transduced through dopamine D1 receptors and in the other half this signal is transduced through dopamine D2 receptors. The efferents of these two classes of MSNs delineate two parallel information processing streams, the direct and indirect striatal output pathways. These two pathways coordinate in the dynamic integration of cortical information with dopamine-coded reward/salience information to select advantageous behaviors while suppressing less advantageous options (Wichmann and DeLong, 1996). Dysfunction in this circuitry is implicated in a range of neuropsychiatric and neurodegenerative conditions (Graybiel, 2000). Notably, inhibition of dopamine D2 receptors on indirect pathway MSNs is putatively the mechanism of antipsychotic action of the D2 receptor antagonists, the standard of care pharmacotherapy for the treatment of psychosis in schizophrenia (Seeman, 2010; McCutcheon et al., 2019). Rodent behavioral studies in mice with genetic deletion of *PDE10A* (Siuciak et al., 2006b; Sano et al., 2008; Piccart et al., 2014) and mice or rats treated with PDE10A inhibitors such as papaverine (Siuciak et al., 2006a), PQ-10 (Chappie et al., 2007), TP-10 (Schmidt et al., 2008), THPP-1 (Smith et al., 2013), and JNJ-42314415 (Megens et al., 2014a) revealed that PDE10A inhibition causes behavioral effects similar to D2 antagonists. In fact, the similarities to D2 antagonists were considered very suggestive of the potential for antipsychotic activity, launching an industry-wide effort to develop PDE10A inhibitors as a new class of antipsychotic agents that regulate striatal function outside of the traditional neurotransmitter/receptor realm. Extensive reviews of the work to identify PDE10A inhibitors have been published (Chappie et al., 2012; Jørgensen et al., 2013; Jankowska et al., 2019). Recent searches have identified >150 PDE10A inhibitor patents with >15 companies represented. Ultimately, these efforts resulted in 12 reported clinical candidates and 4 clinically validated PDE10A PET ligands (Geerts et al., 2017).

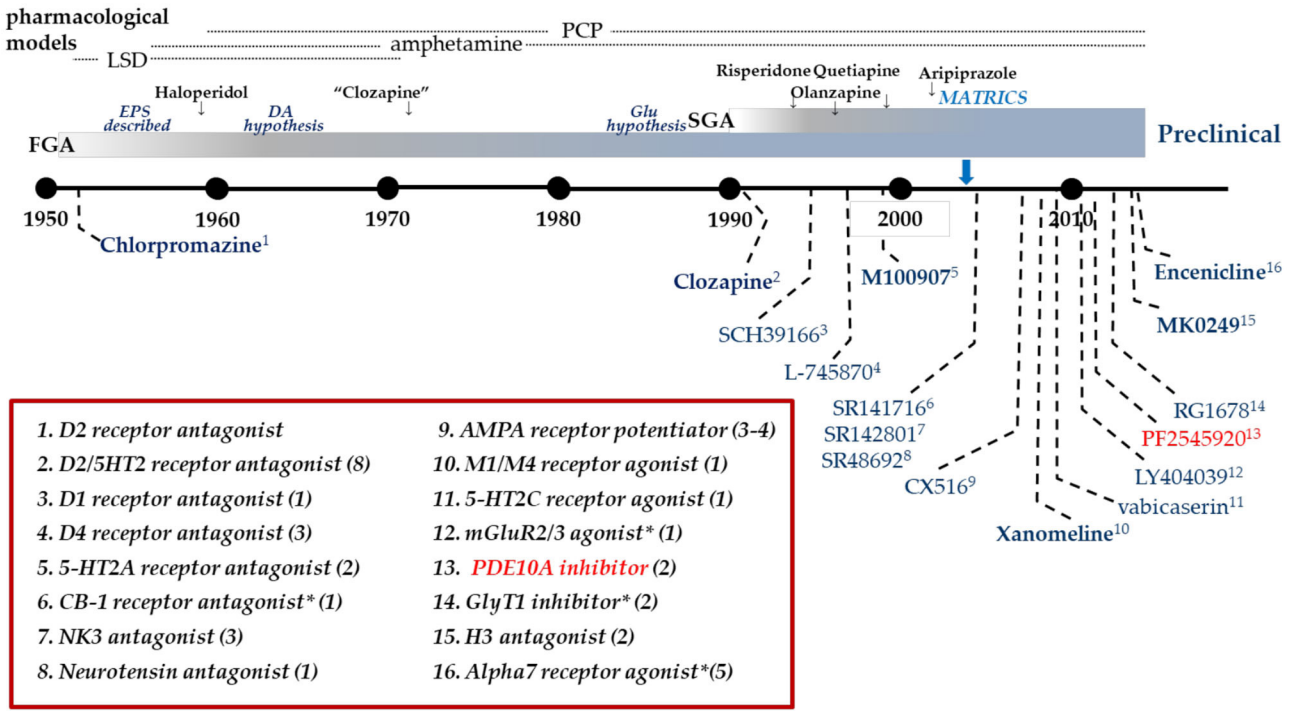

FIGURE 1 | Clinical drug development in Schizophrenia. Timeline of clinical drug development in schizophrenia following the discovery of the antipsychotic activity of chlorpromazine in 1952. Individual compounds are listed in the graphic with their pharmacological mechanism identified in the box. Many mechanisms have been targeted with multiple compounds as identified by the value in parentheses. Dates are an approximation, based on publication dates or drug approval. *Includes allosteric modulators.

In clinical studies to date, PDE10A inhibitors have generally been found to be safe and well-tolerated at doses yielding exposures in the range targeted for efficacy (Tsai et al., 2016). Significantly, PDE10A inhibitors were found to be psychoactive in the targeted exposure ranges, producing a state characterized as "awake sedation" or "conscious sedation," as discussed at a NIMH-sponsored workshop on PDE10A held January 25, 2013 at the NIH Neuroscience Center in Rockville, MD, USA. At higher exposures, PDE10A inhibitors were found to induce sporadic dystonia, particularly of the tongue, head, and neck. This motor side effect is consistent with the compounds modulating basal ganglia circuitry, albeit in a maladaptive fashion.

Two companies, Pfizer and Takeda, have published results of Phase II efficacy studies with PDE10A inhibitors in patients experiencing acute psychosis associated with chronic schizophrenia. Pfizer's PF-02545920 was first characterized for PDE10A enzyme occupancy in healthy volunteers at doses of 10 mg and 20 mg using PET imaging (Delnomdedieu et al., 2017). PDE10A enzyme occupancy was demonstrated to be 14–27% following the 10 mg dose and 45–63% following the 20 mg dose. Both doses were safe and well-tolerated. PF-02545920 was then tested for antipsychotic efficacy in patients with schizophrenia experiencing an acute exacerbation of psychotic symptoms (Walling et al., 2019). The study involved 4 weeks of treatment in patients randomly assigned to receive either 5 mg or 15 mg of PF-02545920 (Q12H, 74 patients per treatment group). Comparator cohorts received placebo (74 patients) or 3 mg of risperidone (Q12H, 37 patients), a D2 antagonist that is a standard of care. Risperidone showed a statistically significant difference from placebo in alleviating symptoms based on the Positive and Negative Syndrome Scale (PANSS) total score at the end of 4 weeks. However, neither dose of PF-02545920 produced a statistical separation from placebo at any time point.

Pre-clinical data suggested that PDE10A inhibition may also augment the antipsychotic activity of D2 antagonists. To investigate this potential therapeutic utility, Pfizer conducted a second clinical study in schizophrenia patients receiving a D2 antagonist but whose symptoms were sub-optimally controlled (DeMartinis et al., 2019). The study involved 3 dose groups: PF-02545920 at 5 mg (Q12H, 78 patients) or 15 mg (Q12H, 82 patients), or placebo (80 patients) with treatment planned for 12 weeks. However, the study was halted due to an interim futility analysis indicating a low probability of any significant additional beneficial response when PF-02545920 was added to standard of care D2 antagonists.

Takeda developed a PDE10A inhibitor designated as TAK-063. Early clinical characterization using PET imaging indicated TAK-063 can be dosed to achieve PDE10A enzyme occupancies from 2.8 to 72.1% with good toleration (Takano et al., 2016). Takeda then conducted a clinical trial in schizophrenia patients experiencing an acute exacerbation of psychotic symptoms

(Macek et al., 2019). The study involved the dosing of TAK-063 (20 mg; 83 patients) or placebo (81 patients) for 6 weeks. Modeling from the PET study indicated this 20 mg dose would yield ∼30% PDE10A enzyme occupancy. Unfortunately, the study did not achieve its primary endpoint of a significant change from baseline in the PANSS score. However, Takeda noted that three secondary endpoints were improved in the TAK-063 group. Those endpoints were the Clinical Global Impression severity (CGI-S) scores, Clinical Global Impression improvement (CGI-I) scores, and the percentage of CGI-I responders.

The positive movements in the secondary endpoints of the TAK-063 study were also measured in the PF-02545920 monotherapy trial and were found to be non-significantly changed at either 5 or 15 mg. Importantly, the positive control in the study, risperidone, was found to have significant positive effects vs. these secondary endpoints suggesting that the study was capable of sensing changes to these measures over the duration of the study. There were also no effects of PF-02545920 on global clinical measures in the study in which this agent was added to D2 antagonist treatment. The enzyme occupancy levels for the 15 mg dose of PF-02545920 was in a similar range to that estimated for the 20 mg dose of TAK-063, suggesting that enzyme occupancy is not a factor in the difference in secondary outcomes measures. Durations of treatments were also in the same range, suggesting this factor also does not account for the difference.

As this review was in preparation, H. Lundbeck A/S announced halting a trial based on an interim futility analysis of the effects of their PDE10A inhibitor Lu AF11167 against persistent prominent negative symptoms in patients with schizophrenia (BNSS). Secondary endpoints in the Lu AF1167 trial were the PANSS and the study protocol called for the drug to be administered for 12 weeks. Although additional PDE10A inhibitors (Geerts et al., 2017) have advanced to early clinical safety studies, searches of company websites suggest that efforts regarding the PDE10A mechanism with respect to schizophrenia have been discontinued.

Pfizer also conducted a proof-of-concept Phase II study of the efficacy of PF-02545920 to improve symptoms in patients with Huntington's disease (Delnomdedieu et al., 2018). Doses of 5 or 20 mg were used in a study of over 200 early-stage symptomatic patients. There was no significant effect of treatment on the primary outcome measure, the Unified-Huntington's-Disease-Rating-Scale Total-Motor-Score (UHDRS-TMS). However, a dose-dependent improvement was observed on an exploratory measure, the Q-motor score, suggesting a possible effect of the drug on motor coordination.

In summary of the clinical trial data currently available, PDE10A inhibitors were demonstrated to be psychoactive in that they produced somnolence or sedation in all clinical studies publicly reported. However, the Pfizer or Takeda PDE10A inhibitors did not produce clinically meaningful improvements in positive symptoms in patients suffering schizophrenia as measured using the PANSS scale, the primary outcome measures in these studies. Based on the preliminary report from Lundbeck, there was apparently no robust effect of this mechanism on negative symptoms. In the Takeda study in schizophrenia patients exhibiting acute exacerbation of symptoms, there was evidence of a favorable change in global clinical impressions; however, this was not replicated in the Pfizer studies. Given the compelling preclinical data and biological rationale suggesting that PDE10A inhibition would positively impact schizophrenia, the clinical results from Pfizer, Takeda, and Lundbeck call for a reevaluation of our hypotheses regarding the mechanism(s) by which PDE10A inhibitors and D2 antagonists may ameliorate psychosis. There is now a wealth of data on the physiology of PDE10A and preclinical data on the effects of PDE10A inhibitors that can be compared to that of D2 receptors and D2 receptor antagonists. Our purpose here is to review and synthesize this extensive data set with an ultimate goal of understanding why these two mechanisms do not produce similar clinical activity and to highlight knowledge gaps that impede full interpretation of the clinical data. Understanding the apparent lack of predicted antipsychotic activity will hopefully inform future efforts to develop new antipsychotic therapies and justify/enable continued drug development research for this indication. We also hope this review may serve in the formulation of new hypotheses around therapeutic uses for PDE10A inhibitors. To set the stage, we first provide a brief review of the physiology of striatal MSNs and dopamine signaling within these neurons.

STRIATAL MSNs–A KEY CELLULAR TARGET OF ANTIPSYCHOTIC DRUGS

As used here, *striatum* refers to the contiguous subcortical nuclei of caudate n., n. accumbens, and olfactory tubercle (rodent nomenclature), the input loci of the cortico-striato-nigral-thalamic loop known as the basal ganglia circuit (Albin et al., 1989; Gerfen, 1992; Haber et al., 2000). The MSNs comprise the major neuronal type in the striatum—in rodents MSNs are estimated to comprise 90–95% of striatal neurons, whereas in humans the percentage is slightly lower. These GABAergic projection neurons receive an extensive, topographically organized, excitatory glutamatergic input from cortex and thalamus (Bolam et al., 2000; Haber et al., 2000; Haber, 2016). The MSNs are also the recipient of a topographically organized dopaminergic input from substantia nigra and ventral tegmentum (Bolam et al., 2000). There are two anatomically and biochemically defined subsets of MSNs (Alexander and Crutcher, 1990; Gertler et al., 2008). MSNs of the *direct* pathway express dopamine D1 receptors and the neuropeptides dynorphin and substance P. Direct pathway MSNs project *directly* to and inhibit the output nuclei of the basal ganglia, the substantia nigra/entopeduncular n., which in turn project to and inhibit the thalamus. Activation of direct pathway MSNs dis-inhibits the excitatory thalamic output to cortex. MSNs of the *indirect* pathway express dopamine D2 receptors, adenosine A2A receptors, and the neuropeptide enkephalin. These MSNs also modulate the activity of the substantia nigra/entopeduncular n., but in this case *indirectly* via a multi-synaptic pathway through the external globus pallidus and subthalamic n. with the end result being dis-inhibition of the output nuclei to suppress thalamic feedback to cortex. In the classical model of the basal ganglia

circuit, the direct striatal output pathway broadly functions to facilitate behavioral responses, whereas the indirect striatal output pathway functions to suppress behavioral responses that compete with those being facilitated through the direct pathway (Alexander and Crutcher, 1990; Calabresi et al., 2014).

The excitatory glutamatergic drive on MSN activity is regulated by the peri-synaptic dopaminergic input arising from substantia nigra and ventral tegmental nucleus (Surmeier et al., 2007). The intracellular signaling triggered by dopamine in the MSN is multi-faceted (Valjent et al., 2019). The most well-studied mechanisms down-stream of dopamine receptor activation are G protein-dependent modulations of cAMP formation. D1 receptors are positively coupled to adenylate cyclase, whereas D2 receptors are negatively coupled to adenylate cyclase (Bibb, 2005). Thus, dopamine release in striatum causes an increase in cAMP in direct pathway neurons while inhibiting cAMP synthesis in indirect pathway neurons. D2 receptor antagonists increase cAMP in indirect pathway neurons by reducing the D2 receptor brake on cyclase activity. D1 receptor stimulation and D2 receptor inhibition also increase cGMP synthesis in striatum (West, 2016). Dopamine-regulated striatal cGMP synthesis is driven by nitric oxide (NO) stimulation of soluble guanylate cyclase, which is expressed by both direct and indirect pathway MSNs. However, NO is delivered by *inter-neuronal* diffusion following stimulation of neuronal nitric oxide synthesis (nNOS) located in a small population of nNOS-positive striatal interneurons. Thus, cGMP signaling may not be as discreetly segregated in direct and indirect pathway neurons as is cAMP signaling. Both D1 and D2 receptors also signal through an interaction with β-arrestin to regulate an Akt/GSK3b signaling cascade independently of G-protein signaling (Del'Guidice et al., 2011). Furthermore, dopamine receptors may form functional heteromeric complexes through dimerization with adenosine, metabotropic glutamate, peptidergic, or serotonin receptors (Perreault et al., 2014; Borroto-Escuela et al., 2020). These dopamine receptor heteromers have unique downstream signaling signatures in the MSNs. Additional mechanisms by which D2 antagonists modulate striatal information processing include effects on D2 receptors on glutamate terminals (Bamford et al., 2004) as well as on striatal interneurons (Centonze et al., 2003). Blockade of non-striatal D2 receptors may also contribute to the therapeutic mechanism of action of this class (O'Donnell, 2012).

It is an open question how the different D2 receptor signaling mechanisms outlined above are impacted by D2 receptor antagonists to mediate the changes in basal ganglia information processing that results in the suppression of psychosis in schizophrenia (Boyd and Mailman, 2012; Martel and Gatti McArthur, 2020). The molecular signaling mechanisms activated by PDE10A inhibitors intersect with those activated by D2 receptor antagonists at the level of cAMP and cGMP signaling. Significant to the perspective of this review, PDE10A inhibitors and dopamine D2 receptor antagonists have many similar effects on MSN activity and basal ganglia function downstream of the respective proximal molecular signaling mechanisms. It is these similarities that supported advancing the PDE10A inhibitors as potential antipsychotics (Menniti et al., 2007). Thus, the next section of this review will focus on the role of PDE10A in regulating cyclic nucleotide signaling and function in MSNs and a comparison of such effects to dopamine receptor modulators.

PDE10A—A PHOSPHODIESTERASE HIGHLY ENRICHED IN STRIATAL MSNs

Discovery of the PDE10A gene in 1999 (Fujishige et al., 1999; Loughney et al., 1999; Soderling et al., 1999) resulted from a bioinformatics search for genes with homology to known PDEs, enabled by the newly available complete sequence of the human genome. While high levels of PDE10A mRNA were detected in brain and testes, high levels of PDE10A protein were detected only in brain (Coskran et al., 2006). Analyses of both PDE10A mRNA and protein expression revealed that the distribution of this phosphodiesterase is further delimited to high expression only in striatal medium spiny neurons (Xie et al., 2006). PDE10A is expressed as 3 major splice variants, PDE10A1, A2, and A3, although as many as 15 minor variants may also exist (Fujishige et al., 2000; MacMullen et al., 2017). Immunohistochemical analyses indicate PDE10A distributes throughout the MSNs, i.e., in soma and throughout the complete dendritic and axonal compartments (Seeger et al., 2003). In biochemical analyses of striatal tissue, which contains MSN cell bodies, dendrites, and axon collaterals, PDE10A is primarily membrane bound (Xie et al., 2006). Membrane localization appears to be the result of irreversible n-terminal palmitoylation (Charych et al., 2010). Electron microscopic analysis revealed the protein to distribute into dendritic spines, including juxtaposed to the post-synaptic density (Xie et al., 2006). Consistent with this observation, biochemical analyses indicate that the enzyme is incorporated into a post-synaptic complex that includes NMDA receptors, PSD95, AKAP150, and PKA (Russwurm et al., 2015). In contrast, there is no information on subcellular localization of PDE10A in MSN axons and terminals in globus pallidus and substantia nigra.

PDE10A mRNA and protein are detected in other neurons throughout the brain, albeit at levels much lower than in MSNs (Seeger et al., 2003; Coskran et al., 2006). In forebrain neurons outside of striatum, PDE10A-like immunoreactivity is confined to cell nuclei and/or the perinuclear compartment. Nuclear localization of PDE10A protein in hippocampus was confirmed in cell fractionation studies (Giralt et al., 2013). PDE10A mRNA levels are upregulated in hippocampus by the induction of LTP (O'Connor et al., 2004), and PDE10A mRNA and protein also vary with a diurnal rhythm in pineal gland (Spiwoks-Becker et al., 2011). Collectively, these data imply function roles for the enzyme in non-striatal brain regions. However, pharmacological inhibition of PDE10A causes no detectable changes in cyclic nucleotide levels or gene expression in non-striatal forebrain tissue (Kleiman et al., 2011), in contrast to robust changes in striatal tissue. Thus, PDE10A appears to have a unique role in regulation of striatal MSN function.

Striatal MSNs contain a high density of cAMP and cGMP signaling components, including the highest levels of phosphodiesterase in brain (Lakics et al., 2010; Kelly, 2014). PDE10A, highly expressed in both direct and indirect

pathway MSNs, is anatomically placed to regulate the activity of both direct and indirect pathways. As a dual substrate phosphodiesterase, PDE10A is capable of regulating both cAMP and cGMP signaling in MSNs of both pathways. Next, we review what is known about the role of PDE10A in regulating cyclic nucleotide signaling in MSNs.

PDE10A REGULATION OF CYCLIC NUCLEOTIDE SIGNALING IN MSNs

Initial characterizations of PDE10A demonstrated that the enzyme hydrolyzes both cAMP and cGMP (Fujishige et al., 1999; Soderling et al., 1999). The affinity of recombinant PDE10A for cAMP is considerably greater than for cGMP, and it was suggested that the enzyme may be a cAMP-regulated cGMP-ase. Subsequent studies of the effects of PDE10A inhibitors on striatal cyclic nucleotide levels in rodents reviewed below indicated that PDE10A, in fact, regulates both cAMP and cGMP signaling in striatum. However, there is no evidence for competitive substrate interactions; i.e., the regulation by PDE10A of cAMP signaling is independent of the regulation of cGMP signaling and *vice versa*.

Systemic administration of PDE10A inhibitors to mice causes a robust increase in striatal cGMP levels relative to levels in the absence of inhibitor (i.e., "basal" levels)[1]. The increase in cGMP levels may be 5-fold or higher than basal levels (Chappie et al., 2007; Schmidt et al., 2008; Malamas et al., 2011; Suzuki et al., 2015). An increase in cAMP over basal level is also observed in striatum (Schmidt et al., 2008; Malamas et al., 2011; Suzuki et al., 2015). The magnitude of the cAMP increases in absolute terms is similar to that for cGMP. However, basal levels of cAMP are about 10-fold higher than for cGMP and so the effect of PDE10A inhibition on cAMP levels as a ratio to basal levels are modest and more difficult to reliably detect. Systemic administration of PDE10A inhibitors also produces robust increases in both cAMP and cGMP levels in rat striatum[2]. These data indicate that PDE10A regulates actively turning over pools of cGMP and cAMP in striatal MSNs, even in the absence of any overt behavioral or pharmacological stimulus to drive cyclic nucleotide synthesis.

cGMP Signaling

The pool of cGMP regulated by PDE10A is derived from soluble guanylate cyclase (sGC) stimulated by nitric oxide (NO) synthesized by neuronal nitric oxide synthase (NOS) (Threlfell et al., 2009; Padovan-Neto et al., 2015). Striatal MSNs express high levels of sGC, an enzyme that, when activated by NO, catalyzes the cyclization of GTP to form cGMP. NO is a diffusible intra- as well as inter-cellular second messenger formed from L-arginine by three different synthases, neuronal NOS, endothelial NOS, or inducible NOS. In striatum of mice with genetic deletion of nNOS, basal cGMP is decreased 80–90%. Furthermore, the effect of PDE10A inhibition to increase cGMP is completely abrogated (Padovan-Neto et al., 2015). Similar effects on both basal and PDE10A inhibitor enhanced cGMP levels are observed after systemic administration of NOS or nNOS-selective inhibitors. Genetic deletion of nNOS or administration of nNOS inhibitors also completely block the increase in cGMP caused by D2 antagonists or dopamine D1 receptor agonists (West, 2016). The locus of nNOS driving this striatal cGMP synthesis is nNOS-positive striatal interneurons. In addition to expressing high levels of nNOS, these interneurons express the neuropeptides somatostatin and NPY, and are characterized electrophysiologically as having a low threshold for induction of Ca^{2+} spikes and for sustaining a prolonged depolarized membrane potential (Kawaguchi, 1993). They are referred to in the literature as SOM+ or PLTS striatal interneurons.

Striatal nNOS positive interneurons integrate a variety of synaptic inputs, including glutamatergic input from cortex, cholinergic input from striatal fast-spiking interneurons and GABAergic inputs from striatal and extra-striatal sources (Tepper et al., 2010). Notably, a principal driver for cell firing is activation of dopamine D1-like (probably D5) receptors expressed by these cells (Centonze et al., 2002). Consistent with this scheme, we observe that systemic administration of the D1 agonist SKF-81297 causes a modest increase in striatal cGMP level, whereas the AMPA receptor antagonist CP-465,022 caused a reduction. However, the D1 antagonist SCH-23390 had no effect on the PDE10A inhibitor-induced increase in striatal cGMP. Furthermore, the increase in striatal cGMP levels caused by a PDE10A inhibitor administered with a D1 agonist were found to be additive, not synergistic. Surprisingly, the D2 antagonist haloperidol was found to cause a more robust increase in striatal cGMP than the D1 agonist, despite the fact that the nNOS positive interneurons do not express D2 receptors (Centonze et al., 2002; Tepper et al., 2010). Furthermore, the D2 agonist quinpirole, while having no effect on cGMP levels when administered alone, attenuated the PDE10A inhibitor induced cGMP increase. Conversely, the increase in cGMP levels caused by PDE10A and D2 inhibition are super-additive. Thus, activation of the nNOS-positive interneurons results in the formation of NO, which diffuses into MSNs to stimulate the formation of cGMP by sGC. Whereas, NO-driven cGMP synthesis is the source of the PDE10A regulated cGMP pool, there appears to be compartmentalization and differential regulation of cGMP pools regulated by D1 and D2 receptor stimulation. Specifically, PDE10A appears to regulate a cGMP pool linked to D2 receptor activity but does not directly regulate the cGMP pool downstream of D1 receptor activation (**Figure 2**).

cAMP Signaling

Systemic administration of PDE10A inhibitors to mice or rats increase striatal cAMP levels (Schmidt et al., 2008; Suzuki et al., 2015). However, as noted above, this effect of the inhibitors is difficult to quantify against the background levels of cAMP, which in turn makes it difficult to study the nature of the upstream signaling mechanisms driving the cAMP pool(s) regulated by PDE10A. To overcome this obstacle, we studied

[1]When microwave irradiation is used to rapidly inactivate tissue enzymatic activity.

[2]In this case measured by microdialysis.

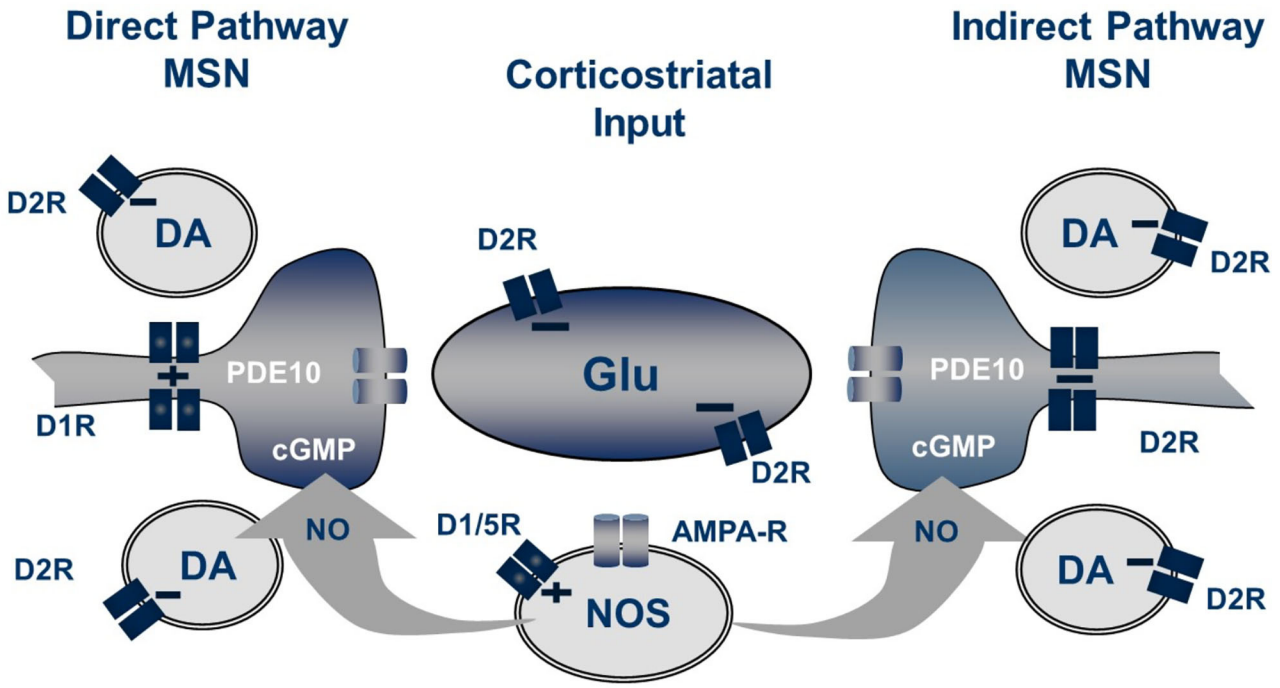

FIGURE 2 | Striatal regulation of cGMP signaling in direct and indirect pathway MSNs. The generation of NO from NOS+ interneurons is dependent upon both D1-like receptors and glutamate acting on AMPA receptors while the concentration of cGMP in MSN is regulated by phosphodiesterase activities including PDE10A. Not shown are the cholinergic interneurons which also play a role in this regulation.

change in the level of CREB phosphorylation as a surrogate for change in cAMP. Systemic administration of PDE10A inhibitors to mice results in a rapid and robust increase in striatal levels of phospho-CREB (Schmidt et al., 2008; Smith et al., 2013; Suzuki et al., 2015). The phospho-CREB response to PDE10A inhibition is downstream of cAMP signaling since it is not affected by genetic deletion of nNOS, which completely eliminates the PDE10A inhibitor-induced increase in striatal cGMP (see above). Furthermore, for TP-10, the dose-response relationship for increasing phospho-CREB paralleled that for the increase in cAMP and increases in phospho-CREB were temporally aligned with plasma drug concentrations (Schmidt et al., 2008). Pharmacological inhibition of dopamine D1 receptors attenuated the increase in phospho-CREB caused by PDE10A inhibition. However, the effect of a dopamine D1 receptor agonist to increase phospho-CREB levels was additive with that of a PDE10A inhibitor, not synergistic as would be predicted if PDE10A was the principal phosphodiesterase regulating the D1 receptor stimulated cAMP pool driving CREB phosphorylation. Stimulation of D2 receptors, which are negatively coupled to adenylyl cyclase, attenuated the PDE10A inhibitor-induced increase in phospho-CREB. Again, however, the effect of PDE10A inhibition and D2 receptor inhibition were additive and not synergistic. Thus, changes in phospho-CREB, as a surrogate for changes in cAMP, indicate that PDE10A plays a role in regulating the cAMP signaling pools downstream of both dopamine D1 and D2 receptor signaling. However, the lack of synergistic effects of PDE10A inhibition with either a D1 agonist or D2 antagonist indicate that PDE10A is but one of several regulatory factors and one of several cAMP phosphodiesterases highly expressed in striatum (Polito et al., 2013).

Indirect pathway MSNs express adenosine A2 (A2A) receptors positively coupled to adenylyl cyclase. Nishi et al. (2008) reported that the PDE10A inhibitor papaverine increased phosphorylation of DARPP-32 at the Thr34 (PKA) site in mouse brain slices containing striatum. This effect of papaverine was potentiated by the A2A agonist CGS21680 and partially inhibited by A2A antagonist ZM241385. These data indicate that PDE10A may play a role in regulating cAMP signaling driven by A2A receptor activation in indirect pathway MSNs. Adenylyl cyclase activity in striatum is also regulated by calcium signaling mechanisms including those triggered by ionotropic glutamate receptor activation. However, we found that systemic administration of NMDA or AMPA receptor antagonists did not attenuate the increase in phospho-CREB induced by administration of a PDE10A inhibitor. Thus, the complex pharmacology of PDE10A inhibitors on cAMP levels likely reflect the complexity and compartmentalization of cAMP signaling in striatum and highlight the limitations of using bulk tissue measurements of signaling molecules to investigate such compartmentalized systems.

Finally, we note that there is virtually no data on the cyclic nucleotide signaling cascades regulated by PDE10A in the axons and terminals of MSNs, where PDE10A is also highly expressed.

CONSEQUENCES OF PDE10A INHIBITION DOWNSTREAM OF STRIATAL CYCLIC NUCLEOTIDES

Consistent with localization of the enzyme in both MSN populations, PDE10A inhibition alters cyclic nucleotide signaling activated by D1 and D2 receptors. There are both qualitative and quantitative differences in these effects. Significant to this discussion, the consequences of PDE10A inhibition appear to be biased for greater activation of indirect pathway MSNs, at least in rodent systems.

Studies by Nishi et al. (2008) in brain slices containing striatum first indicated that PDE10A inhibition has an effect biased toward activation of indirect pathway MSNs. In studies of protein phosphorylation in striatal slices from mouse, Nishi et al. reported that PDE10A inhibition increased phosphorylation of DARRP-32 at Thr34, the AMPA receptor subunit GluR1 at Ser 845, and ERK2 at Thr202/Tyr204. Effects of PDE10A inhibition on DARPP-32 phosphorylation were not affected by inhibition of soluble guanylyl cyclase with ODQ, indicating DARPP-32 phosphorylation is downstream of cAMP and PKA signaling. DARPP-32 phosphorylation in direct and indirect pathway MSNs were further analyzed in slices from mice in which DARRP-32 was differentially tagged with Flag or Myc, respectively. PDE10A inhibition increased phosphorylation of DARRP-32 pulled down with either tag, consistent with effects of PDE10A inhibition on cAMP signaling in both MSN populations. However, the efficacy of PDE10A inhibition to increase DARPP-32 phosphorylation was greater for Myc-tagged protein, i.e., that pulled down from indirect pathway MSNs. Nishi et al. concluded that PDE10A inhibition has a greater impact on signaling in MSNs of the indirect pathway and highlighted that the effects of PDE10A inhibition bore resemblance to those of D2 antagonists.

A similar conclusion was reached by Vincent and colleagues, in this case examining the effects of PDE10A inhibition in striatal slices using cAMP or PKA biosensors (Polito et al., 2015). Biosensor responses in direct and indirect pathway MSNs were distinguished pharmacologically based on MSN responsiveness to dopamine D1 or adenosine A2A agonists, respectively. In slices transfected with the cAMP biosensor Epac-SH150, PDE10A inhibition equivalently increased biosensor signal in both direct and indirect pathway MSNs. In contrast, in slices transfected with PKA biosensor AKAR3, PDE10A inhibition resulted in increased biosensor signal in indirect pathway MSNs but not in direct pathway MSNs. The biased activation of PKA-signaling in indirect pathway MSNs was also observed *in vivo* in mice treated with a PDE10A inhibitor. These studies used mice in which indirect pathway MSNs were identified by expression of EGFP-tagged dopamine D2 receptors. PDE10A administration increased PKA-dependent histone H3 phosphorylation exclusively in EGFP-positive MSNs. Thus, while PDE10A regulates cAMP signaling in MSNs of both the direct and indirect pathway, the downstream consequences are of greater impact in indirect pathway MSNs.

A differential effect of PDE10A inhibition on indirect pathway MSNs is also evident with regard to cGMP signaling, based on *in vivo* electrophysiological studies of PDE10A inhibitors on the excitability of MSNs by West and colleagues (Threlfell et al., 2009; Padovan-Neto et al., 2015). For these studies, MSNs of direct or indirect pathways were identified by whether or not, respectively, they were activated by antidromic stimulation from substantia nigra, the terminal zone for direct pathway MSNs. Strikingly, PDE10A inhibition increased excitability of indirect pathway MSNs to cortical stimulation without effecting excitability of direct pathway MSNs. This effect of PDE10A inhibition was abrogated in nNOS knock out mice, indicating mediation by cGMP signaling (Padovan-Neto et al., 2015). A subtle effect of PDE10A inhibitors on direct pathway MSNs was observed by Threlfell et al.; the number of MSNs activated by antidromic stimulation of substantia nigra was increased in animals treated with a PDE10A inhibitor (Threlfell et al., 2009). This implied that PDE10A inhibition increased axonal excitability of direct pathway MSNs. However, whether this effect was abrogated in the nNOS knock out mice was not tested so it is not known whether this effect was mediated by cGMP. A similar analysis of axonal excitability of indirect pathway MSNs was not technically feasible. Thus, PDE10A regulates cGMP signaling in MSNs of the indirect pathway with consequences biased toward activation of indirect pathway MSNs. Given that NO is a diffusible messenger, we may conjecture that PDE10A also regulates cGMP signaling in direct pathway MSNs, but downstream effectors of such signaling have not been established.

PDE10A plays a significant role in regulation of gene expression changes in MSNs. Strick et al. found that PDE10A inhibition led to increases in expression of both substance P and enkephalin mRNA in striatum (Strick et al., 2010); see also (Suzuki et al., 2015). Since these markers are expressed selectively by direct and indirect pathway MSNs, respectively, it was concluded that PDE10A regulates gene expression in both MSN populations. This conclusion was supported in a more recent study in which TAK-063 was found to induce increases in striatal cfos expression in both direct and indirect pathway MSNs (Nakatani et al., 2017). In the earlier Strick et al. study, the PDE10A inhibitor-induced increase in cfos expression was unaffected by genetic deletion of nNOS, indicating the cfos response is downstream of cAMP signaling. Microarray profiling of mRNA expression indicates that the number of genes under regulation by PDE10A is quite substantial and restricted to striatum (Kleiman et al., 2011). Thus, PDE10A functions as a brake on a complex transcriptional program in direct and indirect pathway MSNs, indicating that inhibitors of the enzyme may have long term consequences to the function of these neurons.

BEHAVIORAL EFFECTS OF PDE10A INHIBITION IN RODENTS

Given the prominent localization of PDE10A to striatal medium spiny neurons, the effects of PDE10A inhibition have been studied in rodent behavioral paradigms that are sensitive to pharmacological manipulation of basal ganglia activity. In many cases, the effects of the PDE10A inhibitors were compared to antipsychotic dopamine D2 receptor antagonists and in some paradigms the effects of these two classes of compounds were

very similar. This similarity is consistent with the physiological data reviewed above indicating a biased efficacy of PDE10A inhibition for activation of indirect pathway MSNs, i.e., those expressing dopamine D2 receptors. These observations served as a significant part of the rationale for advancing PDE10A inhibitors into clinical trials for the treatment of psychosis in schizophrenia. However, clear distinctions between these two classes of compounds have also been noted. These distinctions take on new significance in light of the lack of antipsychotic efficacy reported for PF-02545920, TAK-063 and Lu AF11167.

The most robust effects of PDE10A inhibition in rodents are inhibition of NMDA receptor channel blocker-induced hyperlocomotor activity and inhibition of conditioned avoidance responding. NMDA receptor channel blockers, including phencyclidine, ketamine, and MK-801, cause a spectrum of behavioral effects in humans that are similar to those experienced by patients with schizophrenia (Luby et al., 1959; Lahti et al., 2001). In fact, these drug effects in humans are the foundation for the hypothesis that *NMDA receptor hypofunction* is a primary mechanism underlying the expression of schizophrenia symptoms (Krystal et al., 2003; Javitt et al., 2012). In rodents, this class of compounds induce hyperlocomotor activity, among other behavioral effects. Thus, the ability of pharmacological agents to attenuate NMDA channel blocker-induced hyperlocomotor activity is considered indicative of potential for clinical antipsychotic activity (Jentsch and Roth, 1999). PDE10A inhibitors very effectively block such hyperlocomotor activity—effects are dose dependent and inhibition may be complete (Siuciak et al., 2006a; Chappie et al., 2007; Schmidt et al., 2008; Grauer et al., 2009; Malamas et al., 2011; Smith et al., 2013; Megens et al., 2014a; Suzuki et al., 2015). Furthermore, there is a close correspondence between the dose response of PDE10A inhibitors for inhibition of channel blocker-induced locomotor activity and increases in striatal cGMP levels.

It is hypothesized that aberrant dopamine signaling gives rise to the mis-attribution of stimulus salience, leading to the development of psychotic and delusional symptoms in schizophrenia (Kapur et al., 2005; Winton-Brown et al., 2014). The ability of D2 antagonists to reduce stimulus salience is hypothesized to underly the antipsychotic activity of this class, at least in part. Conditioned avoidance responding is a behavioral assay of stimulus salience and D2 antagonists are effective at inhibiting this behavior in rodents (Wadenberg, 2010). PDE10A inhibitors are also highly efficacious at blocking conditioned avoidance responding (Schmidt et al., 2008; Grauer et al., 2009; Malamas et al., 2011; Smith et al., 2013; Suzuki et al., 2015). The dose response for this effect overlays with that for inhibition of channel blocker-induced hyperlocomotor activity and increasing striatal cyclic nucleotides. Thus, the effectiveness and tight PK/PD relationship for PDE10A inhibitors to block NMDA receptor channel blocker-induced hyperlocomotion and conditioned avoidance responding were foundations of the rationale for investigating this class as antipsychotic agents.

PDE10A inhibitors bear similarity to D2 antagonists in several other assays. PDE10A inhibitors ameliorate apomorphine-induced agitation in rats (Megens et al., 2014b) and deficits in extradimensional set shifting caused by subchronic NMDA antagonist administration in rats (Rodefer et al., 2005; Shiraishi et al., 2016). PDE10A inhibitors also block amphetamine-stimulated locomotor activity, although less effectively than for inhibition of NMDA channel blocker-induced activity (Siuciak et al., 2006a; Schmidt et al., 2008). In an empirical assay phenotyping drug-induced behavior in mouse, the SmartCube™, PDE10A inhibitors were identified as producing an antipsychotic-like profile similar to D2 antagonists (Roberds et al., 2011).

In contrast to the findings outlined above, PDE10A inhibitors lack efficacy in some rodent behavioral assays in which D2 antagonists are effective. These notably include induction of catalepsy and reversal of deficits in prepulse inhibition of startle. The available data suggest that the mechanism for the differences is that activation of direct pathway MSNs by PDE10A inhibitors counters the activation of indirect pathway MSNs underlying the behavioral responses.

Catalepsy in rodents is considered an indicator of liability to produce extrapyramidal side effects by agents that suppress psychosis (Hoffman and Donovan, 1995). Dopamine D2 antagonists produce a robust cataleptic response that monotonically increases with dose and time. This effect is attributable to activation of indirect pathway MSNs, which suppresses the behavioral response of stepping down from an elevated bar without impairing motor function (i.e., the ability to step down). In contrast, PDE10A inhibitors produce relatively little catalepsy (Schmidt et al., 2008; Grauer et al., 2009; Suzuki et al., 2015). In our experience, the cataleptic response to PDE10A inhibition was variable with both dose and time as well as with respect to replication with different compounds under nominally identical experimental conditions (Schmidt et al., 2008). Megens et al. (2014b) found that, whereas low doses of PDE10A inhibitors had no or limited propensity to induce catalepsy when administered alone, these compounds had potent and efficacious cataleptic effects when co-administration with a dopamine D1 receptor antagonist. This group also observed that such cataleptic effects were reversed at high doses of PDE10A inhibitors, which also inhibited the cataleptic effects of D2 antagonists. These data are consistent with a hypothesis that the weak and variable cataleptic effects of PDE10A inhibitors are due to direct pathway MSN activation, which counteracts the catalepsy-producing activation of the indirect pathway MSNs.

A similar scenario appears at play with regard to the effects of PDE10A inhibitors on prepulse inhibition of startle (PPI) in rat and mouse. PPI is a translatable experimental measure of sensorimotor gating (Swerdlow et al., 2008). PPI is deficient in schizophrenia as well as in other neuropsychiatric conditions and PPI deficits can be induced in rodents by manipulations of glutamatergic and dopaminergic neurotransmission that are used to model putative neurochemical abnormalities in schizophrenia (Geyer et al., 2001). D2 receptor antagonists ameliorate PPI deficits in rodents. Consequently, similar activity by new pharmacological agents may form part of the rationale for advancing such agents into clinical trials to test for antipsychotic efficacy. However, in the case of PDE10A inhibitors, inhibition of PPI is inconsistent, with some investigators reporting inhibition

(Grauer et al., 2009; Das et al., 2014; Suzuki et al., 2016) and others reporting no activity (Schmidt et al., 2008; Weber et al., 2009; Suzuki et al., 2016), including for the same compound (i.e., TP-10). This discrepant effect of PDE10A inhibitors on PPI appears to stem from the competing activation of direct and indirect pathway MSNs. Gresack et al. (2014) reported that PDE10A inhibitors were effective at reversing PPI deficits induced by a dopamine D2 antagonist, quinpirole, but not by the mixed dopamine agonist apomorphine. However, PDE10A inhibitors were effective against apomorphine-induced deficits if co-administered with a dopamine D1 receptor antagonist. It was concluded that the activation of direct pathway MSNs attenuates effects on PPI that derive primarily from activation of indirect pathway MSNs.

A similar conclusion was proffered by Suzuki et al. in a study comparing the effects of TAK-063 and PF-02545920 on PPI among other assays (Suzuki et al., 2016). The Takeda group found that these two compounds were representative of two subclasses of PDE10A inhibitors. TAK-063 represented a class with relatively faster enzyme dissociation rate than a class represented by PF-02545920. Significantly, the fast-dissociating compounds had a greater impact on indirect pathway activation relative to the direct pathway, whereas the slow dissociating class had a relatively more balanced activation of the two pathways. This difference was manifest as an ability of TAK-063 to ameliorate PPI deficits, whereas PF-02545920 was ineffective.

In summary of the above, the behavioral effects of PDE10A inhibitors in rodents reflects a unique pharmacology. In some part, PDE10A inhibitors bear resemblance to dopamine D2 receptor antagonists. This can be rationalized from the effects of PDE10A inhibitors on signaling in striatal MSNs, specifically, the apparent preferential effect of PDE10A inhibitors for activation of indirect pathway MSNs. Nonetheless, for some behaviors, PDE10A inhibitors lack the efficacy of D2 antagonists. This appears due to the effect of PDE10A inhibitors to activate direct pathway MSNs, which counters indirect pathway activation. This hypothesis is strengthened by the intriguing observations of Suzuki and Kimura of Takeda on differentiation of the PDE10A inhibitors based on enzyme off rate kinetics (Suzuki et al., 2016). These data clearly indicate that the unique behavioral profiles of PDE10A inhibitors reflects the balance of activity on direct and indirect pathway MSNs.

BEHAVIORAL EFFECTS OF PDE10A INHIBITION NON-HUMAN PRIMATES

While rodent studies clearly reveal a unique pharmacology for PDE10A inhibitors that results from activation of both direct and indirect pathway MSNs, it is speculative as to how this pharmacology might translate to effects on human behavior. In this regard, the behavioral repertoire of non-human primates is obviously more comparable to that of humans. A study by Papa and collaborators in rhesus monkeys (Uthayathas et al., 2014) provides significant insight into the consequences PDE10A inhibition may have in humans and how this may differ from the consequences of D2 receptor inhibition.

The behavioral effects of the PDE10A inhibitor MP-10 (PF-02545920) were compared with that of the D2 antagonist risperidone in rhesus monkey. Doses of both compounds were chosen to mimic exposure ranges relevant to use in humans in clinical trials and clinical practice, respectively. Plasma exposures were verified and pharmacodynamic effects in brain were established by PET imaging of [^{18}F]fluorodeoxyglucose uptake. A within subject design was employed in which each of 4 animals received each dose of MP-10 and risperidone on multiple occasions during which behavior was videotaped. Behavioral changes were scored using a standardized motor disability scale for parkinsonian primates and a newly designed "Drug Effects on Nervous System" scale to assess non-motor effects (Uthayathas et al., 2013). Each scale rated and assigned a score to a series of defined behaviors. Essentially, animals under the influence of these drugs underwent a careful neurological examination similar to what might be given in humans.

Overall, behavioral scores were similar for MP-10 and risperidone, at the level of both summary scores and scores on individually rated tests. However, subtle differences were noted that indicate a critical differentiation of the two compounds. The effects of risperidone were tightly dose responsive and reproducible in individual animals upon repeated exposures. In contrast, the effects of MP-10 were more all-or-none and there was notable variability in response of individuals from test session to test session. This variability was not accounted for by variability in exposures. Thus, both compounds produced qualitatively similar effects across a range of behaviors, but with a difference in dose responsiveness. It was hypothesized that the variable and all-or-none response pattern observed with MP-10 may reflect a "tipping point" in the activities of the direct and indirect pathways. Balanced activation of the two pathways with PDE10A inhibition results in little or no behavioral effect. However, tipping the balance toward indirect pathway activation results in a behavioral response similar to the D2 antagonist risperidone. The variability in response to MP-10 within individual animals is interpreted to indicate that this tipping point is relatively "sharp" and subject to subtle environmental and homeostatic influences that vary across nominally identical test sessions. It is also important to note that there were no emergent behavioral effects of MP-10 that might indicate a balance tipped toward direct pathway activation.

A more significant insight comes from the results of two other tests, the Kluver Board Test and Perch Test. In the Kluver Board Test, animals are required to reach into the openings of a plexiglass box with one finger to retrieve a reward. The difficulty of the task on individual trials is manipulated by varying the size of the opening. The numerical scores on the Kluver Board Test were identical for MP-10 and risperidone—at low doses animals made few errors whereas at high doses animals repeatedly failed to retrieve the reward. Significantly, the reason for the failures were different for the two compounds. Whereas, under risperidone the animals attempted to retrieve the reward but lacked the dexterity. In contrast, under MP-10 the animals stopped attempting to retrieve the reward. Results from the Perch Test further reflects this dichotomy. In this test, animals are required to scale a rod with perches to retrieve a reward at

the top of the test enclosure. Under MP-10, animals showed no disability, whereas under risperidone animals lacked the coordination and balance to perform the task. Thus, these data indicate effects of PDE10A inhibition on motivational aspects of primate behavior that differs from that of D2 receptor inhibition, which is more highly related to motor fluency. As stated by Papa and colleagues—"MP-10- treated animals retained the ability to respond but did not engage tasks, whereas risperidone-treated animals retained the motivation to respond but were unable to perform the intended actions."

DISCUSSION

At present, dopamine D2 receptor inhibition is the only well-proven pharmacology to ameliorate psychosis and delusions in patients with schizophrenia. Dopamine D2 receptors are densely expressed by MSNs of the indirect striatal output pathway. As noted earlier, D2 receptors are deployed in other striatal elements, such as on corticostriatal glutamate terminals and are expressed outside of the striatum. Notwithstanding, inhibition of D2 receptors on indirect pathway MSNs is a principal mechanism of their antipsychotic action. D2 receptor signaling in MSNs is complex. The most well-studied is G-protein mediated signaling to suppress adenylyl cyclase activity in response to dopamine. Thus, D2 antagonists disinhibit cAMP signaling in these neurons. D2 antagonists also increase cGMP signaling in MSNs. Augmentation of glutamatergic signaling likely plays a role in this effect although the coupling mechanisms are not understood in detail. In addition, D2 receptors signal through the β-arrestin/AKT/GSK3β kinase cascade, independently of G-protein-coupling. Further complexity derives from the dimerization of D2 receptors with a variety of other 7-transmembrane receptors that likely have unique signaling roles in the MSNs. The cumulative effect of D2 antagonists on these different signaling cascades is to increase the activity of indirect pathway MSNs and thereby bias striatal output toward the indirect pathway over the direct pathway. It is hypothesized that this biased activation of the indirect pathway suppresses the expression of psychotic symptoms. It is this hypothesis that framed the interest in PDE10A inhibitors as novel antipsychotics.

PDE10A is densely expressed by striatal MSNs and PDE10A inhibition increases both cAMP and cGMP signaling in these neurons. While PDE10A is expressed by both direct and indirect pathways MSNs, the net effect of PDE10A inhibition can be interpreted as a preferential activation of indirect pathway MSNs, based on biochemical and electrophysiological data. In this regard, PDE10A inhibitors bear similarity to D2 receptor antagonists and this suggested that PDE10A inhibitors similarly may be antipsychotic. The cap to this hypothesis was the finding that PDE10A inhibitors are highly efficacious for inhibiting conditioned avoidance responding in rodents, a behavioral assay of stimulus salience and an activity thought to be highly predictive of antipsychotic efficacy. The preclinical data with PDE10A inhibitors is summarized in **Table 1**. Nonetheless, PDE10A inhibitors from Pfizer, Takeda, and Lundbeck failed to exhibit robust antipsychotic efficacy in Phase II clinical studies. While the lack of clinical efficacy is disappointing, it affords a new opportunity to gain insights into the nature of antipsychotic drug action. Given the notable similarities in the effects of PDE10A inhibitors and D2 receptor antagonists across a range of experimental paradigms, why are only the latter compounds efficacious for ameliorating psychosis and delusions? As a first step toward answering this question, we re-visit key tenets supporting the rationale for investigating PDE10A inhibitors as antipsychotics and offer some reinterpretations of the supporting preclinical data. We then discuss some of the gaps in our knowledge that bear further investigation.

TABLE 1 | Summary of the effects of PDE10A inhibitors.

Whole striatum	↑cGMP ↑cAMP ↑Phosphorylation of PKA substrates change in expression of multiple genes	
Physiology	*Indirect Pathway* ↑cAMP biosensor ↑PKA biosensor ↑pDARPP-32 ↑Enk mRNA expression ↑MSN excitability (cGMP)	*Direct Pathway* ↑cAMP biosensor ↑pDARPP-32 ↑SP mRNA expression ↑MSN excitability (axonal)
Rodent behavior	↓NMDA LMA ↓CAR ↓Amphetamine LMA SmartCube phenotype	Suppression of catalepsy Suppression of PPI efficacy
	↓Apomorphine-induced agitation Reversal of NMDA-induced EDS deficits	
NHP behavior	Motoric effects *qualitatively* comparable to D2 antagonist, *quantitatively* more variable and less dose-responsive Suppression of motivation	
Human behavior	Somnolence "Conscious sedation" *Failure to suppress acute psychosis*	

↑ and ↓ indicate increases or decreases in levels or activities, respectively.

At the molecular signaling level, the nominal intersection of PDE10A inhibitors and D2 antagonists is that both classes of compounds increase cAMP and cGMP levels in indirect pathway MSNs. However, based on the wealth of data reviewed above, it can be concluded that PDE10A is not directly coupled to D2 receptor cyclic nucleotide signaling. Instead, the pools of cyclic nucleotides impacted by these two pharmacologies overlap but are not synonymous[3]. Thus, in so far as D2 receptor antagonist modulation of cyclic nucleotide signaling is a "first molecular step" toward antipsychotic activity, then PDE10A inhibitors bear similarity to D2 antagonists but do not precisely activate the same signaling pools. Furthermore, D2 antagonists also impact D2 receptor signaling via the β-arrestin/AKT/GSK3β kinase cascade and by D2 receptor heteromers. There is emerging research suggesting that the modulation of these signaling pathways significantly contribute to antipsychotic activity (Del'Guidice et al., 2011; Borroto-Escuela et al., 2016, 2020; Weiwer et al.,

[3]A similar conclusion can be drawn regarding PDE10A and the cyclic nucleotide pools regulated by D1 receptors in direct pathway MSNs.

2018). There are no documented linkages between PDE10A and these other D2 signaling mechanisms and so in this respect PDE10A inhibitors and D2 antagonists may be even further divergent.

The differences in molecular signaling notwithstanding, there is clear evidence from electrophysiological studies that both D2 antagonists and PDE10A inhibitors increase the activation of indirect pathway MSNs. A key tenet with regard to antipsychotic activity is that activation of indirect pathway MSNs is preferential to the activation of direct pathway MSNs. Such indirect pathway bias has a clear basis for D2 antagonists, given that D2 receptors are restricted to MSNs of this pathway. PDE10A inhibitors also have a greater impact on electrophysiological and biochemical measures in indirect pathway MSNs compared to direct pathway counterparts. Nonetheless, biochemical (Nishi et al., 2008; Strick et al., 2010; Polito et al., 2015) and behavioral (Gresack et al., 2014; Megens et al., 2014b) studies indicate that PDE10A inhibitors also impact direct pathway activity. The rodent behavioral studies of Gresack et al. (2014) on pre-pulse inhibition and Megens et al. (2014b) on catalepsy indicate that the direct pathway activation is consequential. Direct and indirect pathway MSNs are not a uniform neuronal population, beyond the well-recognized differences in dopamine signaling and neuropeptide expression. The two MSN populations have intrinsic differences in excitability, attributed to differences in the morphology of their dendritic trees (Gertler et al., 2008). The cortical inputs to the two MSNs populations also differ, arising from different layer 5 pyramidal neurons and the excitatory synapses formed with the respective MSN subtypes are morphologically and functionally distinct (Reiner et al., 2010). In this context, we raise the possibility that the differences in the effects of PDE10A inhibition on direct and indirect pathway MSNs may be more reflective of intrinsic differences in these two neuronal populations rather than a differential impact of PDE10A inhibition *per se*. Stated another way, biochemical and electrophysiological measures used so far may be overestimating the relative impact of PDE10A inhibition on indirect vs. direct pathway MSN activity, which is more balanced at the wholistic level of behavioral integration. This interpretation is subtle but important in that it further contrasts PDE10A inhibitors and D2 receptor antagonists.

The above discussion is germane to the question of whether increasing the bias of PDE10A inhibition toward indirect pathway activation will yield antipsychotic activity. This question is raised by Suzuki et al. (2016) with their findings regarding differences between TAK-063 and PF-02545920 in relative effects on direct and indirect pathway MSNs. The Takeda group found that these compounds were representative of sub-classes of PDE10A inhibitors differentiated based on enzyme dissociation rate. TAK-063, a fast-dissociating compound, had a greater impact on indirect pathway activation and this difference was manifest behaviorally as an ability of TAK-063 to ameliorate PPI deficits where PF-02545920 was ineffective. This difference becomes intriguing in light of the clinical findings in schizophrenia patients experiencing acute exacerbation of symptoms, where TAK-063 evidenced some efficacy on measures of global clinical impressions (Macek et al., 2019) but PF-02545920 did not have such effects (Walling et al., 2019, see above). This finding suggests that greater biasing PDE10A inhibition toward indirect pathway activation is a potential path toward more robust antipsychotic efficacy. Possibly countering this argument is the fact that there was no efficacy of PF-02545920 when administered with D2 antagonists (DeMartinis et al., 2019), a manipulation that would be expected to yield significant indirect pathway bias. However, a caveat is that the patients in the latter study had an inadequate response to D2 antagonists and so may have been refractory or at a ceiling of efficacy, accounting for the lack of augmentation with the addition of the PDE10A inhibitor. Thus, it will be of interest to further explore the therapeutic potential of more indirect pathway-biased PDE10A inhibitors, if such can be developed, or to investigate the combination of a PDE10A inhibitor with a low dose of D2 antagonist in acute exacerbation patients.

The discussion above is focused on comparison of D2 antagonists and PDE10A on molecular aspects of signaling. Orthogonal to this is a comparison based on behavioral effects in preclinical models believed to be predictive of antipsychotic efficacy. Particularly, PDE10A inhibitors are very effective at inhibiting NMDA receptor channel blocker-induced hyperlocomotor activity and at blocking conditioned avoidance responding in rodents, activities shared with D2 receptor antagonists. In humans, NMDA receptor channel blockers cause behavioral effects remarkably similar to those exhibited by humans with schizophrenia (Luby et al., 1959; Krystal et al., 2003; Javitt et al., 2012). Accordingly, the ability of PDE10A inhibitors to effectively block hyperactivity induced by NMDA receptor channel blockers in rodents was significantly supportive for advancing this class into clinical trials as a therapeutic for schizophrenia. However, the mechanisms by which channel blockers are "schizophrenomimetic" in humans (Luby et al., 1959) or induce hyperactivity in rodents are not well-understood, nor is the mechanism by which PDE10A inhibitors, or D2 antagonists, block their effects in rodents beyond the hypothesis that both activate indirect pathway. Thus, at present, there are limited back-translational learnings from the failure of the PDE10A inhibitors to evidence clinical antipsychotic activity, other than that blockade of channel blocker induced hyperactivity is apparently not predictive of therapeutic efficacy.

A more significant finding supporting the investigation of PDE10A inhibitors as antipsychotics was their very effective blockade of conditioned avoidance responding in rodents. Psychotic and delusional symptoms in schizophrenia are hypothesized to arise from aberrant dopamine signaling resulting in mis-attribution of stimulus salience (Kapur et al., 2005; Winton-Brown et al., 2014). Conditioned avoidance responding is a rodent behavioral assay of stimulus salience (Wadenberg, 2010). D2 antagonists are very effective at blocking conditioned avoidance responding and this effect is interpreted to reflect the ability of these compounds to dampen psychosis and delusions in patients with schizophrenia by dampening the aberrant attachment of salience to innocuous sensory cues. Contributing a strong element of predictive validity to the assay, compounds from a number of pharmacological classes that failed to inhibit conditioned avoidance responding in rodents also failed to prove antipsychotic in clinical trials. Thus, the ability of PDE10A

inhibitors to reduce stimulus salience in the rodent assay was one of the strongest considerations driving the clinical development of these compounds as antipsychotics. Nonetheless, PDE10A inhibitors have not been found to be effective antipsychotics. We offer a possible framework for interpreting this lack of translation. Despite the behavioral phenocopy, it is possible that PDE10A inhibitors suppress conditioned avoidance responding by altering a basal ganglia computation that is distinct from that by which D2 receptor antagonists suppress this behavior. This interpretation is prompted by the findings from the primate studies of Papa and colleagues contrasting effects of the D2 receptor antagonist risperidone and the PDE10A inhibitor MP-10 on a Kluver Board reaching task (Uthayathas et al., 2014). Both compounds disrupted performance; however, risperidone appeared to disrupt motor functions necessary to perform the task without an apparent effect on motivation to perform, whereas MP-10 appeared to impact motivation or the reward value of task performance without impacting the motor ability to perform. Regardless of exact overt behavioral constructs, the effects of PDE10A inhibitors and D2 receptor antagonists on basal ganglia computations is evidently different based on the effects on primate behavior, yet this difference nonetheless yields a behavioral phenocopy in rodent measures such as conditioned avoidance responding. The important point is that whatever the effect of PDE10A inhibition on basal ganglia computation, it is not antipsychotic.

The preceding section of the Discussion outlined a number of key differences between PDE10A inhibitors and D2 antagonists. Unfortunately, it is not clear which, if any, of these are responsible for the difference in clinical antipsychotic efficacy. Nonetheless, we hope this part of the review provides some initial triangulation points for investigating the basis for the differential efficacy. Next we outline some gaps in our knowledge regarding the physiology of PDE10A that may further serve in this regard and as also as starting points for developing new therapeutic uses for PDE10A inhibitors.

The fact that PDE10A inhibition impacts both direct and indirect pathway function suggests that the consequences of PDE10A inhibition may more fruitfully be investigated with respect to their effects on integrated outputs of the direct and indirect pathways acting in concert rather than in opposition. Recent analyses of basal ganglia information processing highlight direct and indirect pathway co-activation and co-ordination during behavior integration (Calabresi et al., 2014; Cox and Witten, 2019). In particular, several groups have found the direct and indirect pathways are activated in concert, not in opposition, at the initiation of movement and action selection in mice (Cui et al., 2013; Tecuapetla et al., 2016; London et al., 2018). Given that PDE10A inhibitors activate the direct and indirect pathways in concert, analyses of their effects may be better framed by what is being learned about how the two MSN populations function as a single network to integrate information. In fact, PDE10A inhibitors may provide an important tool to study such integration. However, the behavioral effects of PDE10A inhibitors suppress action selection, not facilitate this activity as may have been predicted if PDE10A inhibitors promote the concurrent activation of the direct and indirect pathways. This puzzle provides a segue to gaps in our knowledge regarding the effects of PDE10A inhibitors on two key aspects of the basal ganglia computational machinery, timing and plasticity.

An essential aspect of information processing by MSNs is the temporal integration of the corticostriatal input with dopamine signaling. Dopamine signaling has both tonic and phasic aspects (Goto et al., 2007). The timing of phasic dopamine signaling is critical to the assignation of reward value to ensembles of cortical inputs to MSNs as well as to the re-activation of the rewarded ensembles for subsequent action selection (Arbuthnott and Wickens, 2007). Given that the canonical function of phosphodiesterases is to regulate the timing and spatial spread of cyclic nucleotide signaling, PDE10A inhibition undoubtably has an effect on the temporal integration of signaling in MSNs. In one study relevant to this point, Yagishita et al. (2014) reported a role for PDE10A in regulating the timing of PKA activation on a sub-second time scale in distal dendrites of MSNs. PDE10A inhibition disrupted this critical timing and thereby degraded the specificity of the information signaled by cortical input in this compartment. Thus, one avenue for translational research is a more in-depth comparison of the effects of PDE10A and D2 receptor inhibition on short-time scale integration of information by striatal MSNs and the consequences to behavior.

At the other extreme of timing, D2 antagonists are administered chronically, and efficacy as currently measured in clinical trials emerges only after weeks of treatment. Furthermore, long term treatment with these agents induce significant long-time scale changes in striatal information processing, with a clear example being the induction of tardive dyskinesias (Jeste and Caligiuri, 1993). PDE10A inhibitors have a profound effect on gene expression in the MSNs (Kleiman et al., 2011). Such effects may be presumed to impact the functionality of these neurons with chronic treatment over long timescales. However, has not been explored for PDE10A inhibitors or for the effects of such compounds in comparison with D2 antagonists. In so far as such long-term effects contribute to the clinical efficacy of D2 antagonists, such studies may yield valuable insight into mechanisms of antipsychotic action.

In the same vein, different forms of synaptic plasticity are also essential to information processing by MSNs (Calabresi et al., 2007; Surmeier et al., 2009; Wickens, 2009; Lovinger, 2010). Given that cyclic nucleotide signaling is a key regulator of this plasticity (Calabresi et al., 2000), it is undoubtable that PDE10A inhibition impacts these processes. However, this aspect of PDE10A physiology and pharmacology has not yet been studied in depth. Elucidating the effect of PDE10A inhibition on the multiple forms of corticostriatal synaptic plasticity would provide a valuable reference point in inferring how PDE10A inhibitors impact information processing by striatal MSNs. Again, comparison of PDE10A and D2 inhibition in this regard may serve as another point of triangulation for understanding the differences in antipsychotic efficacy.

The gaps in our knowledge regarding the effects of PDE10A inhibitors highlighted above focus on molecular mechanisms. However, perhaps the most significant gap in our knowledge regarding PDE10A inhibitors as well as D2 receptor antagonists is a clearer understanding of the effects of such compounds in humans, both in healthy individuals and those suffering from schizophrenia. There is currently no validated method to assess activation of the indirect striatal output pathway in humans. Nonetheless, advances in functional imaging and the determination of regional connectivity are beginning to shed light on the circuitry that may be dysfunctional in schizophrenia (Tarcijonas and Sarpal, 2019). Reduced corticostriatal connectivity has been associated with psychosis and clinical improvement with antipsychotic therapy is associated with improved connectivity between specific cortical regions and the striatum (Sarpal et al., 2015). Although this effect cannot be definitively localized to indirect pathway neurons, as these neurons express the majority of D2 receptors in the striatum, they are likely to be a significant contributor to the imaging signals. If an increase in cortico-striato-pallidal connectivity is a biomarker of the clinical efficacy of D2 antagonists, the lack of clinical efficacy with PF-2545920 and TAK-063 predicts such connectivity will not be improved by these compounds. Alternatively, enhanced cortico-striatal connectivity by these compounds similar to that caused by D2 receptor inhibition would indicate that improved connectivity alone is not sufficient for a therapeutic response or suggest that PDE10A inhibition uniquely produces additional circuitry effects that confound this benefit. Thus, although PDE10A inhibitors will not be a treatment for schizophrenia, they may still be useful clinical tools in understanding the disorder and in the development of new biomarkers of efficacy and medications.

A simple but essential complimentary step to imaging studies such as discussed above is an in-depth clinical evaluation of the *subjective* effects of PDE10A inhibition in humans. Despite the fact that multiple PDE10A inhibitors have been tested in humans, we lack fundamental information on their subjective effects due to the requirements for conducting and blinding Phase I and Phase II clinical studies. This leaves us to infer behavioral consequences, as on "stimulus salience" or "action selection," from animal data. Obviously, our inferences that PDE10A inhibitors may be antipsychotic based on the animal data were wrong. Given that there are a number of PDE10A inhibitors that have proven to be safe and well-tolerated in humans, our strong recommendation is the conduct and publication of studies on the subjective effects of PDE10A inhibition in people. Ideally, this study would include a D2 receptor antagonist as comparator. A model for this analysis may be the study of Papa and colleagues in rhesus monkeys (Uthayathas et al., 2014). This would be a straightforward way to gain insight into the significance of the differential effects of TAK-063 and PF-02545920 on measures of global clinical impressions observed in the Phase II studies. Such a study may also provide valuable insight into the different cognitive domains tapped by these clinical global measures in comparison to the PANSS. This will provide an essential foundation for framing further back-translational behavioral studies and for interpreting the effects of these compounds on behavior at the molecular level, on the way to developing new and better treatments for schizophrenia and related disorders. Finally, such studies may serve as an important step in considering alternative clinical indication for PDE10A inhibitors and to capitalize on the tremendous investment that has been made in the novel pharmacology.

SUMMARY AND CONCLUSION

Why are D2 antagonists antipsychotic? Nearly 70 years after the first clinical use of chlorpromazine we do not have enough of a molecular understanding to design mechanistically new drugs that have similar, let alone better, efficacy. A potentially powerful approach toward gaining such understanding is the back-translational comparison of the effects of D2 antagonists with different pharmacologies that have been tested in the clinic but failed to evidence comparable antipsychotic efficacy. In this regard, we suggest that PDE10A inhibitors may be particularly useful because of the enzyme's very restricted distribution to striatal MSNs and the relatively straightforward effect of inhibitors to increase cyclic nucleotide levels in these neurons. There is already a wealth of published data on the effects of PDE10A inhibitors, reviewed here, that may enable back-translational efforts. Nonetheless, there remain significant gaps, notably on the effects of PDE10A inhibitors in humans, both healthy and suffering psychosis. The pharmaceutical industry has invested tremendously in the development of high quality PDE10A inhibitors. Rather than consider these efforts a "failure," we suggest using these tools to continue to gain insight into the molecular basis for antipsychotic efficacy. Such work will undoubtedly aid in the development of new, more efficacious, safer antipsychotic agents and, indeed, may even provide insight into the nature of psychosis.

AUTHOR CONTRIBUTIONS

All authors contributed to the preparation of this review.

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Conflict of Interest: TC is currently employed by Pfizer Inc., CS is recently retired from Pfizer Inc., and FM was previously employed by Pfizer Inc. All authors while at Pfizer Inc. participated in the discovery and development of PF-0245920, a PDE10A inhibitor mentioned in the manuscript. However, the review article was prepared independently and the authors will accrue no commercial or financial benefit from publication that may be construed as a conflict of interest.

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