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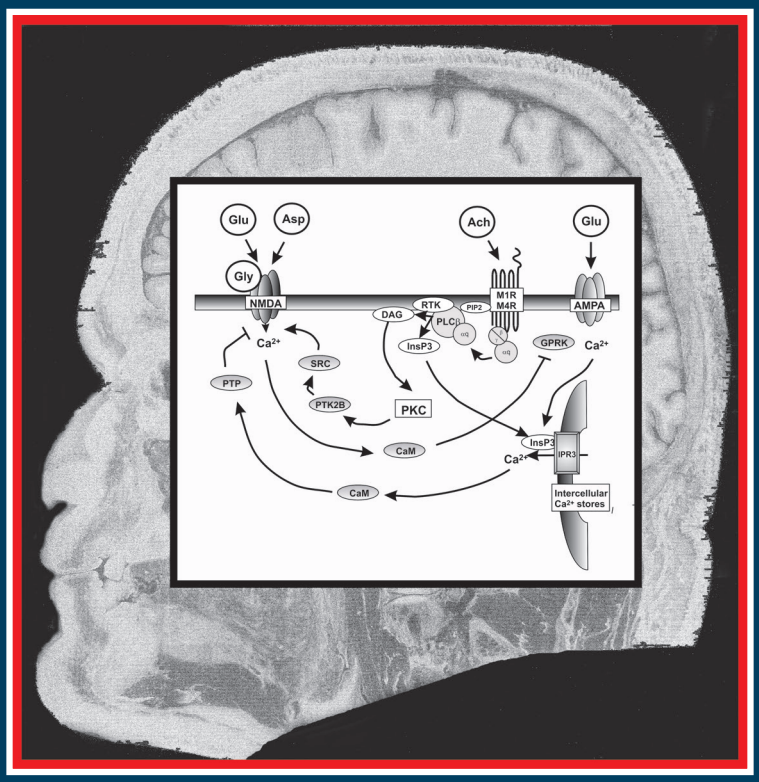

MAPPING THE PATHOPHYSIOLOGY OF SCHIZOPHRENIA: INTERACTIONS BETWEEN MULTIPLE CELLULAR PATHWAYS

Topic Editors
Chao Deng and Brian Dean

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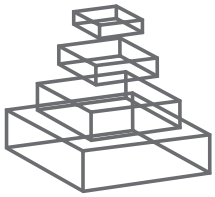

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MAPPING THE PATHOPHYSIOLOGY OF SCHIZOPHRENIA: INTERACTIONS BETWEEN MULTIPLE CELLULAR PATHWAYS

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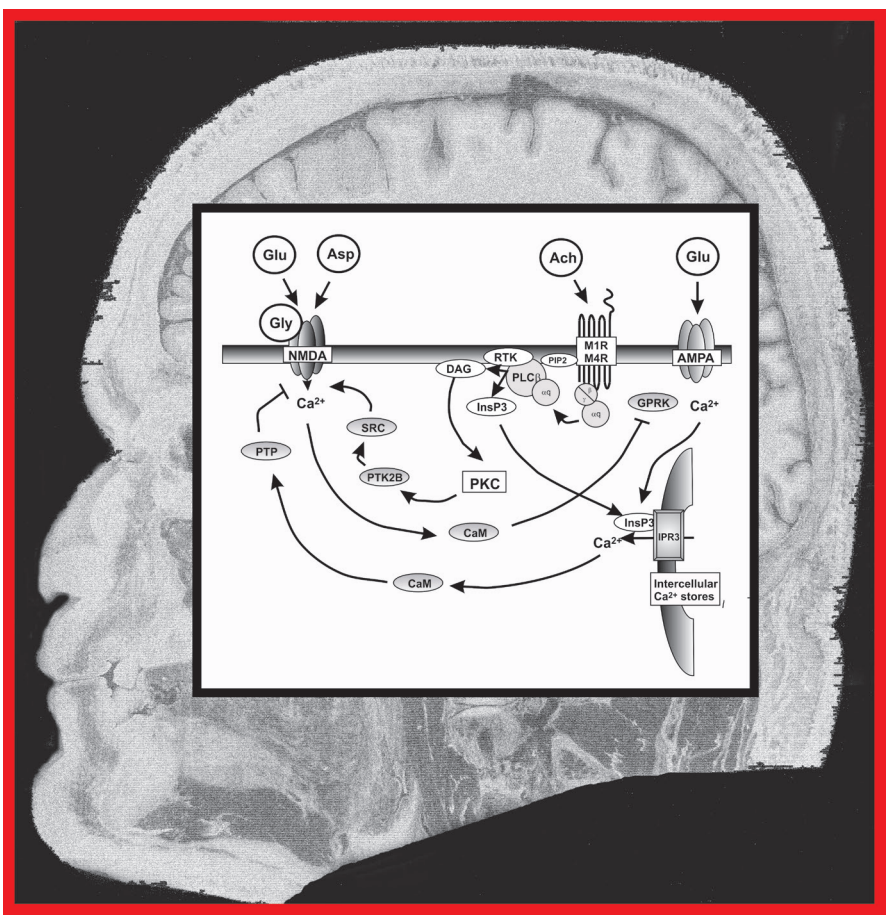

Mapping the pathophysiology of schizophrenia- Illustrated by Brian Dean

Schizophrenia is a complex disorder involving dysregulation of multiple pathways in its pathophysiology. Dopaminergic, glutamatergic, GABAergic and cholinergic neurotransmitter systems are affected in schizophrenia and interactions between these receptors contribute to the pathophysiology of the disease. Inflammation has also been found to play a major role in the development and exacerbation of psychotic symptoms in schizophrenia. Additionally, evidence from genetic, post-mortem and animal studies over the past decade has identified a number of susceptibility factors for schizophrenia, including neuregulin 1 (Nrg1) and its receptor ErbB4, disrupted-in-schizophrenia-1 (DISK1), catechol-O-methyl tranferase (COMT), BDNF, and Akt. These factors and related pathways interact closely with dopaminergic, glutamatergic and GABAergic neurotransmitter systems. A key question is how do these interactions contribute to the pathophysiology of schizophrenia? More specifically, how do these components interact during early brain development based on the view of schizophrenia as a developmental disorder? Therefore, this Research Topic aims to map the pathophysiology of schizophrenia by illuminating the interactive nature of specific pathways on different levels of the brain from cellular pathways and neural circuits to functional deficits.

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EDITORIAL
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Mapping the pathophysiology of schizophrenia: interactions between multiple cellular pathways

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Keywords: schizophrenia, dopamine, GABA, acetylcholine, neurodevelopment, glutamate, neuregulin 1, ErbB4

Schizophrenia is a complex disorder involving dysregulation of multiple pathways in its pathophysiology with strong evidence to support roles for dopaminergic, glutamatergic, GABAergic, and cholinergic neurotransmitter systems and their interactions in the pathophysiology of the disorder (Benes, 2009; Karam et al., 2010; Gibbons et al., 2013). Additionally, evidence from genetic, post-mortem and animal studies over the past decade has identified a number of susceptibility factors for schizophrenia, including neuregulin 1 (Nrg1) and its receptor ErbB4, disrupted-in-schizophrenia-1 (DISK1), catechol-O-methyl transferase (COMT), BDNF, and Akt, along with their related pathways, that interact closely with dopaminergic, glutamatergic, and GABAergic neurotransmitter systems (Karam et al., 2010). Hence a key question is how these neurotransmitter systems and their interactions contribute to the pathophysiology of schizophrenia and whether interactive changes in these pathways occur in early brain development, based on the view of schizophrenia as a developmental disorder?

This Frontier Research Topic has brought together leading experts in the field to address these questions from different angles in nine reviews, one theoretic article and two research articles. The first four articles focus on the roles and interactions of neurotransmitters in the pathophysiology of psychiatric disorders. Snyder and Gao (2013) provide an excellent review of NMDA receptor hypofunction hypothesis, suggesting NMDA receptor hypofunction as a convergence point for progression and symptoms of schizophrenia. They also discuss evidence on altered NMDA receptor subunits in schizophrenia and how these alterations interact with multiple schizophrenia susceptibility genes that lead to NMDA receptor dysfunction during development (Snyder and Gao, 2013). Scarr et al. (2013) present an in depth and very detailed coverage of cholinergic involvement in schizophrenia and how it interacts with other neurotransmitters including glutamate, dopamine, GABA and serotonin, as well as its links with the inflammatory/immune system. The review also provides a frame work for testable hypotheses of the potential outcomes of a dysregulated cholinergic system for research into the pathophysiologies of psychiatric disorders (Scarr et al., 2013). Cognitive deficits are considered core symptoms of schizophrenia, while abnormalities in gamma oscillations have been identified in schizophrenia patients that are associated with deficits in attention, working memory, and other cognitive functions (Uhlhaas and Singer, 2010). Furth et al. (2013) reviewed the central role of dopamine D4 receptor in the generation of gamma frequency synchronization of neural networks and cognitive processes via their influence on parvalbumin-expressing GABAergic interneurons. They also examined their close synergistic relationship with neuregulin/ErbB4 signaling, in particular in the prefrontal cortex and hippocampus two major brain regions implicated in schizophrenia (Furth et al., 2013). Furthermore, there has been increasing evidence linking oxidative stress to the pathophysiology of schizophrenia. In their article, Yao and his team reviewed some of these findings, focusing particularly on their findings on homeostatic imbalance of purine catabolism and its association with monoamine neurotransmitters in first episode antipsychotic-naïve patients with schizophrenia (Yao et al., 2013).

In the following three papers, Chana et al. have provided an excellent review of the current progress in the search for biomarkers for schizophrenia and psychosis, focusing on biomarkers for major neurotransmitter systems in post-mortem brain studies, as well as covering some recent and exciting studies in microRNA dysregulation in both the blood and brain of schizophrenia patients (Chana et al., 2013). It is followed by a very timely review of current knowledge on the features of polysialic acid and their synthesizing enzymes (specially ST8SIA2), their functions in regulations of cell adhesion, ion channels, neurotrophins (such as BDNF) and catecholamine neurotransmitters (particularly dopamine), in light of several recent lines of evidence linking polysialic acid to schizophrenia (Sato and Kitajima, 2013). Iwakura and Nawa provide a very clear overview of the ErbB1-4 dependent EGF/neuregulin signals, their role in regulating the development and function of the central nervous system, and the contribution of deficits in ErbB signaling to schizophrenia and neurological disease (Iwakura and Nawa, 2013).

Four articles discuss the animal model of schizophrenia, two of which address the *Nrg1*-cannabinoid interaction in a hypomorphic *Nrg1* (*Nrg1* HET) mouse model of schizophrenia. In an original study, Spencer et al. investigated *Nrg1*-cannabinoid interaction in the hippocampus using proteomics, in which they identified alterations of some proteins involved in vesicular release of neurotransmitters, serotonergic neurotransmission, and growth factor release in response to *Nrg1* hypomorphism and

Nrg1-cannabinoid interaction (Spencer et al., 2013). In a short review, Karl and Arnold further discussed the complex neurobehavioral effects of *Nrg1*-cannabinoid interaction and its clinical implications (Karl and Arnold, 2013). In a BDNF heterozygous mouse model, Manning and van den Buuse investigated the effects of chronic methamphetamine treatment during late adolescence/early adulthood on a behavioral endophenotype related to the positive symptoms of schizophrenia, prepulse inhibition (PPI) of the acoustic startle reflex (Manning and van den Buuse, 2013). In the fourth animal model paper, altered dopamine ontogeny in the developmentally vitamin D deficient rat model and its relevance to schizophrenia were reviewed (Kesby et al., 2013). This review suggests that early alterations in dopamine ontogeny are a core feature in the pathophysiology of schizophrenia representing a critical aspect useful to a model of this disease.

Finally, in the context of schizophrenia as a neurodevelopmental disorder, Catts et al. (2013) discuss elegantly the normal development of the prefrontal cortex on the molecular and cellular levels in line with cognitive development, as well as the timing of cognitive decline in schizophrenia. They proposed to reconsider schizophrenia as an outcome from a failure to reach the final state of cortical maturation resulting in retainment of an immature cortex rather than resulting from an excess of adolescent synaptic pruning (Catts et al., 2013).

In summary, these studies illustrate clearly the interactive nature of specific pathways on different levels of the brain from molecular and cellular pathways, and neural circuits to functional deficits contributing to the pathophysiology of schizophrenia. We believe that this Frontier Research Topic will stimulate the development of future collaborative and interdisciplinary research to reveal the unknown mechanisms underlying the pathophysiology of schizophrenia.

REFERENCES

Benes, F. M. (2009). Neural circuitry models of schizophrenia: is it dopamine, GABA, glutamate, or something else. *Biol. Psychiatry* 65, 1003–1005. doi: 10.1016/j.biopsych.2009.04.006

Catts, V. S., Fung, S. J., Long, L. E., Joshi, D., Vercammen, A., Allen, K. M., et al. (2013). Rethinking schizophrenia in the context of normal neurodevelopment. *Front. Cell. Neurosci.* 7:60. doi: 10.3389/fncel.2013.00060

Chana, G., Bousman, C. A., Money, T. T., Gibbons, A., Gillett, P., Dean, B., et al. (2013). Biomarker investigations related to pathophysiological pathways in schizophrenia and psychosis. *Front. Cell. Neurosci.* 7:95. doi: 10.3389/fncel.2013.00095

Furth, K. E., Mastwal, S., Wang, K. H., Buonanno, A., and Vullhorst, D. (2013). Dopamine, cognitive function, and gamma oscillations: role of D4 receptors. *Front. Cell. Neurosci.* 7:102. doi: 10.3389/fncel.2013.00102

Gibbons, A. S., Scarr, E., Boer, S., Money, T., Jeon, W.-J., Felder, C., et al. (2013). Widespread decreases in cortical muscarinic receptors in a subset of people with schizophrenia. *Int. J. Neuropsychopharmacol.* 16, 37–46. doi: 10.1017/S1461145712000028

Iwakura, Y., and Nawa, H. (2013). ErbB1-4-dependent EGF/neuregulin signals and their cross talk in the central nervous system: pathological implications in schizophrenia and Parkinson's disease. *Front. Cell. Neurosci.* 7:4. doi: 10.3389/fncel.2013.00004

Karam, C. S., Ballon, J. S., Bivens, N. M., Freyberg, Z., Girgis, R. R., Lizardi-Ortiz, J. E., et al. (2010). Signaling pathways in schizophrenia: emerging targets and therapeutic strategies. *Trends Pharmacol. Sci.* 31, 381–390. doi: 10.1016/j.tips.2010.05.004

Karl, T., and Arnold, J. C. (2013). What does a mouse tell us about neuregulin 1—cannabis interactions. *Front. Cell. Neurosci.* 7:18. doi: 10.3389/fncel.2013.00018

Kesby, J. P., Cui, X., Burne, T. H. J., and Eyles, D. W. (2013). Altered dopamine ontogeny in the developmentally vitamin D deficient rat and its relevance to schizophrenia. *Front. Cell. Neurosci.* 7:111. doi: 10.3389/fncel.2013.00111

Manning, E. E., and van den Buuse, M. (2013). BDNF deficiency and young-adult methamphetamine induce sex-specific effects on prepulse inhibition regulation. *Front. Cell. Neurosci.* 7:92. doi: 10.3389/fncel.2013.00092

Sato, C., and Kitajima, K. (2013). Impact of structural aberrancy of polysialic acid and its synthetic enzyme ST8SIA2 in schizophrenia. *Front. Cell. Neurosci.* 7:61. doi: 10.3389/fncel.2013.00061

Scarr, E., Gibbons, A. S., Neo, J., Udawela, M., and Dean, B. (2013). Cholinergic connectivity: it's implications for psychiatric disorders. *Front. Cell. Neurosci.* 7:55. doi: 10.3389/fncel.2013.00055

Snyder, M. A., and Gao, W.-J. (2013). NMDA hypofunction as a convergence point for progression and symptoms of schizophrenia. *Front. Cell. Neurosci.* 7:31. doi: 10.3389/fncel.2013.00031

Spencer, J. R., Darbyshire, K. M. E., Boucher, A. A., Kashem, M. A., Long, L. E., McGregor, I. S., et al. (2013). Novel molecular changes induced by Nrg1 hypomorphism and Nrg1-cannabinoid interaction in adolescence: a hippocampal proteomic study in mice. *Front. Cell. Neurosci.* 7:15. doi: 10.3389/fncel.2013.00015

Uhlhaas, P. J., and Singer, W. (2010). Abnormal neural oscillations and synchrony in schizophrenia. *Nat. Rev. Neurosci.* 11, 100–113. doi: 10.1038/nrn2774

Yao, J. K., Dougherty, G. G., Reddy, R. D., Matson, W. R., Kaddurah-Daouk, R., and Keshavan, M. S. (2013). Associations between purine metabolites and monoamine neurotransmitters in first-episode psychosis. *Front. Cell. Neurosci.* 7:90. doi: 10.3389/fncel.2013.00090

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NMDA hypofunction as a convergence point for progression and symptoms of schizophrenia

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Schizophrenia is a disabling mental illness that is now recognized as a neurodevelopmental disorder. It is likely that genetic risk factors interact with environmental perturbations to affect normal brain development and that this altered trajectory results in a combination of positive, negative, and cognitive symptoms. Although the exact pathophysiology of schizophrenia is unknown, the N-methyl-D-aspartate receptor (NMDAR), a major glutamate receptor subtype, has received great attention. Proper expression and regulation of NMDARs in the brain is critical for learning and memory processes as well as cortical plasticity and maturation. Evidence from both animal models and human studies implicates a dysfunction of NMDARs both in disease progression and symptoms of schizophrenia. Furthermore, mutations in many of the known genetic risk factors for schizophrenia suggest that NMDAR hypofunction is a convergence point for schizophrenia. In this review, we discuss how disrupted NMDAR function leads to altered neurodevelopment that may contribute to the progression and development of symptoms for schizophrenia, particularly cognitive deficits. We review the shared signaling pathways among the schizophrenia susceptibility genes DISC1, neuregulin1, and dysbindin, focusing on the AKT/GSK3β pathway, and how their mutations and interactions can lead to NMDAR dysfunction during development. Additionally, we explore what open questions remain and suggest where schizophrenia research needs to move in order to provide mechanistic insight into the cause of NMDAR dysfunction, as well as generate possible new avenues for therapeutic intervention.

Keywords: gene, NMDA receptors, psychiatric disorders, neurodevelopment, schizophrenia

INTRODUCTION

Schizophrenia is a devastating psychological disorder that consists of a complex set of positive, negative, and cognitive symptoms. Although the pathophysiological mechanisms associated with this disease remain unclear, the dopamine (DA) hypothesis has dominated the theories of schizophrenia for several decades (Howes and Kapur, 2009; Abi-Dargham, 2012). It was proposed that hyperactivity in the mesolimbic DA pathway is the mediator of positive symptoms of schizophrenia, whereas hypoactivity in the mesocortical DA pathway mediates the negative and cognitive symptoms of schizophrenia. However, focusing on the DA system has led to limited progress in understanding the pathophysiological processes in schizophrenia, and subsequently has led to minimal development of novel therapeutics (Miyamoto et al., 2012). In the past two decades, hypotheses of schizophrenia have progressed beyond the DA hypothesis. In a major paradigm shift on the etiology of schizophrenia, it has been proposed that numerous genetic and environmental risk factors converge on the N-methyl-D-aspartate receptors (NMDAR)-mediated glutamatergic system and result in NMDAR hypofunction in the limbic system during neurodevelopment.

NMDARs are widely thought to be crucial in synaptic plasticity and circuit formation for pre- and early postnatal stages of brain development, otherwise known as the "critical developmental window." Numerous studies have indicated that the maturation of brain circuitry is usually coincident with the NMDAR subunit switch (e.g., NR2B-to-NR2A and NR3A-to-NR3B) that occurs at the onset of the critical period of development (Monyer et al., 1994; Sheng et al., 1994; Quinlan et al., 1999; Wang et al., 2008; Roberts et al., 2009; Wang and Gao, 2009; Snyder et al., 2013). The NMDAR subunit shift therefore marks the transition from juvenile to "adult" neural processing (Dumas, 2005; Henson et al., 2010) and the subunit switch makes the NMDARs extremely vulnerable to genetic and environmental risk factors (Spear, 2000). Because NMDARs regulate DA neurons and DA transmission,

Abbreviations: Akt, also known as Protein Kinase B (PKB), is a serine/threonine-specific protein kinase; AMPAR, α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptor; BDNF, brain derived neurotrophic factor; cAMP, cyclic adenosine monophosphate; CaMKII, Ca2+/calmodulin dependent protein kinase II; cdk5, cyclin-dependent kinase 5; CK2, casein kinase 2; COMT, catechol-o-methyltransferase; DA, dopamine; Dysbindin, also known as dystrobrevin-binding protein 1; DISC1, disrupted in schizophrenia-1; DAOA, D-amino acid oxidase activator; HDAC, histone deacetylase; DNMT1, DNA-methyltransferase 1; ERK, extracellular-signal-regulated kinase; GABA, gamma-aminobutyric acid; GAD65, glutamic acid decarboxylase 65; GAD67, glutamic acid decarboxylase 67; GSK-3β, glycogen synthase kinase 3β; LTP, long-term potentiation; MAGUK, membrane-associated guanylate kinase; mGluR, metabotropic glutamate receptor; MK801, dizocilpine; NMDAR, N-methyl-D-aspartate receptor; NRG1, neuregulin 1; PCP, phencyclidine; PKA, protein kinase A; PKC, protein kinase C; PLC, phospholipase C; PSD95, post synaptic density protein 95; SAP102, synapse associated protein 102; SFK, Src family of kinases; PDE4B, cAMP-specific phosphodiesterase 4B; SR, serine racemase; vGluT, vesicular glutamate transporter.

hypofunction of NMDARs may be responsible for the abnormal DA activity associated with the symptoms of schizophrenia. Indeed, the NMDAR-mediated glutamatergic model provides an alternate approach for conceptualizing the brain abnormalities associated with schizophrenia (Harrison and Weinberger, 2005; Lewis and Moghaddam, 2006; Lisman et al., 2008). Although it remains unclear what changes induce the onset of cognitive dysfunction, NMDAR dysfunction appears to be a convergence point for progression and symptoms of schizophrenia, especially for cognitive deficits. There have been several elegant review articles; some issues on a specific topic, such as neuregulin1, circuit-level glutamatergic hypothesis and metabotropic glutamate receptors, can be found in these references (Moghaddam, 2003; Coyle, 2006; Lisman et al., 2008; Banerjee et al., 2010; Marek et al., 2010; Niswender and Conn, 2010; Geddes et al., 2011; Lin et al., 2012; Millan et al., 2012; Vinson and Conn, 2012). Below we focus on the current literature and explain how the hypothesis of NMDA hypofunction is formulated, why NMDA hypofunction could be a convergence point for the progression and symptoms of schizophrenia, what mechanisms are associated with regulation of NMDAR function, as well as possible signaling pathways related to the regulation of NMDAR function by high-risk genes for schizophrenia. It is likely that convergent mechanisms target NMDAR, which in turn contribute to negative symptoms and neurocognitive dysfunction directly (Lau and Zukin, 2007), as well as to positive symptoms via dysregulation of brain DA systems indirectly (Howes and Kapur, 2009; Abi-Dargham, 2012).

EVIDENCE FOR ABNORMAL GLUTAMATE TRANSMISSION AND NMDAR HYPOFUNCTION IN SCHIZOPHRENIA

In the past two decades, the abnormalities found in human subjects with schizophrenia and the various animal models for schizophrenia all point to an important contribution of the glutamatergic system to the disease (Moghaddam and Jackson, 2003; Javitt, 2004; Millan, 2005). Accumulating studies have shown that aberrant NMDAR function, namely NMDAR hypofunction, in the limbic brain region, may underlie many aspects of molecular, cellular, and behavioral abnormalities associated with schizophrenia (Mohn et al., 1999; Olney et al., 1999; Tamminga, 1999; Dracheva et al., 2001; Krystal et al., 2002; Moghaddam and Jackson, 2003; Javitt, 2004; Coyle, 2006). First, mice with reduced NMDAR expression display behaviors related to schizophrenia (Mohn et al., 1999). Second, NMDAR antagonists, such as phencyclidine (PCP), dizocilpine (MK-801), and ketamine, produce "schizophrenia like" symptoms in healthy individuals (Javitt and Zukin, 1991; Krystal et al., 1994; Lahti et al., 1995). Compelling evidence has suggested that the NMDAR antagonist PCP and its analog compounds can produce a pattern of metabolic, neurochemical, and behavioral changes that reproduce almost exactly those seen in patients with schizophrenia, with remarkable regional specificity (Morris et al., 2005). This finding has provided considerable insight into the processes that lead to the development of the disease, emphasizing the potential importance of NMDAR hypofunction. Third, a majority of the genes that are associated with an increased risk for schizophrenia can influence the function of NMDARs or related receptor-interacting proteins and signal transduction pathways (Moghaddam, 2003; Harrison and Weinberger, 2005) (see below for detail). Fourth, dysregulated NMDAR subunits are usually seen in postmortem tissue from patients with schizophrenia (Akbarian et al., 1996; Gao et al., 2000; Kristiansen et al., 2007; Geddes et al., 2011; Weickert et al., 2012) and in animal models of NMDAR antagonism (Lisman et al., 2008; Gunduz-Bruce, 2009). Postmortem studies also show changes in glutamate receptor binding, transcription, and subunit protein expression in the prefrontal cortex (Akbarian et al., 1996; Kristiansen et al., 2006; Beneyto and Meador-Woodruff, 2008), thalamus (Ibrahim et al., 2000; Clinton and Meador-Woodruff, 2004; Clinton et al., 2006; Dracheva et al., 2008), and hippocampus (Gao et al., 2000; Beneyto et al., 2007; McCullumsmith et al., 2007) of subjects with schizophrenia (Geddes et al., 2011). These changes include decreased NR1, increased excitatory amino-acid transporter, and altered NMDA receptor-affiliated intracellular proteins such as post synaptic density protein 95 (PSD95) and synapse associated protein 102 (SAP102) in the prefrontal cortex and thalamus [see (Geddes et al., 2011) Table 1 for detail]. Fifth, glutamatergic neurons also interact with other neurons that have been strongly implicated in the pathophysiology of schizophrenia, including morphologically altered GABAergic interneurons (Lewis et al., 2005) and antipsychotic drug-targeted DA neurons (Howes and Kapur, 2009; Abi-Dargham, 2012; Grace, 2012).

On the basis of these observations, it has been postulated that the glutamatergic disturbances may involve hypofunctioning of NMDARs on gamma-aminobutyric acid (GABA) interneurons in the limbic circuit (Olney and Farber, 1995; Olney et al., 1999; Lindsley et al., 2006; Lisman et al., 2008). How might this be achieved? Activity in the corticolimbothalamic circuit is strongly regulated by local GABAergic interneurons, especially basket and chandelier cells. Output from the cortical pyramidal neurons is suppressed and coordinated by GABAergic interneurons. These cells are activated by recurrent collaterals from the pyramidal neurons and exert a powerful feedback inhibitory action on pyramidal cells via synapses onto the soma and axon hillock (**Figure 1**). Both basket and chandelier cells are particularly important for restraining excessive pyramidal neuron activity, the impairment of these cells leads to dramatic disinhibition of the pyramidal neuron efferent activity and elevated uncoordinated firing throughout the corticolimbic circuit. Considering the dysfunction of NMDAR subunits in patients with schizophrenia (Akbarian et al., 1996; Eastwood et al., 1997; Goff and Wine, 1997; Grimwood et al., 1999; Gao et al., 2000; Clinton et al., 2003; Clinton and Meador-Woodruff, 2004; Weickert et al., 2012), it has been speculated that NMDAR subunits distributed on interneurons may be responsible for NMDAR hypofunction (Nakazawa et al., 2012). The central pathological characteristics seem to be caused by NMDAR hypofunction acting on GABAergic interneurons, followed by the disinhibition of glutamatergic transmission and an overstimulation of non-NMDARs on pyramidal neurons (**Figure 1**) (Olney and Farber, 1995; Olney et al., 1999; Lindsley et al., 2006; Lisman et al., 2008). The postulated existence of disinhibited glutamatergic transmission and the subsequent cascade of excitotoxic events resulting from NMDAR hypofunction, degeneration of GABAergic interneurons, or a combination of both, have suggested diverse experimental therapeutic interventions for schizophrenia, such as facilitation of NMDA receptor-mediated

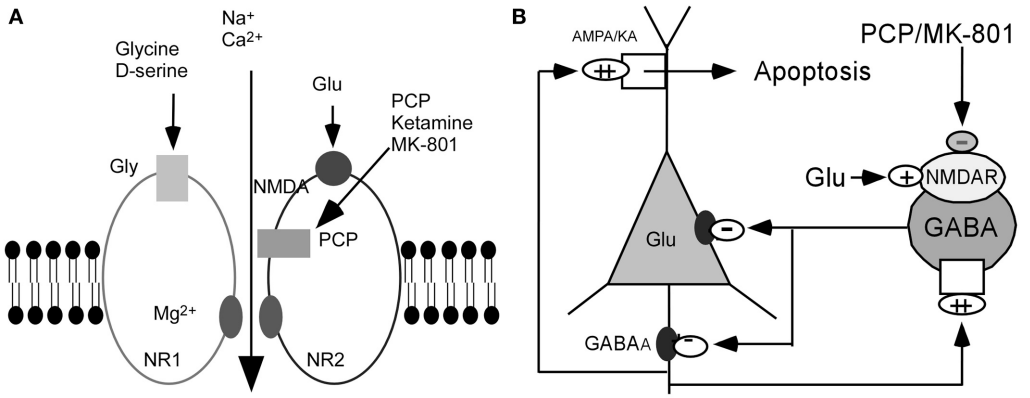

FIGURE 1 | Hypothesis of NMDAR hypofunction. (A) Schematic diagram of NMDAR complex. **(B)** NMDAR hypoactivity and glutamate neurotoxicity. PCP/MK801 ⇒ NMDAR hypofunction on GABAergic neurons ⇒ disinhibition of pyramidal neurons ⇒ more glutamate release ⇒ AMPA/KA receptors excessively stimulated ⇒ excitotoxic damage [**Figure 1B** was modified from (Olney et al., 1999)].

neurotransmission and potentiation of GABAergic inhibition (Coyle and Tsai, 2004; Javitt, 2004). Recently, a heuristic model for the pathophysiology of schizophrenia that attempts to reconcile the neuropathological and neurocognitive features of the disorder has been proposed (Lisman et al., 2008).

When does the hypofunction of NMDAR occur and what are the mechanisms involved? Specifically, it is crucial to understand which neurons express altered glutamate receptor subtypes, whether these neurons are inhibitory or excitatory, and how the circuitries are affected. It is possible that the hypofunction of the NMDAR on GABAergic interneurons disrupts the functional integrity of the corticolimbic circuit, causing cognitive impairments and negative symptoms. Based on this hypothesis, it is reasonable to speculate that the NMDARs on frontal cortical and limbic GABAergic interneurons are most sensitive to these antagonists and therefore may be an important site of pathology resulting in NMDAR dysfunction. To address these possibilities, we have examined the developmental changes and functions of NMDARs in identified prefrontal neurons. Interestingly, we found that the development of NR2 subunits in pyramidal neurons and GABAergic interneurons of rat prefrontal cortex is cell type-specific (Wang et al., 2008; Wang and Gao, 2009). NR2B levels remain high until adulthood, without significant NR2B-to-NR2A subunit switch, in layer 5 pyramidal neurons in the prefrontal cortex (Wang et al., 2008); however, they are gradually replaced by NR2A subunits in fast-spiking interneurons (Wang and Gao, 2009). Particularly, fast-spiking interneurons in the prefrontal cortex undergo dramatic changes in glutamatergic receptors during the adolescent period (Wang and Gao, 2009, 2010) and consequently, a cell type-specific change of NMDAR subunits in parvalbumin-positive interneurons is clearly evidenced (Xi et al., 2009). These findings strongly suggested that fast-spiking or parvalbumin-positive interneurons are more sensitive to pharmacological or environmental stimulation. Indeed, we found that MK-801 induces distinct changes of AMPA and NMDARs in the fast-spiking interneurons and pyramidal cells in adolescent rat prefrontal cortex (Wang and Gao, 2012). Furthermore, when the NR1 subunit was selectively eliminated in parvalbumin-positive interneurons in forebrain cortices and hippocampus in early (neonatal) development, the rats exhibited reduced glutamic acid decarboxylase 67 (GAD67) and parvalbumin as well as distinct schizophrenia-related symptoms that emerged after adolescence; in contrast, post-adolescent deletion of NR1 did not result in such abnormalities (Belforte et al., 2010). These basic studies in NMDAR development in the prefrontal cortex have been extremely useful in the formulation of an NMDAR hypofunction hypothesis. The high vulnerability of corticolimbic fast-spiking interneurons to genetic predispositions and early environmental insults such as excitotoxicity and oxidative stress could help to better explain their significant contribution to the development of schizophrenia (Nakazawa et al., 2012). Given that both DA and GABA systems are indeed the targets of NMDAR disruption, it is plausible to propose that dysfunction of NMDARs in the DA neurons and GABAergic cells induce DA hyperactivity or GABA downregulation, which in turn results in psychosis.

Still, this does not completely explain the pathophysiology of schizophrenia, as there is evidence of NMDAR dysfunction in other key brain areas, especially during development. In addition to the prefrontal cortex, the hippocampus is a brain region that is consistently implicated in schizophrenia (Bogerts et al., 1990; Medoff et al., 2001; Harrison, 2004; Witthaus et al., 2009). In hippocampus, like other cortical regions, proper NMDAR subunit expression and function is necessary for hippocampal development, with NMDAR misregulation affecting synaptogenesis and circuit maturation (Roberts et al., 2009; Brigman et al., 2010; Gambrill and Barria, 2011; John Gray et al., 2011). Therefore, misregulation of NMDAR subunit composition and function during hippocampal development may contribute to the pathogenesis in schizophrenia. Indeed, we recently found in the MAM neurodevelopmental schizophrenia model, that NMDAR function is disrupted in CA1 pyramidal neurons early in hippocampal development (Snyder et al., 2013). Understanding when and how NMDAR function is disrupted in regards to schizophrenia progression is a key area of research.

SCHIZOPHRENIA IS A NEURODEVELOPMENTAL DISORDER WITH MULTIPLE SUSCEPTIBILITY GENES CONVERGING ON NMDARs

It is increasingly recognized that schizophrenia is a neurodevelopmental disorder that involves disrupted alterations in brain circuits (Weinberger, 1987; Lewis and Gonzalez-Burgos, 2008; Jaaro-Peled et al., 2009). Although psychosis usually emerges in late adolescence or early adulthood, we still do not understand all of the changes in normal or abnormal development prior to and during this period. It is particularly unclear what factors alter the excitatory-inhibitory synaptic balance in the juvenile brain and what changes induce the onset of cognitive dysfunction. Current studies suggest that problems related to schizophrenia are evident much earlier than the juvenile stage of development. The emerging picture from genetic and epigenetic studies indicates that early brain development is affected. However, after many years of intensive investigations, no single gene has been found to be responsible for schizophrenia. Although recent findings have generated great interest in the copy number variations of genes in schizophrenia patients, they are rare and are unlikely to account for the majority of cases of the disorder (Allen et al., 2008; O'Donovan et al., 2008; Stefansson et al., 2008). Rather, a number of high-risk genes have been identified as increasing susceptibility for schizophrenia (Allen et al., 2008), including the catechol-o-methyltransferase gene (COMT) (Weinberger et al., 2001; Bilder et al., 2004; Cannon, 2005; Harrison and Weinberger, 2005; Savitz et al., 2006; Tunbridge et al., 2006; Tan et al., 2009), neuregulin 1 (NRG1) (Roy et al., 2007; Mei and Xiong, 2008; Kato et al., 2011), disrupted in schizophrenia-1 (DISC-1) (Lipina et al., 2010; Niwa et al., 2010), and dystrobrevin-binding protein 1 (dysbindin) (Iizuka et al., 2007; Ji et al., 2009; Papaleo and Weinberger, 2011; Papaleo et al., 2012), among others. Many of these genetic variants associated with schizophrenia are involved with neurodevelopment that is related to the glutamatergic system in the brain (Hahn et al., 2006; Allen et al., 2008; Shi et al., 2008; Papaleo et al., 2012).

Recent studies indicate that single genes may not be sufficient to cause schizophrenia. Instead, multiple "susceptibility" genes could possibly work together to trigger disease onset with each susceptibility gene coding for a subtle molecular abnormality in transmitter receptors, enzymes, protein kinases, transcription, and translation (Harrison and Weinberger, 2005). These subtle changes could disrupt neurodevelopment, intracellular signaling pathways and neurotransmission, consequently resulting in disturbed information processing in brain circuits that mediate the symptoms of schizophrenia. It is therefore not surprising that many of the susceptibility genes for schizophrenia regulate not only neuronal proliferation, neuronal migration, and synaptogenesis during early development, but also have functions linked to glutamate neurotransmission, especially the NMDA receptor, in postnatal development (Straub and Weinberger, 2006; Karam et al., 2010).

Numerous susceptibility genes have been shown to be able to regulate various elements of NMDAR mediated signaling. Dysbindin, neuregulin, and DISC1 all function to affect NMDAR function through a variety of mechanisms. Both dysbindin and neuregulin regulate the formation and function of the postsynaptic density (PSD), a set of proteins that interacts with the postsynaptic membrane to provide structural and functional regulatory elements for neurotransmission and for NMDARs (Numakawa et al., 2004; Hahn et al., 2006). Neuregulin also activates an Erb signaling system that is co-localized with NMDARs (Hahn et al., 2006). This Erb signaling system is a member of the receptor tyrosine kinase and neurotrophin signal transduction system, interacts with PSD, and is involved in neuroplasticity mediated by NMDARs (Huang et al., 2000). Furthermore, neuregulin has been shown to alter NMDAR expression (Ozaki et al., 1997; Li et al., 2007; Mei and Xiong, 2008; Banerjee et al., 2010) [see (Geddes et al., 2011) for detail]. Preventing NRG1/ErbB4 signaling leads to loss of NMDA synaptic currents and dendritic spines (Li et al., 2007). Dysbindin also regulates the activity of the vesicular glutamate transporter, vGluT (Fanous et al., 2005), and may contribute to NMDAR dysfunction (Karlsgodt et al., 2011). Furthermore, the degree of dysbindin-induced NR1 degradation correlates with impairment in spatial working memory performance (Karlsgodt et al., 2011). This is strong evidence that dysbindin's effects on NMDAR expression could contribute to the cognitive symptoms of schizophrenia.

DISC1 affects presynaptic glutamate release from axonal terminals (Maher and LoTurco, 2012), and regulates cyclic adenosine monophosphate (cAMP) signaling, which would affect the functions of glutamate neurotransmission mediated by metabotropic glutamate receptors (mGluR) (Millar et al., 2005). DISC1 also binds to and stabilizes serine racemase (SR), the enzyme that generates D-serine, an endogenous co-agonist of the NMDA receptor. In a mouse model of selective and inducible expression of mutant DISC1 in astrocytes, the main source of D-serine in the brain, Ma et al. found that mutant DISC1 leads to SR degradation, resulting in D-serine deficiency that coincides with behavioral changes indicative of altered NMDAR neurotransmission (Ma et al., 2012). While not yet specifically tested, these changes would likely lead to reduced function of NMDARs at synapses. In addition, the DAOA gene encodes a protein that activates the enzyme D-amino acid oxidase, which degrades the co-transmitter D-serine that acts at glutamate synapses and at NMDARs. DAOA activates this enzyme, so abnormalities in this gene would be expected to alter the metabolism of D-serine, which in turn would alter glutamate neurotransmission at NMDARs (Stahl, 2007a).

Thus, there is strong evidence that the known susceptibility genes for schizophrenia converge on glutamate synapses, specifically at NMDARs. These observations support the notion that the NMDAR hypofunction hypothesis is a plausible theory for schizophrenia (Stahl, 2007a) and NMDAR dysfunction is a convergence point for schizophrenia (Kantrowitz and Javitt, 2010). Genes that code for any subtle molecular abnormalities linked to NMDAR function in specific brain circuits theoretically could create inefficient information processing at glutamate synapses that can produce the symptoms of schizophrenia, especially cognitive dysfunctions. If these genetically mediated abnormalities occur simultaneously in a permissive environment, the syndrome of schizophrenia could be induced and onset of symptoms will be triggered (Stahl, 2007b).

MOLECULAR MECHANISMS ASSOCIATED WITH NMDAR REGULATION AND NMDAR HYPOFUNCTION IN SCHIZOPHRENIA

As discussed above, there are many risk genes associated with schizophrenia. However, changes in their expression and function are unlikely to entirely account for the pathophysiology of schizophrenia. A fundamental question is what causes the alteration of NMDAR during neurodevelopment in schizophrenia. In addition to genetic modifications, there are several possible mechanisms, including altered transcription/translation and posttranslational modifications that could contribute to NMDAR hypofunction in schizophrenia. For example, NMDAR hypofunction could result from reduced levels of mRNA and translation and in fact, there is evidence of reduced mRNA levels of some NMDAR subunits in postmortem tissue of schizophrenics (Dracheva et al., 2001; Beneyto and Meador-Woodruff, 2008; Weickert et al., 2012) but plenty of evidence also suggests an increase or no change in some subunits (Akbarian et al., 1996; Geddes et al., 2011; Weickert et al., 2012). Given the complexity of the disorder and the numerous risk genes involved, it is likely that several mechanisms work in concert. Fortunately, substantial knowledge exists as to how NMDARs are translated, trafficked to synaptic membranes, stabilized, exocytosed, and removed for recycling or degradation (Sans et al., 2003; Wenthold et al., 2003; Perez-Otano and Ehlers, 2004; Lau and Zukin, 2007). However, any disruption of this well-regulated process can lead to NMDAR hypofunction and contribute to altered development and symptomatology seen in schizophrenia. Thus, it becomes a daunting challenge to understand the pathophysiological processes involved.

An exciting avenue of research in schizophrenia and other psychiatric disorders is evaluating the epigenetic changes that occur in these illnesses. Epigenetics is a broad term that describes changes to chromatin which alter the frequency of gene transcription without changing the genetic sequence. These changes include DNA methylation and a variety of histone modifications. In general, increasing DNA methylation, particularly at CpG islands of promoter sequences, will decrease gene expression (Bird, 2002). Therefore, even if a gene is not found to be definitively altered in human schizophrenic patients by standard genome-wide association study (GWAS), it is possible that epigenetic changes are contributing to altered neurodevelopment and cognitive symptoms in schizophrenia (Borrelli et al., 2008; Day and Sweatt, 2011; Rodenas-Ruano et al., 2012). Indeed, a role for histone acetylation and methylation in cognition is increasingly being appreciated (Jeremy Day and Sweatt, 2011). Other data suggest that chromatin modifications by histone deacetylases (HDACs) may underlie cognitive dysfunctions in a variety of mental disorders (Fischer et al., 2010). Thus far, epigenetic modulation of several genes, including GAD1 and RELN, has been found to be altered in schizophrenia (Abdolmaleky et al., 2005; Ruzicka et al., 2007). Additionally, the DNA methylating enzyme, DNA-methyltransferase 1 (DNMT1), showed increased expression in cortical interneurons in postmortem tissue from schizophrenics (Veldic et al., 2005). This change in DNMT1 correlated with the alterations in GAD1 and RELN. However, it is possible that other genes and associated interacting proteins are also similarly affected. For example, animal research has shown that NMDAR subunit expression can be altered through various epigenetic changes (Stadler et al., 2005; Jiang et al., 2010; Rodenas-Ruano et al., 2012). Furthermore, DNA methylation changes have been found in the promoter sequence for NR3B in major psychosis (Mill et al., 2008). These studies suggest that epigenetic regulation of NMDARs could contribute to the pathophysiology of schizophrenia. Still, it is unclear how epigenetic factors control the expression of NMDARs, particularly mRNA expression of individual subunits. It is possible that CpG islands in the promoter region of a NMDAR subunit are regulated by chromatin modification (Rodenas-Ruano et al., 2012). Gene mutation or environmental risk factors could alter gene promoter sequences via either DNA methylation or histone modification and thus result in mis-expression of NMDARs.

Furthermore, NMDAR subunits undergo several post-translation modifications including phosphorylation, palmitoylation, and polyubiquitination. Dysregulation of any of these processes can greatly impact channel function and expression and consequently contribute to NMDAR hypofunction. The most-studied posttranslational modification of NMDARs is phosphorylation, which is a well-characterized means for regulating synaptic localization, stabilization, and channel kinetics. Therefore, changes in NMDAR phosphorylation have important implications both for synaptic plasticity and cognitive symptoms in schizophrenia (Rosenblum et al., 1996; Lu et al., 1998; Li et al., 2009). This dynamic process not only involves the direct phosphorylation of NMDARs, but also kinase activation and subsequent phosphorylation of other synaptic proteins (Lau and Zukin, 2007; Lau et al., 2010). Moreover, the NR2 subunit's large C-terminus has many putative sites for phosphorylation which can affect channel gating and stabilization at the synapse (Monyer et al., 1992; Kornau et al., 1995). NMDAR subunits are phosphorylated at serine or threonine and at tyrosine residues (Raymond et al., 1994; Wang and Salter, 1994; Kohr and Seeburg, 1996; Tingley et al., 1997). These sites are substrates for phosphorylation by a variety of kinases including the Src family of kinases (SFK), cAMP-dependent protein kinase A (PKA), protein kinase C (PKC), cyclin-dependent kinase 5 (Cdk5), casein kinase 2 (CK2), and CaMKII (Omkumar et al., 1996; Raman et al., 1996; Li et al., 2001; Chung et al., 2004). In fact, the activity and expression of many of these kinases are altered in postmortem tissue from human schizophrenic patients (Aksenova et al., 1991; Engmann et al., 2011; Funk et al., 2012). This provides strong evidence that altered kinase signaling likely plays a role in NMDAR function in schizophrenia.

It is clear that the interaction between synaptic scaffolding proteins and the NR2 subunit C-terminal tails are critical for NMDAR synaptic targeting and thus could contribute to NMDAR hypofunction. PDZ-containing proteins can bind directly to NR2 subunits via PDZ recognition sequences in the distal portions of their C-termini, and this association is critical for targeting NDMARs to the synapse (Mori et al., 1998; Steigerwald et al., 2000; Lin et al., 2004). Further, both NR2A and NR2B are known to interact with membrane-associated guanylate kinase (MAGUK) family of proteins, including PSD-95, PSD-93, and SAP102 (Al-Hallaq et al., 2007). Interestingly,

the neuregulin receptor ErbB4 also associates with similar PDZ domains, positioning NRG-Erb signaling to affect NMDAR function (Garcia et al., 2000). Furthermore, ErbB4 interacts with FYN, a member of SFKs. SFKs phosphorylate tyrosine residues on both NR2A and NR2B subunits affecting channel gating and increasing NMDAR currents (Wang and Salter, 1994; Kohr and Seeburg, 1996; Hisatsune et al., 1999; Nakazawa et al., 2001; Takasu et al., 2002). NRG1-Erb signaling can prevent Src upregulation of NMDAR-mediated currents by inhibiting NR2B phosphorylation (Li et al., 2001; Bjarnadottir et al., 2007; Pitcher et al., 2011). Additionally, NMDAR tyrosine phosphorylation is important for synaptic plasticity. NR2B tyrosine phosphorylation is increased following long-term potentiation (LTP) and inhibiting Src activation prevents LTP induction (Grant et al., 1992; Rosenblum et al., 1996; Rostas et al., 1996; Lu et al., 1998). In hippocampus, NRG-Erb signaling can suppress LTP (Kwon et al., 2005; Pitcher et al., 2008). Therefore, NRG1 could contribute to cognitive dysfunction in schizophrenia by altering NMDAR function and/or affecting synaptic plasticity (Mei and Xiong, 2008). Similarly, DISC1 is a known binding partner of PDE4B, which regulates cAMP activity and thus PKA activity (Millar et al., 2005; Clapcote et al., 2007). PKA-mediated phosphorylation of NMDARs can affect their release from the endoplasmic reticulum, and regulate expression levels of NR2B (Scott et al., 2003; Llansola et al., 2004). However, it has not been directly tested whether mutations in DISC1 affect NMDAR expression and function. Additionally, it remains an open question if disruption of dysbindin would produce similar modifications in NMDARs. If and how the schizophrenia risk genes affect NMDAR phosphorylation and thus expression and function is an area of research that needs to be further explored.

Another crucial mechanism for proper NMDAR function is the maintenance of appropriate levels of NMDARs in the synapse. This process requires a balance between NMDAR insertion and endocytosis. Specialized endocytic zones involving clathrin-coated pits have been described lateral to the PSD for glutamatergic synapses, and serve to internalize NMDARs (Blanpied et al., 2002; Petralia et al., 2003; Nong et al., 2004). Altered dysbindin expression can alter NMDAR surface expression through clathrin-dependent endocytosis (Jeans et al., 2011). Further, palmitoylation and ubiquitination can also regulate NMDAR synaptic numbers. Palmitoylation is a reversible process that involves the covalent attachment of palmitate group to proteins via thioester bonds at cysteine residues. Palmitoylation is a critical regulator of many cellular processes involved in neuronal development and synaptic plasticity (Fukata and Fukata, 2010). Therefore, dysregulation of palmitoylation could contribute to synaptic dysfunction and cognitive symptoms in schizophrenia. Furthermore, key proteins implicated in schizophrenia, including GAD65 and PSD-95 are known to be regulated dynamically through palmitoylation (El-Husseini Ael et al., 2002; Kanaani et al., 2008). More recently, it was discovered that palmitoylation can regulate NR2A and NR2B trafficking (Hayashi et al., 2009). In fact, palmitoylation can promote synaptic stabilization or sequestering of NMDARs in the Golgi apparatus to affect the level of NMDARs at synapses. Interestingly, altered protein palmitoylation was found in a mouse model of 22q11.2 deletion, a high risk factor of developing schizophrenia (Madry et al., 2008). However, it remains unknown if NMDAR palmitoylation is disrupted in schizophrenia and if or how other schizophrenia risk genes may be involved.

Equally as important as trafficking and stabilizing proteins in the synapse is the process of targeting proteins for removal and degradation. It is known that ubiquitin-based protein degradation of NMDARs is an important homeostatic regulator of NMDAR levels at synapses (Ehlers, 2003). For example, downregulation of synaptic NR1 has been associated with polyubiquitination (Groblewski and Stafford, 2010; Bangash et al., 2011). Additionally, ubiquitination of scaffolding proteins, such as Shank3, is linked to NR2B downregulation (Mao et al., 2009a). Also, NR2B itself is ubiquitinated in a Fyn dependent manner (Jurd et al., 2008). Given NRG1-ErbB4 interactions with Fyn, it is possible that their signaling could contribute to ubiquitination of NR2B. However, this relationship has not been tested experimentally. Therefore, while there is evidence that the ubiquitin proteasome pathway is disrupted in schizophrenia (Nilsson et al., 2007), it is currently unknown how ubiquitination of NMDARs and other synaptic proteins contribute to the disease process. Exploring this relationship as well as how schizophrenia risk genes could alter these processes is an important line of research.

Given the diverse set of mechanisms that could contribute to NMDAR hypofunction, it is not surprising that multiple signaling pathways are implicated in schizophrenia. For example, both PLC/IP3R/Ca^{2+} and Ras/MEK/ERK (extracellular signal-regulated kinase) signaling pathways are involved in the neuregulin-induced reduction of NMDAR currents, which likely occurs through enhancing NR1 internalization via an actin-dependent mechanism (Gu et al., 2005). While the candidate genes discussed activate many signaling cascades to affect neurodevelopment and NMDAR function, the AKT (also known as protein kinase B) signaling pathway, and its downstream target glycogen synthase kinase 3β (GSK-3β) may serve as a convergence point or common pathway. AKT is a serine/threonine kinase that serves in a variety of processes including regulation of protein synthesis, neurodevelopment, and neuronal plasticity (Sanna et al., 2002; Jiang et al., 2005; Balu et al., 2012). Further, DISC1, NRG1, and dysbindin all contribute to these cellular processes, and are all known regulators of AKT and GSK3β (Lemke, 1996; Huang et al., 2000; Kamiya et al., 2005; Ghiani et al., 2010; Lee et al., 2011). DISC1 regulates the AKT-GSK3β signaling pathway to affect neurodevelopment and adult neurogenesis (Kim et al., 2009; Mao et al., 2009b). Furthermore, knockdown of DISC1 with siRNA caused a decrease in AKT phosphorylation, which would in turn increase GSK3β activity (Hashimoto et al., 2006). Interestingly, reducing GSK3β activity was able to correct behavioral deficits in DISC1 mutant mice, strongly implicating DISC1 affects GSK3β in schizophrenia pathogenesis (Lipina et al., 2011, 2012). Similarly, both NRG1 and dysbindin can regulate AKT phosphorylation (Numakawa et al., 2004; Guo et al., 2010). Additionally, AKT protein levels and phosphorylation of GSK3β are altered (Emamian et al., 2004) and NRG1-stimulated phosphorylation of AKT is reduced in schizophrenia (Keri et al., 2009). Yet, how would regulation of the AKT/GSK3β signaling pathway by DISC1, NRG1, and dysbindin affect NMDAR function? It was

recently demonstrated that GSK3β activity can regulate NMDAR expression and function (Li et al., 2009; Xi et al., 2011). While this evidence provides a possible common link between schizophrenia risk genes and NMDAR hypofunction, direct experimental evidence is still needed.

CONCLUSION AND FUTURE PERSPECTIVE

In this review, we have summarized the current literature and discussed the various mechanisms that are associated with NMDAR regulation in schizophrenia. All of the findings derived from the known genetic risk factors for schizophrenia suggest that NMDARs may serve as a convergence point for the progression and symptoms of schizophrenia. Despite such progress, there are still many questions that need to be answered to confirm this intriguing hypothesis. For example, it is unclear how gene mutations in neurons and/or astrocytes and their interaction can lead to NMDAR dysfunction during development. It is also unknown how disrupted NMDAR function leads to altered neurodevelopment, which contributes to the progression and development of this devastating disease. The vast majority of schizophrenia research has focused on changes in adulthood, leaving neurodevelopmental alterations relatively unexplored. So, while it is known that proper expression and regulation of NMDARs is critical for cortical maturation and synaptic plasticity that underlie cognitive functioning, it is unknown if there is a common signaling pathway, such as AKT/GSK-3β pathway, mediates this pathophysiological process among the schizophrenia susceptibility genes. If yes, what are the downstream target substrates of AKT and/or GSK-3β that contribute to the regulation of NMDAR functions? It is possible that AKT/GSK-3β act directly upon NMDARs as our recent research suggests (Li et al., 2009; Xi et al., 2011). However, given their diverse targets (Kockeritz et al., 2006; Peineau et al., 2008; Karam et al., 2010; Li and Gao, 2011), it is also possible they indirectly affect NMDARs by acting on other targets, such as β-catenin (Mao et al., 2009b), β-arrestin (Beaulieu et al., 2005), DISC1 and/or PDE4 interaction (Mao et al., 2009b; Lipina et al., 2012), as well as the AKT/mTOR signaling pathway. Activation of mTOR has been functionally linked with local protein synthesis in synapses, resulting in the production of proteins required for synaptic formation and maturation (Kelleher et al., 2004; Hoeffer and Klann, 2010).

In addition, although psychosis manifests primarily in late adolescence or early adulthood, the emerging picture from genetic and epigenetic studies indicates that early brain development is affected, and cognitive symptoms, such as learning and memory deficits, are evident much earlier. Specifically, schizophrenia may progress from risk to prodrome in the early stage until onset of psychosis and chronic disability in the late stage (Insel, 2010). Therefore, theoretically, the key to forestall the disorder is to detect and prevent early stages of risk and prodrome with novel therapeutic targets for early treatment (Lieberman et al., 2006; Insel, 2010). However, in general, schizophrenia-related research has focused on how NMDAR function in adults contributes to psychosis and cognitive symptoms. These findings, although intriguing, are limited in that they do not reveal the changes before psychosis, specifically during neurodevelopment. In fact, there is no consensus among animal models to what changes occur pre-pubertally and how the genetic susceptibilities interact. Does the process occur simultaneously or sequentially, with various changes culminating in altered development? If it is a sequential process, when do these changes occur and is there a point of no return in terms of preventing cognitive symptoms and psychosis? It appears that adolescence is a critical period for onset of psychosis, but how and by what mechanisms? Therefore, in studying molecular mechanisms that underlie the pathophysiology of schizophrenia and related disorders, a sharp focus on the specific neurodevelopmental trajectory, especially in early development and adolescent brain maturation, is vitally important (Jaaro-Peled et al., 2009; Insel, 2010). Animal studies, particularly developmental models, will certainly help to reveal the neurodevelopmental trajectory of schizophrenia, yield disease mechanisms, and eventually offer opportunities for the development of new treatments, especially for early treatment of cognitive deficits. Utilizing multiple animal models to address similar questions will provide the greatest opportunity for determining consistent changes that most likely contribute to the progression of schizophrenia. It would also be important to definitively determine which neurons express altered glutamate receptor subtypes, whether these neurons are inhibitory or excitatory, and how the circuitries are affected by these high-risk genes.

Furthermore, it is critical to determine if there comes a point during neurodevelopment where brain circuitry is sufficiently altered that no therapeutics will halt the progression of the disease. At present, there are no approved medications for the treatment of either negative symptoms or cognitive dysfunction in schizophrenia (Ibrahim and Tamminga, 2011). However, new pharmacological and behavioral approaches aimed at potentiating glutamatergic neurotransmission, particularly at NMDARs, offer new hope for future clinical development. Although many studies support the theory of NMDAR hypofunction, they do not address the very important conceptual question of whether early treatment with mGluR agonists or other agents is able to prevent the progression or reverse the cognitive deficits and even psychosis that occur in the late stage of the disease. A failure to correct mutant phenotypes with treatment administered after symptom onset would suggest a missed critical period window and indicate that schizophrenia is a terminally differentiated phenotype of altered brain development. Earlier theories supported the notion that effective treatment for developmental disorders such as schizophrenia and autism could only occur during the critical developmental window, after which the brain would be hard wired. Indeed, recent studies demonstrated that a comprehensive phenotype correction is possible with pharmacological intervention (mGluR5 inhibitor) starting in young (3–5 postnatal weeks) animals, after development of the phenotype, in both a Fragile X syndrome model (Michalon et al., 2012) and Shank-2 knockout mice (Won et al., 2012). In addition, adolescent administration of mGluR5 PAMs not only reverse adult-onset deficits, but also prevent the emergence of cognitive impairment induced by neonatal treatment with PCP in a developmental model of schizophrenia (Clifton et al., 2013). These findings certainly offer fresh hope for schizophrenia treatment, suggesting that NMDARs could be critical targets for treatment. Currently, our experiments

are under way to test this hypothesis in rats with gestational methylazoxymethanol exposure (Snyder et al., 2013) and other animal models.

Finally, if NMDAR dysfunction in schizophrenia is relative, rather than absolute, enhanced practice might be able to overcome reduced plasticity. Given the number of convergent mechanisms that may contribute to impaired NMDAR function, ideal treatment in schizophrenia may engage optimizing function within a number of convergent pathways. Combinatorial pharmacological and behavioral interventions, rather than simply targeting the point of convergence, may prove to be the most successful strategy in treating schizophrenia symptoms. Nevertheless, focusing on NMDAR hypofunction provides a wonderful opportunity for correcting cognitive impairment in schizophrenia disease progression.

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REFERENCES

Abdolmaleky, H. M., Cheng, K. H., Russo, A., Smith, C. L., Faraone, S. V., Wilcox, M., et al. (2005). Hypermethylation of the reelin (RELN) promoter in the brain of schizophrenic patients: a preliminary report. *Am. J. Med. Genet. B Neuropsychiatr. Genet.* 134B, 60–66.

Abi-Dargham, A. (2012). *The Dopamine Hypothesis of Schizophrenia*. Schizophrenia Research Forum. Available online at: http://www.schizophreniaforum.org/for/curr/AbiDargham/default.asp

Akbarian, S., Sucher, N. J., Bradley, D., Tafazzoli, A., Trinh, D., Hetrick, W. P., et al. (1996). Selective alterations in gene expression for NMDA receptor subunits in prefrontal cortex of schizophrenics. *J. Neurosci.* 16, 19–30.

Aksenova, M. V., Burbaeva, G. S., Kandror, K. V., Kapkov, D. V., and Stepanov, A. S. (1991). The decreased level of casein kinase 2 in brain cortex of schizophrenic and Alzheimer's disease patients. *FEBS Lett.* 279, 55–57.

Al-Hallaq, R. A., Conrads, T. P., Veenstra, T. D., and Wenthold, R. J. (2007). NMDA di-heteromeric receptor populations and associated proteins in rat hippocampus. *J. Neurosci.* 27, 8334–8343.

Allen, N. C., Bagade, S., McQueen, M. B., Ioannidis, J. P., Kavvoura, F. K., Khoury, M. J., et al. (2008). Systematic meta-analyses and field synopsis of genetic association studies in schizophrenia: the SzGene database. *Nat. Genet.* 40, 827–834.

Balu, D. T., Carlson, G. C., Talbot, K., Kazi, H., Hill-Smith, T. E., Easton, R. M., et al. (2012). Akt1 deficiency in schizophrenia and impairment of hippocampal plasticity and function. *Hippocampus* 22, 230–240.

Banerjee, A., Macdonald, M. L., Borgmann-Winter, K. E., and Hahn, C. G. (2010). Neuregulin 1-erbB4 pathway in schizophrenia: from genes to an interactome. *Brain Res. Bull.* 83, 132–139.

Bangash, M. A., Park, J. M., Melnikova, T., Wang, D., Jeon, S. K., Lee, D., et al. (2011). Enhanced polyubiquitination of Shank3 and NMDA receptor in a mouse model of autism. *Cell* 145, 758–772.

Beaulieu, J. M., Sotnikova, T. D., Marion, S., Lefkowitz, R. J., Gainetdinov, R. R., and Caron, M. G. (2005). An Akt/beta-arrestin 2/PP2A signaling complex mediates dopaminergic neurotransmission and behavior. *Cell* 122, 261–273.

Belforte, J. E., Zsiros, V., Sklar, E. R., Jiang, Z., Yu, G., Li, Y., et al. (2010). Postnatal NMDA receptor ablation in corticolimbic interneurons confers schizophrenia-like phenotypes. *Nat. Neurosci.* 13, 76–83.

Beneyto, M., Kristiansen, L. V., Oni-Orisan, A., McCullumsmith, R. E., and Meador-Woodruff, J. H. (2007). Abnormal glutamate receptor expression in the medial temporal lobe in schizophrenia and mood disorders. *Neuropsychopharmacology* 32, 1888–1902.

Beneyto, M., and Meador-Woodruff, J. H. (2008). Lamina-specific abnormalities of NMDA receptor-associated postsynaptic protein transcripts in the prefrontal cortex in schizophrenia and bipolar disorder. *Neuropsychopharmacology* 33, 2175–2186.

Bilder, R. M., Volavka, J., Lachman, H. M., and Grace, A. A. (2004). The catechol-O-methyltransferase polymorphism: relations to the tonic-phasic dopamine hypothesis and neuropsychiatric phenotypes. *Neuropsychopharmacology* 29, 1943–1961.

Bird, A. (2002). DNA methylation patterns and epigenetic memory. *Genes Dev.* 16, 6–21.

Bjarnadottir, M., Misner, D. L., Haverfield-Gross, S., Bruun, S., Helgason, V. G., Stefansson, H., et al. (2007). Neuregulin1 (NRG1) signaling through Fyn modulates NMDA receptor phosphorylation: differential synaptic function in NRG1+/- knock-outs compared with wild-type mice. *J. Neurosci.* 27, 4519–4529.

Blanpied, T. A., Scott, D. B., and Ehlers, M. D. (2002). Dynamics and regulation of clathrin coats at specialized endocytic zones of dendrites and spines. *Neuron* 36, 435–449.

Bogerts, B., Ashtari, M., Degreef, G., Alvir, J. M., Bilder, R. M., and Lieberman, J. A. (1990). Reduced temporal limbic structure volumes on magnetic resonance images in first episode schizophrenia. *Psychiatry Res.* 35, 1–13.

Borrelli, E., Nestler, E. J., Allis, C. D., and Sassone-Corsi, P. (2008). Decoding the epigenetic language of neuronal plasticity. *Neuron* 60, 961–974.

Brigman, J. L., Wright, T., Talani, G., Prasad-Mulcare, S., Jinde, S., Seabold, G. K., et al. (2010). Loss of GluN2B-containing NMDA Receptors in CA1 hippocampus and cortex impairs long-term depression, reduces dendritic spine density, and disrupts learning. *J. Neurosci.* 30, 4590–4600.

Cannon, T. D. (2005). The inheritance of intermediate phenotypes for schizophrenia. *Curr. Opin. Psychiatry* 18, 135–140.

Chung, H. J., Huang, Y. H., Lau, L. F., and Huganir, R. L. (2004). Regulation of the NMDA receptor complex and trafficking by activity-dependent phosphorylation of the NR2B subunit PDZ ligand. *J. Neurosci.* 24, 10248–10259.

Clapcote, S. J., Lipina, T. V., Millar, J. K., Mackie, S., Christie, S., Ogawa, F., et al. (2007). Behavioral phenotypes of Disc1 missense mutations in mice. *Neuron* 54, 387–402.

Clifton, N. E., Morisot, N., Girardon, S., Millan, M. J., and Loiseau, F. (2013). Enhancement of social novelty discrimination by positive allosteric modulators at metabotropic glutamate 5 receptors: adolescent administration prevents adult-onset deficits induced by neonatal treatment with phencyclidine. *Psychopharmacology (Berl.)* 225, 579–594.

Clinton, S. M., Haroutunian, V., Davis, K. L., and Meador-Woodruff, J. H. (2003). Altered transcript expression of NMDA receptor-associated postsynaptic proteins in the thalamus of subjects with schizophrenia. *Am. J. Psychiatry* 160, 1100–1109.

Clinton, S. M., Haroutunian, V., and Meador-Woodruff, J. H. (2006). Up-regulation of NMDA receptor subunit and post-synaptic density protein expression in the thalamus of elderly patients with schizophrenia. *J. Neurochem.* 98, 1114–1125.

Clinton, S. M., and Meador-Woodruff, J. H. (2004). Abnormalities of the NMDA receptor and associated intracellular molecules in the thalamus in schizophrenia and bipolar disorder. *Neuropsychopharmacology* 29, 1353–1362.

Coyle, J. T. (2006). Glutamate and schizophrenia: beyond the dopamine hypothesis. *Cell. Mol. Neurobiol.* 26, 365–384.

Coyle, J. T., and Tsai, G. (2004). The NMDA receptor glycine modulatory site: a therapeutic target for improving cognition and reducing negative symptoms in schizophrenia. *Psychopharmacology* 174, 32–38.

Day, J. J., and Sweatt, J. D. (2011). Epigenetic modifications in neurons are essential for formation and storage of behavioral memory. *Neuropsychopharmacology* 36, 357–358.

Dracheva, S., Byne, W., Chin, B., and Haroutunian, V. (2008). Ionotropic glutamate receptor mRNA expression in the human thalamus: absence of change in schizophrenia. *Brain Res.* 1214, 23–34.

Dracheva, S., Marras, S. A. E., Elhakem, S. L., Kramer, F. R., Davis, K. L., and Haroutunian, V. (2001). N-methyl-D-aspartic acid receptor expression in the dorsolateral prefrontal cortex of elderly patients with

schizophrenia. *Am. J. Psychiatry* 158, 1400–1410.

Dumas, T. C. (2005). Developmental regulation of cognitive abilities: modified composition of a molecular switch turns on associative learning. *Prog. Neurobiol.* 76, 189–211.

Eastwood, S. L., Kerwin, R. W., and Harrison, P. J. (1997). Immunoautoradiographic evidence for a loss of alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionate-preferring non-N-methyl-D-aspartate glutamate receptors within the medial temporal lobe in schizophrenia. *Biol. Psychiatry* 41, 636–643.

Ehlers, M. D. (2003). Activity level controls postsynaptic composition and signaling via the ubiquitin-proteasome system. *Nat. Neurosci.* 6, 231–242.

El-Husseini Ael, D., Schnell, E., Dakoji, S., Sweeney, N., Zhou, Q., Prange, O., et al. (2002). Synaptic strength regulated by palmitate cycling on PSD-95. *Cell* 108, 849–863.

Emamian, E. S., Hall, D., Birnbaum, M. J., Karayiorgou, M., and Gogos, J. A. (2004). Convergent evidence for impaired AKT1-GSK3beta signaling in schizophrenia. *Nat. Genet.* 36, 131–137.

Engmann, O., Hortobagyi, T., Pidsley, R., Troakes, C., Bernstein, H. G., Kreutz, M. R., et al. (2011). Schizophrenia is associated with dysregulation of a Cdk5 activator that regulates synaptic protein expression and cognition. *Brain* 134, 2408–2421.

Fanous, A. H., van den Oord, E. J., Riley, B. P., Aggen, S. H., Neale, M. C., O'Neill, F. A., et al. (2005). Relationship between a high-risk haplotype in the DTNBP1 (dysbindin) gene and clinical features of schizophrenia. *Am. J. Psychiatry* 162, 1824–1832.

Fischer, A., Sananbenesi, F., Mungenast, A., and Tsai, L.-H. (2010). Targeting the correct HDAC(s) to treat cognitive disorders. *Trends Pharmacol. Sci.* 31, 605–617.

Fukata, Y., and Fukata, M. (2010). Protein palmitoylation in neuronal development and synaptic plasticity. *Nat. Rev. Neurosci.* 11, 161–175.

Funk, A. J., McCullumsmith, R. E., Haroutunian, V., and Meador-Woodruff, J. H. (2012). Abnormal activity of the MAPK- and cAMP-associated signaling pathways in frontal cortical areas in postmortem brain in schizophrenia. *Neuropsychopharmacology* 37, 896–905.

Gambrill, A. C., and Barria, A. (2011). NMDA receptor subunit composition controls synaptogenesis and synapse stabilization. *Proc. Natl. Acad. Sci. U.S.A.* 108, 5855–5860.

Gao, X. M., Sakai, K., Roberts, R. C., Conley, R. R., Dean, B., and Tamminga, C. A. (2000). Ionotropic glutamate receptors and expression of N-methyl-D-aspartate receptor subunits in subregions of human hippocampus: effects of schizophrenia. *Am. J. Psychiatry* 157, 1141–1149.

Garcia, R. A., Vasudevan, K., and Buonanno, A. (2000). The neuregulin receptor ErbB-4 interacts with PDZ-containing proteins at neuronal synapses. *Proc. Natl. Acad. Sci. U.S.A.* 97, 3596–3601.

Geddes, A. E., Huang, X. F., and Newell, K. A. (2011). Reciprocal signalling between NR2 subunits of the NMDA receptor and neuregulin1 and their role in schizophrenia. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 35, 896–904.

Ghiani, C. A., Starcevic, M., Rodriguez-Fernandez, I. A., Nazarian, R., Cheli, V. T., Chan, L. N., et al. (2010). The dysbindin-containing complex (BLOC-1) in brain: developmental regulation, interaction with SNARE proteins and role in neurite outgrowth. *Mol. Psychiatry* 15, 115–215.

Goff, D. C., and Wine, L. (1997). Glutamate in schizophrenia: clinical and research implications. *Schizophr. Res.* 27, 157–168.

Grace, A. A. (2012). Dopamine system dysregulation by the hippocampus: implications for the pathophysiology and treatment of schizophrenia. *Neuropharmacology* 62, 1342–1348.

Grant, S. G., O'Dell, T. J., Karl, K. A., Stein, P. L., Soriano, P., and Kandel, E. R. (1992). Impaired long-term potentiation, spatial learning, and hippocampal development in fyn mutant mice. *Science* 258, 1903–1910.

Grimwood, S., Slater, P., Deakin, J. F., and Hutson, P. H. (1999). NR2B-containing NMDA receptors are up-regulated in temporal cortex in schizophrenia. *Neuroreport* 10, 461–465.

Groblewski, P. A., and Stafford, J. M. (2010). When the medial prefrontal cortex fails: implications for extinction and posttraumatic stress disorder treatment. *J. Neurosci.* 30, 7124–7126.

Gu, Z., Jiang, Q., Fu, A. K., Ip, N. Y., and Yan, Z. (2005). Regulation of NMDA receptors by neuregulin signaling in prefrontal cortex. *J. Neurosci.* 25, 4974–4984.

Gunduz-Bruce, H. (2009). The acute effects of NMDA antagonism: from the rodent to the human brain. *Brain Res. Rev.* 60, 279–286.

Guo, W. P., Fu, X. G., Jiang, S. M., and Wu, J. Z. (2010). Neuregulin-1 regulates the expression of Akt, Bcl-2, and Bad signaling after focal cerebral ischemia in rats. *Biochem. Cell Biol.* 88, 649–654.

Hahn, C. G., Wang, H. Y., Cho, D. S., Talbot, K., Gur, R. E., Berrettini, W. H., et al. (2006). Altered neuregulin 1-erbB4 signaling contributes to NMDA receptor hypofunction in schizophrenia. *Nat. Med.* 12, 824–828.

Harrison, P. J. (2004). The hippocampus in schizophrenia: a review of the neuropathological evidence and its pathophysiological implications. *Psychopharmacology (Berl.)* 174, 151–162.

Harrison, P. J., and Weinberger, D. R. (2005). Schizophrenia genes, gene expression, and neuropathology: on the matter of their convergence. *Mol. Psychiatry* 10, 40–68.

Hashimoto, R., Numakawa, T., Ohnishi, T., Kumamaru, E., Yagasaki, Y., Ishimoto, T., et al. (2006). Impact of the DISC1 Ser704Cys polymorphism on risk for major depression, brain morphology and ERK signaling. *Hum. Mol. Genet.* 15, 3024–3033.

Hayashi, T., Thomas, G. M., and Huganir, R. L. (2009). Dual palmitoylation of NR2 subunits regulates NMDA receptor trafficking. *Neuron* 64, 213–226.

Henson, M. A., Roberts, A. C., Pérez-Otaño, I., and Philpot, B. D. (2010). Influence of the NR3A subunit on NMDA receptor functions. *Prog. Neurobiol.* 91, 23–37.

Hisatsune, C., Umemori, H., Mishina, M., and Yamamoto, T. (1999). Phosphorylation-dependent interaction of the N-methyl-D-aspartate receptor epsilon 2 subunit with phosphatidylinositol 3-kinase. *Genes Cells* 4, 657–666.

Hoeffer, C. A., and Klann, E. (2010). mTOR signaling: at the crossroads of plasticity, memory and disease. *Trends Neurosci.* 33, 67–75.

Howes, O. D., and Kapur, S. (2009). The dopamine hypothesis of schizophrenia: version III–the final common pathway. *Schizophr. Bull.* 35, 549–562.

Huang, Y. Z., Won, S., Ali, D. W., Wang, Q., Tanowitz, M., Du, Q. S., et al. (2000). Regulation of neuregulin signaling by PSD-95 interacting with ErbB4 at CNS synapses. *Neuron* 26, 443–455.

Ibrahim, H. M., Hogg, A. J. Jr., Healy, D. J., Haroutunian, V., Davis, K. L., and Meador-Woodruff, J. H. (2000). Ionotropic glutamate receptor binding and subunit mRNA expression in thalamic nuclei in schizophrenia. *Am. J. Psychiatry* 157, 1811–1823.

Ibrahim, H. M., and Tamminga, C. A. (2011). Schizophrenia: treatment targets beyond monoamine systems. *Annu. Rev. Pharmacol. Toxicol.* 51, 189–209.

Iizuka, Y., Sei, Y., Weinberger, D. R., and Straub, R. E. (2007). Evidence that the BLOC-1 protein dysbindin modulates dopamine D2 receptor internalization and signaling but not D1 internalization. *J. Neurosci.* 27, 12390–12395.

Insel, T. R. (2010). Rethinking schizophrenia. *Nature* 468, 187–193.

Jaaro-Peled, H., Hayashi-Takagi, A., Seshadri, S., Kamiya, A., Brandon, N. J., and Sawa, A. (2009). Neurodevelopmental mechanisms of schizophrenia: understanding disturbed postnatal brain maturation through neuregulin-1-ErbB4 and DISC1. *Trends Neurosci.* 32, 485–495.

Javitt, D. C. (2004). Glutamate as a therapeutic target in psychiatric disorders. *Mol. Psychiatry* 9, 984–997.

Javitt, D. C., and Zukin, S. R. (1991). Recent advances in the phencyclidine model of schizophrenia. *Am. J. Psychiatry* 148, 1301–1308.

Jeans, A., Malins, R., Padamsey, Z., Reinhart, M., and Emptage, N. (2011). Increased expression of dysbindin-1A leads to a selective deficit in NMDA receptor signaling in the hippocampus. *Neuropharmacology* 61, 1345–1353.

Jeremy Day, J., and Sweatt, J. D. (2011). Epigenetic mechanisms in cognition. *Neuron* 70, 813–829.

Ji, Y., Yang, F., Papaleo, F., Wang, H.-X., Gao, W.-J., Weinberger, D. R., et al. (2009). Role of dysbindin in dopamine receptor trafficking and cortical GABA function. *Proc. Natl. Acad. Sci. U.S.A.* 106, 19593–19598.

Jiang, H., Guo, W., Liang, X., and Rao, Y. (2005). Both the establishment and the maintenance of neuronal polarity require active mechanisms: critical roles of GSK-3beta and its upstream regulators. *Cell* 120, 123–135.

Jiang, Y., Jakovcevski, M., Bharadwaj, R., Connor, C., Schroeder, F. A., Lin, C. L., et al. (2010). Setdb1 histone methyltransferase regulates mood-related behaviors and expression of the NMDA receptor subunit NR2B. *J. Neurosci.* 30, 7152–7167.

John Gray, A., Shi, Y., Usui, H., Matthew During, J., Sakimura,

K., and Roger Nicoll, A. (2011). Distinct modes of AMPA receptor suppression at developing synapses by GluN2A and GluN2B: single-cell NMDA receptor subunit deletion *in vivo*. *Neuron* 71, 1085–1101.

Jurd, R., Thornton, C., Wang, J., Luong, K., Phamluong, K., Kharazia, V., et al. (2008). Mind bomb-2 is an E3 ligase that ubiquitinates the N-methyl-D-aspartate receptor NR2B subunit in a phosphorylation-dependent manner. *J. Biol. Chem.* 283, 301–310.

Kamiya, A., Kubo, K., Tomoda, T., Takaki, M., Youn, R., Ozeki, Y., et al. (2005). A schizophrenia-associated mutation of DISC1 perturbs cerebral cortex development. *Nat. Cell Biol.* 7, 1167–1178.

Kanaani, J., Patterson, G., Schaufele, F., Lippincott-Schwartz, J., and Baekkeskov, S. (2008). A palmitoylation cycle dynamically regulates partitioning of the GABA-synthesizing enzyme GAD65 between ER-Golgi and post-Golgi membranes. *J. Cell. Sci.* 121, 437–449.

Kantrowitz, J. T., and Javitt, D. C. (2010). N-methyl-d-aspartate (NMDA) receptor dysfunction or dysregulation: the final common pathway on the road to schizophrenia? *Brain Res. Bull.* 83, 108–121.

Karam, C. S., Ballon, J. S., Bivens, N. M., Freyberg, Z., Girgis, R. R., Lizardi-Ortiz, J. E., et al. (2010). Signaling pathways in schizophrenia: emerging targets and therapeutic strategies. *Trends Pharmacol. Sci.* 31, 381–390.

Karlsgodt, K. H., Robleto, K., Trantham-Davidson, H., Jairl, C., Cannon, T. D., Lavin, A., et al. (2011). Reduced dysbindin expression mediates N-methyl-D-aspartate receptor hypofunction and impaired working memory performance. *Biol. Psychiatry* 69, 28–34.

Kato, T., Abe, Y., Sotoyama, H., Kakita, A., Kominami, R., Hirokawa, S., et al. (2011). Transient exposure of neonatal mice to neuregulin-1 results in hyperdopaminergic states in adulthood: implication in neurodevelopmental hypothesis for schizophrenia. *Mol. Psychiatry* 16, 307–320.

Kelleher, R. J. 3rd., Govindarajan, A., and Tonegawa, S. (2004). Translational regulatory mechanisms in persistent forms of synaptic plasticity. *Neuron* 44, 59–73.

Keri, S., Seres, I., Kelemen, O., and Benedek, G. (2009). Neuregulin 1-stimulated phosphorylation of AKT in psychotic disorders and its relationship with neurocognitive functions. *Neurochem. Int.* 55, 606–609.

Kim, J. Y., Duan, X., Liu, C. Y., Jang, M. H., Guo, J. U., Pow-anpongkul, N., et al. (2009). DISC1 regulates new neuron development in the adult brain via modulation of AKT-mTOR signaling through KIAA1212. *Neuron* 63, 761–773.

Kockeritz, L., Doble, B., Patel, S., and Woodgett, J. R. (2006). Glycogen synthase kinase-3–an overview of an over-achieving protein kinase. *Curr. Drug Targets* 7, 1377–1388.

Kohr, G., and Seeburg, P. H. (1996). Subtype-specific regulation of recombinant NMDA receptor-channels by protein tyrosine kinases of the src family. *J. Physiol.* 492 (Pt 2), 445–452.

Kornau, H. C., Schenker, L. T., Kennedy, M. B., and Seeburg, P. H. (1995). Domain interaction between NMDA receptor subunits and the postsynaptic density protein PSD-95. *Science* 269, 1737–1740.

Kristiansen, L. V., Beneyto, M., Haroutunian, V., and Meador-Woodruff, J. H. (2006). Altered NMDA receptor expression in schizophrenia. *Mol. Psychiatry* 11, 705.

Kristiansen, L. V., Huerta, I., Beneyto, M., and Meador-Woodruff, J. H. (2007). NMDA receptors and schizophrenia. *Curr. Opin. Pharmacol.* 7, 48–55.

Krystal, J. H., Anand, A., and Moghaddam, B. (2002). Effects of NMDA receptor antagonists: implications for the pathophysiology of schizophrenia. *Arch. Gen. Psychiatry* 59, 663–664.

Krystal, J. H., Karper, L. P., Seibyl, J. P., Freeman, G. K., Delaney, R., Bremner, J. D., et al. (1994). Subanesthetic effects of the noncompetitive NMDA antagonist, ketamine, in humans. Psychotomimetic, perceptual, cognitive, and neuroendocrine responses. *Arch Gen. Psychiatry* 51, 199–214.

Kwon, O. B., Longart, M., Vullhorst, D., Hoffman, D. A., and Buonanno, A. (2005). Neuregulin-1 reverses long-term potentiation at CA1 hippocampal synapses. *J. Neurosci.* 25, 9378–9383.

Lahti, A. C., Koffel, B., LaPorte, D., and Tamminga, C. A. (1995). Subanesthetic doses of ketamine stimulate psychosis in schizophrenia. *Neuropsychopharmacology* 13, 9–19.

Lau, C. G., Takayasu, Y., Rodenas-Ruano, A., Paternain, A. V., Lerma, J., Bennett, M. V. L., et al. (2010). SNAP-25 is a target of protein kinase C phosphorylation critical to NMDA receptor trafficking. *J. Neurosci.* 30, 242–254.

Lau, C. G., and Zukin, R. S. (2007). NMDA receptor trafficking in synaptic plasticity and neuropsychiatric disorders. *Nat. Rev. Neurosci.* 8, 413–426.

Lee, F. H., Fadel, M. P., Preston-Maher, K., Cordes, S. P., Clapcote, S. J., Price, D. J., et al. (2011). Disc1 point mutations in mice affect development of the cerebral cortex. *J. Neurosci.* 31, 3197–3206.

Lemke, G. (1996). Neuregulins in development. *Mol. Cell. Neurosci.* 7, 247–262.

Lewis, D. A., and Gonzalez-Burgos, G. (2008). Neuroplasticity of neocortical circuits in schizophrenia. *Neuropsychopharmacology* 33, 141–165.

Lewis, D. A., Hashimoto, T., and Volk, D. W. (2005). Cortical inhibitory neurons and schizophrenia. *Nat. Rev. Neurosci.* 6, 312–324.

Lewis, D. A., and Moghaddam, B. (2006). Cognitive dysfunction in schizophrenia: convergence of gamma-aminobutyric acid and glutamate alterations. *Arch. Neurol.* 63, 1372–1376.

Li, B., Devidze, N., Barengolts, D., Prostak, N., Sphicas, E., Apicella, A. J., et al. (2009). NMDA receptor phosphorylation at a site affected in schizophrenia controls synaptic and behavioral plasticity. *J. Neurosci.* 29, 11965–11972.

Li, B., Woo, R.-S., Mei, L., and Malinow, R. (2007). The neuregulin-1 receptor erbB4 controls glutamatergic synapse maturation and plasticity. *Neuron* 54, 583–597.

Li, B. S., Sun, M. K., Zhang, L., Takahashi, S., Ma, W., Vinade, L., et al. (2001). Regulation of NMDA receptors by cyclin-dependent kinase-5. *Proc. Natl. Acad. Sci. U.S.A.* 98, 12742–12747.

Li, Y. C., and Gao, W. J. (2011). GSK-3beta activity and hyperdopamine-dependent behaviors. *Neurosci. Biobehav. Rev.* 35, 645–654.

Li, Y. C., Xi, D., Roman, J., Huang, Y. Q., and Gao, W. J. (2009). Activation of glycogen synthase kinase-3 beta is required for hyperdopamine and D2 receptor-mediated inhibition of synaptic NMDA receptor function in the rat prefrontal cortex. *J. Neurosci.* 29, 15551–15563.

Lieberman, J. A., Jarskog, L. F., and Malaspina, D. (2006). Preventing clinical deterioration in the course of schizophrenia: the potential for neuroprotection. *J. Clin. Psychiatry* 67, 983–990.

Lin, C. H., Lane, H. Y., and Tsai, G. E. (2012). Glutamate signaling in the pathophysiology and therapy of schizophrenia. *Pharmacol. Biochem. Behav.* 100, 665–677.

Lin, Y., Skeberdis, V. A., Francesconi, A., Bennett, M. V., and Zukin, R. S. (2004). Postsynaptic density protein-95 regulates NMDA channel gating and surface expression. *J. Neurosci.* 24, 10138–10148.

Lindsley, C. W., Shipe, W. D., Wolkenberg, S. E., Theberge, C. R., Williams, D. L. Jr., Sur, C., et al. (2006). Progress towards validating the NMDA receptor hypofunction hypothesis of schizophrenia. *Curr. Top. Med. Chem.* 6, 771–785.

Lipina, T. V., Kaidanovich-Beilin, O., Patel, S., Wang, M., Clapcote, S. J., Liu, F., et al. (2011). Genetic and pharmacological evidence for schizophrenia-related Disc1 interaction with GSK-3. *Synapse* 65, 234–248.

Lipina, T. V., Niwa, M., Jaaro-Peled, H., Fletcher, P. J., Seeman, P., Sawa, A., et al. (2010). Enhanced dopamine function in DISC1-L100P mutant mice: implications for schizophrenia. *Genes Brain Behav.* 9, 777–789.

Lipina, T. V., Wang, M., Liu, F., and Roder, J. C. (2012). Synergistic interactions between PDE4B and GSK-3: DISC1 mutant mice. *Neuropharmacology* 62, 1252–1262.

Lisman, J. E., Coyle, J. T., Green, R. W., Javitt, D. C., Benes, F. M., Heckers, S., et al. (2008). Circuit-based framework for understanding neurotransmitter and risk gene interactions in schizophrenia. *Trends Neurosci.* 31, 234–242.

Llansola, M., Sanchez-Perez, A. M., Montoliu, C., and Felipo, V. (2004). Modulation of NMDA receptor function by cyclic AMP in cerebellar neurones in culture. *J. Neurochem.* 91, 591–599.

Lu, Y. M., Roder, J. C., Davidow, J., and Salter, M. W. (1998). Src activation in the induction of long-term potentiation in CA1 hippocampal neurons. *Science* 279, 1363–1367.

Ma, T. M., Abazyan, S., Abazyan, B., Nomura, J., Yang, C., Seshadri, S., et al. (2012). Pathogenic disruption of DISC1-serine racemase binding elicits schizophrenia-like behavior via D-serine depletion. *Mol. Psychiatry*. doi: 10.1038/mp.2012.97. [Epub ahead of print].

Madry, C., Betz, H., Geiger, J. R. P., and Laube, B. (2008). Supralinear

potentiation of NR1/NR3A excitatory glycine receptors by Zn2+ and NR1 antagonist. *Proc. Natl. Acad. Sci. U.S.A.* 105, 12563–12568.

Maher, B. J., and LoTurco, J. J. (2012). Disrupted-in-schizophrenia (DISC1) functions presynaptically at glutamatergic synapses. *PLoS ONE* 7:e34053. doi: 10.1371/journal.pone.0034053

Mao, L.-M., Wang, W., Chu, X.-P., Zhang, G.-C., Liu, X.-Y., Yang, Y.-J., et al. (2009a). Stability of surface NMDA receptors controls synaptic and behavioral adaptations to amphetamine. *Nat. Neurosci.* 12, 602–610.

Mao, Y., Ge, X., Frank, C. L., Madison, J. M., Koehler, A. N., Doud, M. K., et al. (2009b). Disrupted in schizophrenia 1 regulates neuronal progenitor proliferation via modulation of GSK3beta/beta-catenin signaling. *Cell* 136, 1017–1031.

Marek, G. J., Behl, B., Bespalov, A. Y., Gross, G., Lee, Y., and Schoemaker, H. (2010). Glutamatergic (N-methyl-D-aspartate receptor) hypofrontality in schizophrenia: too little juice or a miswired brain? *Mol. Pharmacol.* 77, 317–326.

McCullumsmith, R. E., Kristiansen, L. V., Beneyto, M., Scarr, E., Dean, B., and Meador-Woodruff, J. H. (2007). Decreased NR1, NR2A, and SAP102 transcript expression in the hippocampus in bipolar disorder. *Brain Res.* 1127, 108–118.

Medoff, D. R., Holcomb, H. H., Lahti, A. C., and Tamminga, C. A. (2001). Probing the human hippocampus using rCBF: contrasts in schizophrenia. *Hippocampus* 11, 543–550.

Mei, L., and Xiong, W.-C. (2008). Neuregulin 1 in neural development, synaptic plasticity and schizophrenia. *Nat. Rev. Neurosci.* 9, 437–452.

Michalon, A., Sidorov, M., Ballard, T. M., Ozmen, L., Spooren, W., Wettstein, J. G., et al. (2012). Chronic pharmacological mGlu5 inhibition corrects fragile X in adult mice. *Neuron* 74, 49–56.

Mill, J., Tang, T., Kaminsky, Z., Khare, T., Yazdanpanah, S., Bouchard, L., et al. (2008). Epigenomic profiling reveals DNA-methylation changes associated with major psychosis. *Am. J. Hum. Genet.* 82, 696–711.

Millan, M. J. (2005). N-Methyl-D-aspartate receptors as a target for improved antipsychotic agents, novel insights and clinical perspectives. *Psychopharmacol (Berl.)* 179, 30–53.

Millan, M. J., Agid, Y., Brüne, M., Bullmore, E. T., Carter, C. S., Clayton, N. S., et al. (2012). Cognitive dysfunction in psychiatric disorders: characteristics, causes and the quest for improved therapy. *Nat. Rev. Drug Discov.* 11, 141–168.

Millar, J. K., Pickard, B. S., Mackie, S., James, R., Christie, S., Buchanan, S. R., et al. (2005). DISC1 and PDE4B are interacting genetic factors in schizophrenia that regulate cAMP signaling. *Science* 310, 1187–1191.

Miyamoto, S., Miyake, N., Jarskog, L. F., Fleischhacker, W. W., and Lieberman, J. A. (2012). Pharmacological treatment of schizophrenia: a critical review of the pharmacology and clinical effects of current and future therapeutic agents. *Mol. Psychiatry* 17, 1206–1227.

Moghaddam, B. (2003). Bringing order to the glutamate chaos in schizophrenia. *Neuron* 40, 881–884.

Moghaddam, B., and Jackson, M. E. (2003). Glutamatergic animal models of schizophrenia. *Ann. N.Y. Acad. Sci.* 1003, 131–137.

Mohn, A. R., Gainetdinov, R. R., Caron, M. G., and Koller, B. H. (1999). Mice with reduced NMDA receptor expression display behaviors related to schizophrenia. *Cell* 98, 427–436.

Monyer, H., Burnashev, N., Laurie, D. J., Sakmann, B., and Seeburg, P. H. (1994). Developmental and regional expression in the rat brain and functional properties of four NMDA receptors. *Neuron* 12, 529–540.

Monyer, H., Sprengel, R., Schoepfer, R., Herb, A., Higuchi, M., Lomeli, H., et al. (1992). NMDA receptors: molecular and functional distinction of subtypes. *Science* 256, 1217–1221.

Mori, H., Manabe, T., Watanabe, M., Satoh, Y., Suzuki, N., Toki, S., et al. (1998). Role of the carboxy-terminal region of the GluR epsilon2 subunit in synaptic localization of the NMDA receptor channel. *Neuron* 21, 571–580.

Morris, B. J., Cochran, S. M., and Pratt, J. A. (2005). PCP: from pharmacology to modelling schizophrenia. *Curr. Opin. Pharmacol.* 5, 101–106.

Nakazawa, K., Zsiros, V., Jiang, Z., Nakao, K., Kolata, S., Zhang, S., et al. (2012). GABAergic interneuron origin of schizophrenia pathophysiology. *Neuropharmacology* 62, 1574–1583.

Nakazawa, T., Komai, S., Tezuka, T., Hisatsune, C., Umemori, H., Semba, K., et al. (2001). Characterization of Fyn-mediated tyrosine phosphorylation sites on GluR epsilon 2 (NR2B) subunit of the N-methyl-D-aspartate receptor. *J. Biol. Chem.* 276, 693–699.

Nilsson, A., Duan, J., Mo-Boquist, L. L., Benedikz, E., and Sundström, E. (2007). Characterisation of the human NMDA receptor subunit NR3A glycine binding site. *Neuropharmacology* 52, 1151–1159.

Niswender, C. M., and Conn, P. J. (2010). Metabotropic glutamate receptors: physiology, pharmacology, and disease. *Annu. Rev. Pharmacol. Toxicol.* 50, 295–322.

Niwa, M., Kamiya, A., Murai, R., Kubo, K., Gruber, A. J., Tomita, K., et al. (2010). Knockdown of DISC1 by in utero gene transfer disturbs postnatal dopaminergic maturation in the frontal cortex and leads to adult behavioral deficits. *Neuron* 65, 480–489.

Nong, Y., Huang, Y. Q., and Salter, M. W. (2004). NMDA receptors are movin' in. *Curr. Opin. Neurobiol.* 14, 353–361.

Numakawa, T., Yagasaki, Y., Ishimoto, T., Okada, T., Suzuki, T., Iwata, N., et al. (2004). Evidence of novel neuronal functions of dysbindin, a susceptibility gene for schizophrenia. *Hum. Mol. Genet.* 13, 2699–2708.

O'Donovan, M. C., Craddock, N., Norton, N., Williams, H., Peirce, T., Moskvina, V., et al. (2008). Identification of loci associated with schizophrenia by genome-wide association and follow-up. *Nat. Genet.* 40, 1053–1055.

Olney, J. W., and Farber, N. B. (1995). Glutamate receptor dysfunction and schizophrenia. *Arch. Gen. Psychiatry* 52, 998–1007.

Olney, J. W., Newcomer, J. W., and Farber, N. B. (1999). NMDA receptor hypofunction model of schizophrenia. *J. Psychiat. Res.* 33, 523–533.

Omkumar, R. V., Kiely, M. J., Rosenstein, A. J., Min, K. T., and Kennedy, M. B. (1996). Identification of a phosphorylation site for calcium/calmodulindependent protein kinase II in the NR2B subunit of the N-methyl-D-aspartate receptor. *J. Biol. Chem.* 271, 31670–31678.

Ozaki, M., Sasner, M., Yano, R., Lu, H. S., and Buonanno, A. (1997). Neuregulin-beta induces expression of an NMDA-receptor subunit. *Nature* 390, 691–694.

Papaleo, F., and Weinberger, D. R. (2011). Dysbindin and Schizophrenia: it's dopamine and glutamate all over again. *Biol. Psychiatry* 69, 2–4.

Papaleo, F., Lipska, B. K., and Weinberger, D. R. (2012). Mouse models of genetic effects on cognition: relevance to schizophrenia. *Neuropharmacology* 62, 1204–1220.

Peineau, S., Bradley, C., Taghibiglou, C., Doherty, A., Bortolotto, Z. A., Wang, Y. T., et al. (2008). The role of GSK-3 in synaptic plasticity. *Br. J. Pharmacol.* 153(Suppl. 1), S428–S437.

Perez-Otano, I., and Ehlers, M. D. (2004). Learning from NMDA receptor trafficking: clues to the development and maturation of glutamatergic synapses. *Neurosignals* 13, 175–189.

Petralia, R. S., Wang, Y. X., and Wenthold, R. J. (2003). Internalization at glutamatergic synapses during development. *Eur. J. Neurosci.* 18, 3207–3217.

Pitcher, G. M., Beggs, S., Woo, R. S., Mei, L., and Salter, M. W. (2008). ErbB4 is a suppressor of long-term potentiation in the adult hippocampus. *Neuroreport* 19, 139–143.

Pitcher, G. M., Kalia, L. V., Ng, D., Goodfellow, N. M., Yee, K. T., Lambe, E. K., et al. (2011). Schizophrenia susceptibility pathway neuregulin 1-ErbB4 suppresses Src upregulation of NMDA receptors. *Nat. Med.* 17, 470–478.

Quinlan, E. M., Olstein, D. H., and Bear, M. F. (1999). Bidirectional, experience-dependent regulation of N-methyl-D-aspartate receptor subunit composition in the rat visual cortex during postnatal development. *Proc. Natl. Acad. Sci. U.S.A.* 96, 12876–12880.

Raman, I. M., Tong, G., and Jahr, C. E. (1996). Beta-adrenergic regulation of synaptic NMDA receptors by cAMP-dependent protein kinase. *Neuron* 16, 415–421.

Raymond, L. A., Tingley, W. G., Blackstone, C. D., Roche, K. W., and Huganir, R. L. (1994). Glutamate receptor modulation by protein phosphorylation. *J. Physiol. Paris* 88, 181–192.

Roberts, A. C., Díez-García, J., Rodriguiz, R. M., López, I. P., Luján, R., Martínez-Turrillas, R., et al. (2009). Downregulation of NR3A-containing NMDARs is required for synapse maturation and memory consolidation. *Neuron* 63, 342–356.

Rodenas-Ruano, A., Chavez, A. E., Cossio, M. J., Castillo, P. E., and Zukin, R. S. (2012). REST-dependent epigenetic remodeling promotes the developmental switch in synaptic NMDA receptors. *Nat. Neurosci.* 15, 1382–1390.

Rosenblum, K., Dudai, Y., and Richter-Levin, G. (1996). Long-term potentiation increases tyrosine phosphorylation of the N-methyl-D-aspartate receptor subunit 2B in rat dentate gyrus *in vivo*.

Proc. Natl. Acad. Sci. U.S.A. 93, 10457–10460.

Rostas, J. A., Brent, V. A., Voss, K., Errington, M. L., Bliss, T. V., and Gurd, J. W. (1996). Enhanced tyrosine phosphorylation of the 2B subunit of the N-methyl-D-aspartate receptor in long-term potentiation. *Proc. Natl. Acad. Sci. U.S.A.* 93, 10452–10456.

Roy, K., Murtie, J. C., El-Khodor, B. F., Edgar, N., Sardi, S. P., Hooks, B. M., et al. (2007). Loss of erbB signaling in oligodendrocytes alters myelin and dopaminergic function, a potential mechanism for neuropsychiatric disorders. *Proc. Natl. Acad. Sci. U.S.A.* 104, 8131–8136.

Ruzicka, W. B., Zhubi, A., Veldic, M., Grayson, D. R., Costa, E., and Guidotti, A. (2007). Selective epigenetic alteration of layer I GABAergic neurons isolated from prefrontal cortex of schizophrenia patients using laser-assisted microdissection. *Mol. Psychiatry* 12, 385–397.

Sanna, P. P., Cammalleri, M., Berton, F., Simpson, C., Lutjens, R., Bloom, F. E., et al. (2002). Phosphatidylinositol 3-kinase is required for the expression but not for the induction or the maintenance of long-term potentiation in the hippocampal CA1 region. *J. Neurosci.* 22, 3359–3365.

Sans, N., Prybylowski, K., Petralia, R. S., Chang, K., Wang, Y. X., Racca, C., et al. (2003). NMDA receptor trafficking through an interaction between PDZ proteins and the exocyst complex. *Nat. Cell Biol.* 5, 520–530.

Savitz, J., Solms, M., and Ramesar, R. (2006). The molecular genetics of cognition: dopamine, COMT and BDNF. *Genes Brain Behav.* 5, 311–328.

Scott, D. B., Blanpied, T. A., and Ehlers, M. D. (2003). Coordinated PKA and PKC phosphorylation suppresses RXR-mediated ER retention and regulates the surface delivery of NMDA receptors. *Neuropharmacology* 45, 755–767.

Sheng, M., Cummings, J., Roldan, L. A., Jan, Y. N., and Jan, L. Y. (1994). Changing subunit composition of heteromeric NMDA receptors during development of rat cortex. *Nature* 368, 144–147.

Shi, J., Gershon, E. S., and Liu, C. (2008). Genetic associations with schizophrenia: meta-analyses of 12 candidate genes. *Schizophr. Res.* 104, 96–107.

Snyder, M. A., Adelman, A. E., and Gao, W.-J. (2013). Gestational methylazoxymethanol exposure leads to NMDAR dysfunction in hippocampus during early development and lasting deficits in learning. *Neuropsychopharmacology* 38, 328–340.

Spear, L. P. (2000). The adolescent brain and age-related behavioral manifestations. *Neurosci. Biobehav. Rev.* 24, 417–463.

Stadler, F., Kolb, G., Rubusch, L., Baker, S. P., Jones, E. G., and Akbarian, S. (2005). Histone methylation at gene promoters is associated with developmental regulation and region-specific expression of ionotropic and metabotropic glutamate receptors in human brain. *J. Neurochem.* 94, 324–336.

Stahl, S. M. (2007a). Beyond the dopamine hypothesis to the NMDA glutamate receptor hypofunction hypothesis of schizophrenia. *CNS Spectr.* 12, 265–268.

Stahl, S. M. (2007b). Novel therapeutics for schizophrenia: targeting glycine modulation of NMDA glutamate receptors. *CNS Spectr.* 12, 423–427.

Stefansson, H., Rujescu, D., Cichon, S., Pietilainen, O. P., Ingason, A., Steinberg, S., et al. (2008). Large recurrent microdeletions associated with schizophrenia. *Nature* 455, 232–236.

Steigerwald, F., Schulz, T. W., Schenker, L. T., Kennedy, M. B., Seeburg, P. H., and Kohr, G. (2000). C-Terminal truncation of NR2A subunits impairs synaptic but not extrasynaptic localization of NMDA receptors. *J. Neurosci.* 20, 4573–4581.

Straub, R. E., and Weinberger, D. R. (2006). Schizophrenia genes – famine to feast. *Biol. Psychiatry* 60, 81–83.

Takasu, M. A., Dalva, M. B., Zigmond, R. E., and Greenberg, M. E. (2002). Modulation of NMDA receptor-dependent calcium influx and gene expression through EphB receptors. *Science* 295, 491–495.

Tamminga, C. (1999). Glutamatergic aspects of schizophrenia. *Br. J. Psychiatry Suppl.* 37, 12–15.

Tan, H. Y., Callicott, J. H., and Weinberger, D. R. (2009). Prefrontal cognitive systems in schizophrenia: towards human genetic brain mechanisms. *Cogn. Neuropsychiatry* 14, 277–298.

Tingley, W. G., Ehlers, M. D., Kameyama, K., Doherty, C., Ptak, J. B., Riley, C. T., et al. (1997). Characterization of protein kinase A and protein kinase C phosphorylation of the N-methyl-D-aspartate receptor NR1 subunit using phosphorylation site-specific antibodies. *J. Biol. Chem.* 272, 5157–5166.

Tunbridge, E. M., Harrison, P. J., and Weinberger, D. R. (2006). Catechol-o-methyltransferase, cognition, and psychosis: Val158Met and beyond. *Biol. Psychiatry* 60, 141–151.

Veldic, M., Guidotti, A., Maloku, E., Davis, J. M., and Costa, E. (2005). In psychosis, cortical interneurons overexpress DNA-methyltransferase 1. *Proc. Natl. Acad. Sci. U.S.A.* 102, 2152–2157.

Vinson, P. N., and Conn, P. J. (2012). Metabotropic glutamate receptors as therapeutic targets for schizophrenia. *Neuropharmacology* 62, 1461–1472.

Wang, H. X., and Gao, W. J. (2009). Cell type-specific development of NMDA receptors in the interneurons of rat prefrontal cortex. *Neuropsychopharmacology* 34, 2028–2040.

Wang, H. X., and Gao, W. J. (2010). Development of calcium-permeable AMPA receptors and their correlation with NMDA receptors in fast-spiking interneurons of rat prefrontal cortex. *J. Physiol.* 588, 2823–2838.

Wang, H. X., and Gao, W. J. (2012). Prolonged exposure to NMDAR antagonist induces cell-type specific changes of glutamatergic receptors in rat prefrontal cortex. *Neuropharmacology* 62, 1808–1822.

Wang, H. X., Stradtman, G. G. 3rd., Wang, X. J., and Gao, W. J. (2008). A specialized NMDA receptor function in layer 5 recurrent microcircuitry of the adult rat prefrontal cortex. *Proc. Nat. Acad. Sci. U. S. A.* 105, 16791–16796.

Wang, Y. T., and Salter, M. W. (1994). Regulation of NMDA receptors by tyrosine kinases and phosphatases. *Nature* 369, 233–235.

Weickert, C. S., Fung, S. J., Catts, V. S., Schofield, P. R., Allen, K. M., Moore, L. T., et al. (2012). Molecular evidence of N-methyl-D-aspartate receptor hypofunction in schizophrenia. *Mol. Psychiatry*. doi: 10.1038/mp.2012.137. [Epub ahead of print].

Weinberger, D. R. (1987). Implications of normal brain development for the pathogenesis of schizophrenia. *Arch. Gen. Psychiatry* 44, 660–669.

Weinberger, D. R., Egan, M. F., Bertolino, A., Callicott, J. H., Mattay, V. S., Lipska, B. K., et al. (2001). Prefrontal neurons and the genetics of schizophrenia. *Biol. Psychiatry* 50, 825–844.

Wenthold, R. J., Prybylowski, K., Standley, S., Sans, N., and Petralia, R. S. (2003). Trafficking of NMDA receptors. *Annu. Rev. Pharmacol. Toxicol.* 43, 335–358.

Witthaus, H., Kaufmann, C., Bohner, G., Ozgurdal, S., Gudlowski, Y., Gallinat, J., et al. (2009). Gray matter abnormalities in subjects at ultra-high risk for schizophrenia and first-episode schizophrenic patients compared to healthy controls. *Psychiatry Res.* 173, 163–169.

Won, H., Lee, H.-R., Gee, H. Y., Mah, W., Kim, J.-I., Lee, J., et al. (2012). Autistic-like social behaviour in Shank2-mutant mice improved by restoring NMDA receptor function. *Nature* 486, 261–265.

Xi, D., Keeler, B., Zhang, W., Houle, J. D., and Gao, W. J. (2009). NMDA receptor subunit expression in GABAergic interneurons in the prefrontal cortex: application of laser micro dissection technique. *J. Neurosci. Methods* 176, 172–181.

Xi, D., Li, Y. C., Snyder, M. A., Gao, R. Y., Adelman, A. E., Zhang, W., et al. (2011). Group II metabotropic glutamate receptor agonist ameliorates MK801-induced dysfunction of NMDA receptors via the Akt/GSK-3beta pathway in adult rat prefrontal cortex. *Neuropsychopharmacology* 36, 1260–1274.

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Cholinergic connectivity: it's implications for psychiatric disorders

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Acetylcholine has been implicated in both the pathophysiology and treatment of a number of psychiatric disorders, with most of the data related to its role and therapeutic potential focusing on schizophrenia. However, there is little thought given to the consequences of the documented changes in the cholinergic system and how they may affect the functioning of the brain. This review looks at the cholinergic system and its interactions with the intrinsic neurotransmitters glutamate and gamma-amino butyric acid as well as those with the projection neurotransmitters most implicated in the pathophysiologies of psychiatric disorders; dopamine and serotonin. In addition, with the recent focus on the role of factors normally associated with inflammation in the pathophysiologies of psychiatric disorders, links between the cholinergic system and these factors will also be examined. These interfaces are put into context, primarily for schizophrenia, by looking at the changes in each of these systems in the disorder and exploring, theoretically, whether the changes are interconnected with those seen in the cholinergic system. Thus, this review will provide a comprehensive overview of the connectivity between the cholinergic system and some of the major areas of research into the pathophysiologies of psychiatric disorders, resulting in a critical appraisal of the potential outcomes of a dysregulated central cholinergic system.

Keywords: acetylcholine, psychiatric disorders, glutamate, GABA, dopamine, serotonin, cytokines

INTRODUCTION

The central cholinergic system has been implicated in the pathophysiology of schizophrenia (Raedler et al., 2006; Scarr and Dean, 2008, 2009) as well as mood disorders (Dilsaver, 1986; Cannon et al., 2006; Gibbons et al., 2009) and is a target for drug development aimed at improving treatments for these disorders (Furey and Drevets, 2006; Freedman et al., 2008; Scarr, 2012). Whilst efforts have been made to fully understand the changes that occur in the cholinergic system with these disorders, the impact of these changes are rarely considered in the context of their effects on other systems considered pertinent to the pathophysiologies of the disorders, or conversely the influence of other systems on cholinergic functionality. Thus, this review will briefly describe the central cholinergic system and the changes reported for the cholinergic system in schizophrenia and, to a lesser extent, mood disorders. The changes in the cholinergic system will then be considered in the context of documented changes that occur in other central neurotransmitter systems in people with schizophrenia or mood disorders and how such changes may have influenced, or been influenced by, the cholinergic system. Thus, this is not a comprehensive review of either the human cholinergic system (for this see Perry et al., 1999) or of all data relating to the pathophysiologies of schizophrenia and mood disorders. Whilst the contemplations on the interactions between the human cholinergic system and other central systems are, by necessity, somewhat speculative they take as given the concept that the brain is attempting to maintain a stable environment (homeostasis) using various feedback mechanisms. Thus, this review will give a solid theoretical framework for conceptualizing the pathophysiologies of psychiatric disorders as a breakdown of complex systems rather than a single self-contained gene or biological pathway.

THE CENTRAL CHOLINERGIC SYSTEM

In the human central nervous system, the cholinergic system has evolved into a complex network with three principle components,(i) projections from nuclei of the basal forebrain; these include the medial septal nucleus, the nucleus basalis of Meynert, the vertical nucleus of the diagonal band and the horizontal limb of the diagonal band nucleus, which innervate the hippocampus, most cortical regions and some subcortical nuclei, (ii) the pedunculopontine-lateral dorsal tegmental projections from the brainstem to the thalamus, midbrain and other brainstem regions and (iii) interneurons in the striatum (most abundant) and the nucleus accumbens (Everitt and Robbins, 1997; Perry et al., 1999) (see **Figure 1**). Given the complex nature of the cholinergic system in the human central nervous system, it is not surprising that it controls critical, diverse functions such as sleep, cognition, motor control, and sensory processing. Importantly, all functions of the cholinergic system are controlled by the interaction of acetylcholine with two families of receptors; the nicotinic and muscarinic receptors (Dale, 1914). The nicotinic receptors are cation permeable ligand-gated ion

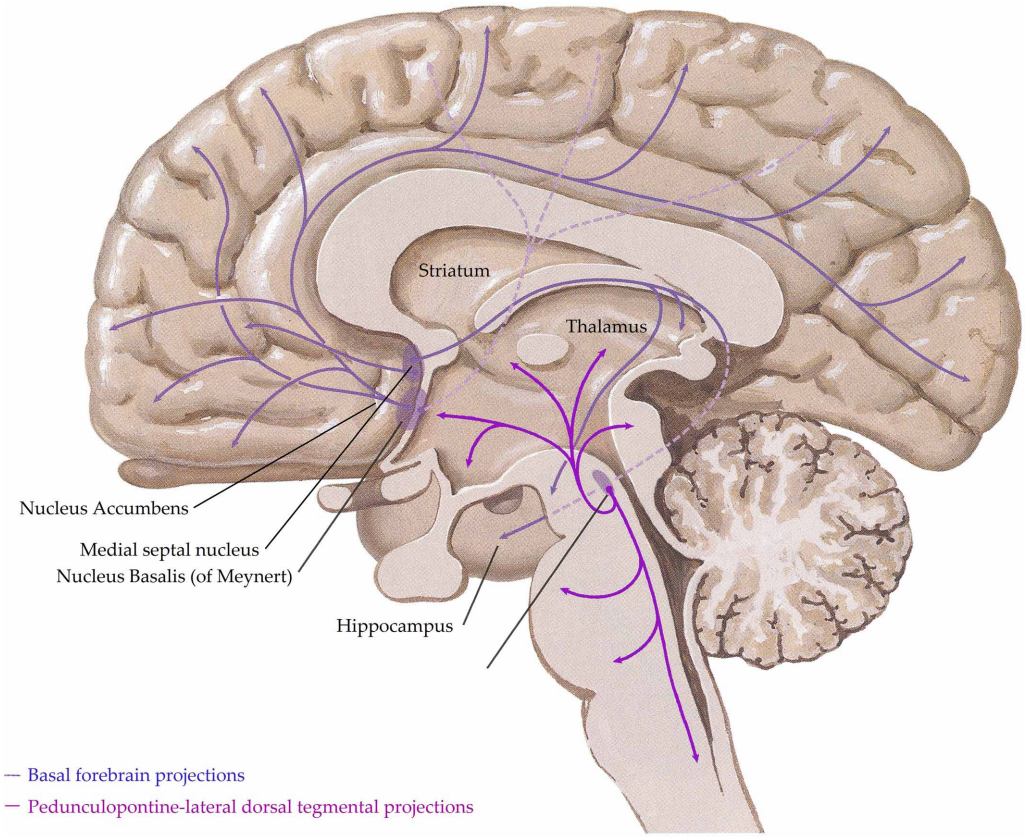

FIGURE 1 | A schematic representation of the human central cholinergic system—striatal interneurons not shown. Adapted from (Felten and Shetty, 2010).

channels, in the central nervous system the receptors consist of alpha (α1–7 and 9–10) and beta (β2–4) subunits which can be combined to form either homomeric (α7–10) or heteromeric (α2–6 and β2–4 or α7 with α9 or 10) pentameric receptors, which are named after their component subunits and appear to have distinct properties (Millar et al., 2011). By contrast, the muscarinic receptors are metabotropic, consisting of the M1–M5 receptors. M1, 3 and 5 all couple canonically to $G_{q/11}$ proteins; stimulating hydrolysis of inositol phosphate, whilst M2 and 4 couple to $G_{i/o}$ proteins, decreasing cyclic adenosine monophosphate(cAMP) levels. All five receptors are found in the human brain, with discreet distribution patterns, implying different functions (Challiss and Tobin, 2009). Ultimately, the functional outcome of central cholinergic stimulation depends on the balance between activation of both receptor families (Lucas-Meunier et al., 2003).

THE CENTRAL CHOLINERGIC SYSTEM IN SCHIZOPHRENIA AND MOOD DISORDERS

The cholinergic system has been proposed to contribute to the pathophysiology of schizophrenia as a result of either an imbalance between central cholinergic and dopaminergic systems (Tandon and Greden, 1989) or an over activation of the pedunculopontine-lateral dorsal tegmental nuclei (Yeomans, 1995). More recently, it has been shown that adjunctive acetylcholinesterase inhibitors can be of use in treating visual hallucinations (Patel et al., 2010; Abad et al., 2011), suggesting a hypo-cholinergic milieu may underlie these symptoms. However, a number of trials have failed to show that cholinesterases offer any significant improvement in the symptoms of schizophrenia (Buchanan et al., 2003, 2008; Friedman, 2004; Dyer et al., 2008; Keefe et al., 2008), suggesting that the problems in the cholinergic system in schizophrenia are not simply due to changes in levels of acetylcholine.

The perturbations of the central cholinergic system have been thoroughly reviewed previously (Raedler et al., 2006; Scarr and Dean, 2008, 2009; Jones et al., 2012; Scarr, 2012) so the main points will simply be summarized:

1. The most reproduced finding is a widespread decrease in levels of muscarinic receptors in the brains of people with schizophrenia, this has been replicated in four separate post-mortem collections (Mancama et al., 2003; Zavitsanou et al., 2004; Newell et al., 2007; Gibbons et al., 2013) and a neuroimaging study (Raedler et al., 2003).
2. Epibatidine binding, predominantly to the α4β2 nicotinic receptor, has been reported to be increased in people with schizophrenia (Martin-Ruiz et al., 2003).

3. The most investigated nicotinic receptor is the α7 nicotinic receptor which is associated with a sensory gating deficit present in people with schizophrenia (Adler et al., 1992) and other psychiatric disorders, although animal studies suggest that a lack of α7 receptors does not affect sensory gating (Paylor et al., 1998). In tissue from people with schizophrenia, levels of hippocampal α7 receptors have been reported to be decreased (Freedman et al., 1995) and unchanged (Thomsen et al., 2011), using a-bungarotoxin [which binds predominantly to α7 (Couturier et al., 1990)]. However, α7 mRNA expression is deceased in lymphocytes (Perl et al., 2003) and the expression of a particular splice variant is decreased in the brains from people with the disorder (Severance and Yolken, 2008), maintaining interest in this site as a potential drug target.

The first indication that the cholinergic system was involved in the pathophysiology of mood disorders came from the development of depressive symptoms in people who had been exposed to cholinesterase inhibitors (Rowntree et al., 1950; Gershon and Shaw, 1961). More recently a number of studies have implicated the muscarinic system, in particular the M2 receptor, in the mood disorders (Cannon et al., 2006; Furey and Drevets, 2006; Gibbons et al., 2009). One aspect of the pathophysiology of psychiatric disorders that is often not explored is how these changes may either arise from changes in other systems or affect the functionality of those systems. This review will explore these interactions theoretically, using data available from the literature.

INTERACTIONS WITH INTRINSIC NEUROTRANSMITTERS

For the purpose of this review, the term intrinsic has been used to describe neurotransmitters that predominantly act locally throughout the central nervous system, although they may have some neurons that project across different brain regions. These neurotransmitters include the excitatory amino acid glutamate and the inhibitory amino acid gamma-amino butyric acid (GABA).

GLUTAMATE

Glutamate in the central nervous system

Glutamate is the most abundant excitatory neurotransmitter in the human central nervous system, the effects of which are mediated via two classes of receptors; ionotropic[N-methyl-D-aspartate (NMDA), 2-amino-3-(3-hydroxy-5-methyl-isoxazol-4-yl)propanoic acid (AMPA), and kainate receptors] and metabotropic ($mGluR_{1-8}$) receptors (Traynelis et al., 2010). Like other ligand gated ion channels, the ionotropic glutamatergic receptors consist of combinations of subunits, in this instance creating tetramers, which give the receptors distinct properties. NMDA receptors are also voltage dependant and consist of two constitutive NR1 subunits, of which there are eight variants, and two NR2 subunits, of which there are four variants. AMPA receptors consist of combinations of the GluR1-4 subunits whilst the kainate receptor exists as combinations of GluR5-7 and KA1-2. Glutamate can also signal through metabotropic receptors; the Group I ($mGluR_1$ and $mGLUR_5$) which couple to G_q protein; stimulating inositol phosphate hydrolysis or Group II ($mGluR_2$ and $mGluR_3$) and Group III ($mGluR_4$, $mGluR_6$, $mGluR_7$,and $mGluR_8$), both of which couple to G_i/G_o protein and decrease levels of cAMP (Niswender and Conn, 2010).

Glutamate in schizophrenia

Magnetic resonance spectroscopy studies have reported elevated glutamate levels in the hippocampus and prefrontal cortex of patients with schizophrenia (van Elst et al., 2005), highlighting these areas as major regions of glutamatergic dysfunction in the disorder. The ability of NMDA receptor antagonists, such as ketamine and phencyclidine, to induce psychotic symptoms in healthy individuals and exacerbate symptoms in people with schizophrenia (Lahti et al., 2001) led to a focus on the role of the ionotropic glutamate receptors in the pathophysiology of schizophrenia. However, the data regarding NMDA and AMPA receptor levels in schizophrenia is inconsistent (Gao et al., 2000; Dean et al., 2001; Scarr et al., 2005; Beneyto et al., 2007; McCullumsmith et al., 2007). For example, binding of [^{3}H]MK-801, which binds to open NMDA receptors, in hippocampal tissue from individuals with schizophrenia has been reported to be both decreased (Beneyto et al., 2007) and unaltered (Gao et al., 2000; McCullumsmith et al., 2007). The lack of altered hippocampal gene expression (Beneyto et al., 2007) also contrasts with the report of decreased NR1 and increased NR2B subunit mRNA levels in the absence of altered [^{3}H]MK-801 binding (Gao et al., 2000). NR1 protein levels are reportedly unaltered in the hippocampus (Toro and Deakin, 2005), suggesting that overall levels of the NR1 subunit are not altered. Increased expression of NR2C mRNA and an increased proportion of NR2D mRNA relative to other NR2 subunits have been reported in the prefrontal cortex from people with schizophrenia (Akbarian et al., 1996), suggesting that NDMA receptor subunit ratios may be altered in the disorder, which would impact receptor function. This possibility gains some support from the finding of increased NR1 and NR2A, but not NR2B, mRNA levels in the dorsolateral prefrontal and occipital cortices from elderly subjects with schizophrenia (Dracheva et al., 2001). However, the fact that different subunits are over expressed could either suggest that changes in NMDA receptor composition vary with age or may simply reflect the heterogeneity of the disorder.

While small decreases in AMPA receptor radio ligand binding are reported in CA2 of the hippocampus (Gao et al., 2000), other studies have failed to detect changes in hippocampal AMPA receptors (Noga and Wang, 2002; Beneyto et al., 2007). Although [^{3}H]MK-801 and [^{3}H]AMPA densities have generally not been altered in the prefrontal cortex in schizophrenia (Healy et al., 1998; Scarr et al., 2005), at least one study has reported increased AMPA receptor levels (Noga et al., 2001). However, this group failed to replicate their original finding in a larger cohort, reporting decreases in striatal and accumbal AMPA receptors, highlighting the heterogeneity of changes in the glutamatergic system in the disorder.

With regards to the kainate receptor, a reduction in radioligand binding density and a reduction in $GluR_5$ mRNA expression have been reported in the prefrontal cortex from people with schizophrenia (Scarr et al., 2005). Whilst hippocampal kainate receptor levels are reportedly unchanged in schizophrenia

(Noga and Wang, 2002; Beneyto et al., 2007), decreased GluR6 and KA2 mRNA expression has been reported in some (Porter et al., 1997) but not all (Beneyto et al., 2007) studies, suggesting that the composition of kainate receptors may also be altered in some people with the disorder.

There is increasing awareness of the potential for targeting metabotropic glutamate receptors as modulators of glutamate release, ionotropic receptor response, and glutamatergic signal transduction, in the treatment of schizophrenia (Vinson and Conn, 2012). Their prospective usefulness is supported by the report of decreased mRNA levels of the $mGluR_{1\alpha}$ isoform in the dorsolateral prefrontal cortex in schizophrenia (Volk et al., 2010). Although the cortical binding density of the $mGluR_2/mGluR_3$ selective ligand, [^{3}H]LY354740, is reported to be unaltered in schizophrenia (Frank et al., 2011), cortical binding of [^{3}H]LY341495, another $mGluR_2/mGluR_3$ selective ligand, and mGluR2 but not mGluR3 mRNA has been reported to be decreased in subjects with schizophrenia, 84% of whom died by suicide (Gonzalez-Maeso et al., 2008). LY341495 has recently been shown to be efficacious in the tail suspension test and novelty suppressed feeding test in mice (Koike et al., 2013), suggesting mGluR2/mGluR3 may be involved in mood state. Therefore, the contribution of suicide to these findings needs to be further explored. Increased hippocampal and amygdala levels of the endogenous $mGluR_3$ agonist, N-acetylaspartylglutamate, have been reported in people with schizophrenia (Reynolds and Reynolds, 2011), suggesting that both ionotropic and metabotropic arms of the glutamatergic system may be affected by the disorder.

Cholinergic modulation of glutamatergic function

Acetylcholine has been shown to modulate glutamatergic excitatory postsynaptic potentials in several brain regions (Li and Pan, 2001; Zhang and Warren, 2002; Hamam et al., 2007), with the effects being either inhibitory or stimulatory. For example, acetylcholine has been found to increase excitatory postsynaptic potentials via nicotinic receptor signaling in the hippocampus (Radcliffe et al., 1999), hypothalamus (Li and Pan, 2001), and nucleus accumbens (Zhang and Warren, 2002). By contrast, acetylcholine or carbachol administration produce long lasting reductions of stimulus-evoked excitatory postsynaptic potential amplitude in the bed nucleus of the stria terminalis and in basal forebrain neurons (Allen et al., 2006; Guo et al., 2012), an effect supporting the finding that endogenous application of acetylcholine to hippocampal synaptosomes reduced glutamate levels (Marchi et al., 1989). The ability of the muscarinic antagonist atropine, but not nicotinic antagonists, to ameliorate these effects, combined with the ability of oxotremorine to inhibit glutamatergic currents in auditory cortical slices suggest that muscarinic receptors mediate the inhibition of glutamate release (Marchi et al., 1989; Atzori et al., 2005; Allen et al., 2006; Guo et al., 2012). Furthermore, in the nucleus accumbens, the inhibitory effects of atropine on excitatory postsynaptic potentials can be replicated with pirenzepine (Zhang and Warren, 2002), suggesting that the M1 and/or M4 are involved in regulating glutamate neurotransmission in this region. Significantly, the effect of acetylcholine on glutamatergic transmission appears to depend on the timing of the acetylcholine release relative to activating the glutamatergic neuron (Gu and Yakel, 2011). In the hippocampus, acetylcholine release prior to glutamatergic activation results in nicotinic α7 receptor-mediated long term potentiation or depression, whilst glutamatergic activation followed by acetylcholine release resulted in muscarinic receptor-mediated long term potentiation.

In the hippocampus, M1 and M3 have been shown to potentiate kainate receptor currents, increasing mossy fiber axon excitability. This modulation is subunit dependant, for example; muscarinic receptor activation potentiates heteromeric GluR6/KA1 and GluR6/KA2 receptors, but not homomeric GluR6 receptors (Benueniste et al., 2010). Thus, in schizophrenia, with reports of decreased hippocampal GluR6 and KA2 mRNA levels (Porter et al., 1997), abnormal kainate subunit ratios could affect receptor functionality. However, it is unclear whether muscarinic receptors affect signaling through kainate receptors composed of the GluR5 subunit, which is thought to underpin the reduction in cortical[^{3}H]kainate density in individuals with schizophrenia (Scarr et al., 2005).

Glutamatergic regulation of cholinergic function

Glutamatergic signaling has been shown to modulate acetylcholine release, predominantly via the ionotropic receptors. For instance, cortical microinjections of the NMDA receptor antagonist 3-(2-Carboxypiperazin-4-yl)propyl-1-phosphonic acid (CCP) increased acetylcholine release in the nucleus accumbens, an effect blocked by local perfusions of both CCP and the AMPA receptor antagonist 6,7-Dinitroquinoxaline-2,3-dione (DNQX) (Del Arco et al., 2008). By contrast, AMPA and NMDA increase acetylcholine release in the basal forebrain (Fournier et al., 2004), where AMPA is more effective, and striatum (Anderson et al., 1994; Ishida et al., 2005), where the NMDA antagonist MK-801, but not the AMPA/kainate antagonist 2, 3-dihydroxyl-6-nitro-7-sulfamoylbenzo(f)quinoxaline (NQBX), reduced acetylcholine efflux (Anderson et al., 1994); suggesting NMDA receptors may be more potent at regulating striatal acetylcholine release. NMDA and AMPA receptors work in concert to mediate glutamatergic signaling (Maeng et al., 2008), therefore, these differences may reflect the relative contributions of the receptors in eliciting a response in different brain regions.

The respective modulation of glutamate and acetylcholine release by cholinergic and glutamatergic pathways respectively depend on the co-expression of appropriate receptors within neurons and their synaptic connections. Microdialysis of AMPA into rat cortex facilitated acetylcholine release in the parietal and prefrontal cortices, an effect attenuated by DNQX (Nelson et al., 2005). Furthermore, DNQX partially attenuated the release of acetylcholine in the parietal cortex caused by carbachol administration to prefrontal cortex. These data suggest that cholinergic signaling in the parietal cortex is co-regulated by cholinergic and glutamatergic input from the prefrontal cortex. However, prefrontal cortical cholinergic afferents were not regulated by AMPA signaling from the parietal cortex, suggesting that the glutamatergic control is unidirectional. Further evidence for co-regulation comes from Group1 mGluRs acting in conjunction with muscarinic receptors to produce long lasting increases in excitatory postsynaptic potentials (Park and Spruston, 2012), possibly via

protein kinase C (PKC)-mediated activation of Src tyrosine kinase (Lu et al., 1999). This co-regulation is supported by reports that co-administrating carbachol and rolipram, a phosphodiesterase inhibitor which prevents cAMP inhibition, produces long lasting increases in hippocampal excitatory postsynaptic potentials associated with brain derived neurotrophic factor-dependant long term potentiation (Navakkode and Korte, 2012). Further support for interactions between the two systems come from studies demonstrating that M1 receptors suppress NMDA receptor function in cornu ammonis (CA) 3 pyramidal cells (Grishin et al., 2004, 2005), by inducing tyrosine phosphatase-mediated suppression of NMDAR activity (Grishin et al., 2005) and that activation of NMDA receptor can lead to the phosphorylation and desensitization of muscarinic receptors. These data provide the basis for a proposed feedback regulatory mechanism for glutamatergic/cholinergic signaling (Butcher et al., 2009) (see **Figure 2**).

Studies have also shown that ventral tegmental presynaptic metabotropic glutamate and muscarinic receptors preferentially inhibit the NMDA mediated component of synaptic transmission (Zheng and Johnson, 2003). In CA1 and CA3 pyramidal cells muscarinic receptors and mGluRs can be simultaneously coupled to inhibitory and stimulatory pathways to modulate NDMAR activity in a calcium-dependent (Grishin et al., 2004), cell specific manner. Thus, these systems appear to rely on cooperation to regulate ionotropic receptor function. Hippocampal M1 and M4 are predominantly responsible for the direct cholinergic modulation of the excitatory CA1-CA3 circuit (Dasari and Gulledge, 2011). CA1 slices from mice lacking CA3 M1 have reduced mGluR mediated long term depression compared to mice with normal CA3 M1 levels (Kamsler et al., 2010), this effect was reversed by activating PKC. Together, these data led to the proposal that normal M1 levels are necessary to maintain baseline PKC activity and that additional PKC stimulation by Group 1 mGluR's facilitates mGluR-mediated long term depression at CA3 presynaptic terminals. Thus, it is possible that in schizophrenia, where deficits in M1 have been reported (Scarr et al., 2009; Gibbons et al., 2013), the PKC activity mediated by the combined signaling of M1 and mGluRs may be insufficient to maintain normal synaptic functionality.

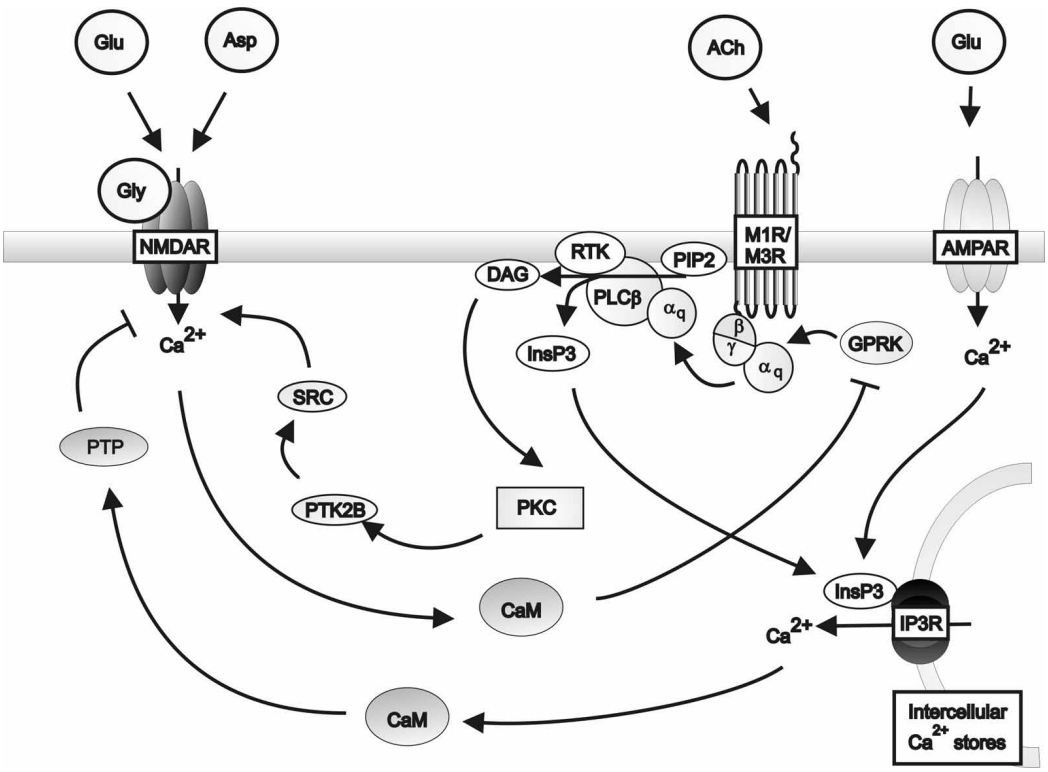

FIGURE 2 | A schematic diagram of the regulation of NMDA receptor activity by G_q protein-coupled muscarinic receptors in the hippocampus. Muscarinic receptors inhibit NMDA receptor activity via the activation of protein tyrosine phosphatase mediated by inositol triphosphate receptor pathways in conjunction with AMPA receptor induced calcium release from intracellular calcium stores. Muscarinic receptors can stimulate NMDA receptor activity via the activation of Src family tyrosine kinase in response to PKC signaling. Activation of the NMDA receptor by glutamate or aspartate and the co-agonist glycine, in turn inhibits muscarinic receptor activity via calmodulin inhibition of G protein coupled receptor kinases. α_q: $G_{q\alpha}$ subunit; β: Gβ subunit; γ: Gγ subunit; ACh: Acetylcholine; AMPAR; AMPA receptor; Asp: Aspartate; CaM: Calmodulin DAG: Diacyl glycerol; Glu: Glutamate; Gly: Glycine; GPRK: G protein coupled receptor kinase; InsP3: Inositol 1,4,5-trisphosphate; IP3R: Inositol triphosphate receptor; M1R: Muscarinic M1 receptor; M3R: Muscarinic M3 receptor; MEK**:** Mitogen-activated protein kinase kinase; NMDAR: NMDA receptor; PLCβ: Phospholipase C β; PIP2; Phosphatidylinositol 4,5-bisphosphate; PKC: Phosphokinase C; PTK2B: Protein tyrosine kinase 2β; PTP: Protein tyrosine phosphatase; RTK: Tyrosine kinase receptor; SRC: Src family tyrosine kinase.

Acetylcholine and glutamate in schizophrenia

The dual role of the cholinergic system, activating and inhibiting glutamatergic signaling, presents challenges in predicting the effects of (i) the M1 deficits associated with and (ii) the NMDA receptor hypofunction predicted in schizophrenia. However, animal studies have shown that inhibitory avoidance memory consolidation can be repressed by co-administration of muscarinic and NMDA antagonists to the ventral tegmentum, at doses that were ineffective when used alone (Mahmoodi et al., 2010), indicating a synergistic interaction. Thus, it is possible that the disturbances in central function seen in schizophrenia could be underpinned by a loss of synaptic plasticity due to suppression of both glutamatergic and cholinergic signaling.

Importantly, the processes governing acetylcholine and glutamate release in turn regulate and are regulated by additional neurotransmitters. For example, stimulating nicotinic receptors reduces AMPA -evoked synaptosomal dopamine overflow (Grilli et al., 2012). In addition, the co-administration of dopamine and muscarinic agonists to rat cortical slices inhibits the muscarinic receptor mediated reduction in excitatory postsynaptic potentials (Atzori et al., 2005). Therefore, the alterations in cholinergic signaling that occur in schizophrenia need to be regarded as a component of a much broader breakdown of central neurotransmission.

GAMMA-AMINO BUTYRIC ACID

Gamma-amino butyric acid in the central nervous system

GABA is the major central inhibitory neurotransmitter, in mammals 25–50% of central synapses utilize GABA (Petroff and Rothman, 1998), making it essential for the balance between neuronal excitation and inhibition that underpins normal brain function (Johnston, 2005). The central effects of GABA are mediated by two receptor families, the $GABA_A$ and $GABA_B$ receptors (Steiger and Russek, 2004). $GABA_A$ receptors are ionotropic, regulating chloride channels. The receptors are pentameric, although there are 19 different subunits within the $GABA_A$ receptor family; α1–6, β1–3, γ1–3, δ, ϵ, π, ρ1–3, and θ, the minimum requirement for an active receptor are an α and β subunit (Whiting, 2003). While a $GABA_C$ receptor was postulated, this receptor consists exclusively of rho (ρ) subunits and, because of their similarity to $GABA_A$ subunits, is now viewed as a $GABA_A$ variant (Barnard et al., 1998). $GABA_B$ receptors are metabotropic, coupled to $G_{i/o}$ proteins, and consist of 2 subunits, $GABA_{B1}$ and $GABA_{B2}$, both of which are necessary for functional receptors (Hyland and Cryan, 2010). As expected, given the diverse nature of the neurotransmitter, GABAergic receptors are widely distributed throughout the brain and highly expressed in cortical, hippocampal, thalamic, basal ganglia, and cerebellar structures.

GABA in schizophrenia

There is strong evidence to support the theory that schizophrenia is associated with deficits in GABAergic neurotransmission [see (Blum and Mann, 2002) for a detailed review]. Briefly, postmortem studies suggest that GABAergic neurons are providing insufficient inhibitory modulation in corticolimbic regions of people with schizophrenia (Benes et al., 1991, 1992, 1996b; Heckers and Konradi, 2010). Similar abnormalities have also been observed in the dorsolateral prefrontal cortex (Benes et al., 1991, 1996b) suggesting the effect could be widespread. This theory is supported by reports of pervasive increased binding densities for the $GABA_A$ ligand, [^{3}H]muscimol, in tissue held in a number of CNS repositories. The areas affected include the cingulate cortex, dorsolateral prefrontal cortex (Benes et al., 1996b; Dean et al., 1999a), caudate nucleus (Hanada et al., 1987), superior temporal gyrus (Deng and Huang, 2006) and hippocampus (Benes et al., 1996a) from people with schizophrenia. Further, more direct, support for the theory comes from reports of increased $GABA_A$ receptor proteins in the prefrontal cortex (Ishikawa et al., 2004) of people with schizophrenia as well as increases in α1 and 5 (Impagnatiello et al., 1998) and α2 (Volk et al., 2002) subunits. The increase in $GABA_A$ expression has been postulated to reflect receptor upregulation, compensating for decreased GABAergic release (Benes et al., 1996a). It is possible that the decreased activity could contribute to working memory deficits, a core cognitive problem in schizophrenia, since $GABA_A$ agonists have been shown to improve performance on working memory and cognitive control tasks in people with the disorder (Lewis et al., 2008). In contrast to these increases in the $GABA_A$ receptor, there have been reports of decreases in $GABA_B$ receptors (Mizukami et al., 2000, 2002) and one of the subunits, $GABA_{B1a}$, (Ishikawa et al., 2005), further implicating the neurotransmitter in the pathophysiology of the disorder and suggesting that the impact of the neurochemical balance depends upon the location and function of the GABAergic receptors.

Glutamic acid decarboxylase (GAD) 67 is essential for GABA synthesis and is used as a marker for GABAergic cells. Cortical expression of mRNA for both GAD67 and the GABA transporter, GAT1, are reported to be decreased in tissue from people with schizophrenia (Volk et al., 2000, 2001), as is cortical GAD67 protein (Curley et al., 2011). Decreased GAD67 expression has also been reported in the anterior cingulate (Woo et al., 2004, 2007) and hippocampus (Benes et al., 2007). However, two studies have reported increased cortical GAD67 mRNA and protein in people with schizophrenia (Hakak et al., 2001; Dracheva et al., 2004), suggesting that cortical dysfunction in schizophrenia is not consistently accompanied by altered expression of GAD67 mRNA. Furthermore, decreases in GAD67 occur in cortical tissue (Guidotti et al., 2000; Thompson et al., 2009) from people with bipolar disorder and cerebellum from people with mood disorders (Fatemi et al., 2005) as well as that from people with schizophrenia, raising the possibility that dysfunction of a subset of GABAergic interneurons may underpin some of the pathophysiology of major psychiatric disorders.

Cholinergic modulation of GABAergic function

The striatum is the major input structure of the basal ganglia and has been implicated in the pathophysiology of schizophrenia (Lester et al., 2010). GABAergic medium sized spiny projection neurons comprise more than 74% of the striatal cell population in humans (DiFiglia et al., 1976) and project almost equally to (i) nuclei that interface between the basal ganglia and the rest of the brain and (ii) other basal ganglia nuclei (Gerfen and Surmeier, 2011). These projection neurons represent the main target of the cholinergic interneurons, the predominant source of striatal

acetylcholine (Izzo and Bolam, 1988; Graybiel, 1990). Although the cholinergic interneurons only constitute 1–2% of striatal cells (Graveland et al., 1985), they are vital for modulating the activity of both striatal projection neurons and GABAergic interneurons. The GABAergic interneurons make up approximately 5% of the striatal cells and are comprised of three populations, distinguishable by their expression of calcium binding proteins (Tepper et al., 2010). A striatal microcircuit has been proposed, where cholinergic interneurons communicate to one another through GABAergic interneurons (Sullivan et al., 2008), thus interactions between cholinergic and GABAergic systems would be fundamental for striatal functioning. Muscarinic receptors are thought to be expressed pre-synaptically by striatal GABAergic neurons (Grilli et al., 2009), directly inhibiting GABA release (Marchi et al., 1990; Sugita et al., 1991; Koos and Tepper, 2002). In particular, muscarine decreased GABA release (Nakamura and Jang, 2012), possibly by activating pre-synaptic M4 receptors. Investigations in the amygdala, nucleus accumbens and striatum confirmed that acetylcholine and muscarine inhibit GABA release, an effect attenuated by pirenzepine, an M1/M4 antagonist (Sugita et al., 1991).

Nicotinic receptors, on the other hand, appear to facilitate GABA release (Lena et al., 1993; Wonnacott et al., 2006). For example, nicotine increased the frequency, but not amplitude of spontaneous inhibitory post-synaptic potentials of hippocampal neurons (Fisher et al., 1998). It was also shown to increase the amplitude of evoked inhibitory post-synaptic potentials (Radcliffe et al., 1999). This effect may account for the activation of choline acetyl transferase expressing neurons in the nucleus accumbens increasing the frequency of $GABA_A$ – mediated inhibitory post-synaptic potentials (Witten et al., 2010). However, the nicotinic mediated release of GABA was prevented by activation of M4 receptors (Grilli et al., 2009), suggesting that both muscarinic and nicotinic receptors may coexist on GABAergic terminals and that the impact of nicotinic receptors on GABA release can be modulated by muscarinic receptors. Finally, studies have reported that the nicotinic effect appears to be indirect, involving either dopamine (Kayadjanian et al., 1994) or serotonin (Bianchi et al., 1995) as the intermediary. Together, these data indicate that the consequence of acetylcholine will depend on the relative distribution of muscarinic and nicotinic receptors and that the effects may be mediated by a second system.

GABAergic regulation of cholinergic function

To obtain insight into GABA-acetylcholine interactions, a number of studies investigated the effects of GABA agonists, such as; muscimol, progabide, SL75102, δ-aminovaleric acid, and 2-pyrrolidone, on acetylcholine levels. In a number of brain regions, low doses of GABA agonists increased acetylcholine levels (Scatton and Bartholini, 1982), probably via stimulation of $GABA_A$ receptors located on cholinergic cells. Earlier studies had suggested that the action of GABA was indirect, with dopamine suggested as an intermediary (Ladinsky et al., 1976; Javoy et al., 1977). However, lesions of the dopaminergic and serotonergic pathways did not affect GABA mediated responses (Scatton and Bartholini, 1982), indicating that they could play a minor role. The same study found that lesions of the glutamatergic cortico-striatal projections ablated the GABAergic inhibition of cholinergic transmission (Scatton and Bartholini, 1982), indicating that GABA may indirectly modulate acetylcholine release by inhibiting the excitatory input to the cholinergic interneurons. Together, these studies illustrate the complexity of interactions between the cholinergic and GABAergic systems, which could affect a diverse set of central functions, including cognitive processes which may be relevant to schizophrenia (Lewis et al., 2008).

Acetylcholine and GABA in schizophrenia

The number of striatal cholinergic interneurons has been shown to be decreased in people with schizophrenia (Holt et al., 1999), this could disrupt the normal function of GABAergic projection neurons thereby contributing to the prefrontal cortical dysfunction associated with schizophrenia. With respect to the neurochemical changes associated with schizophrenia, the widely replicated increase in binding to the $GABA_A$ receptors (Benes et al., 1996b; Dean et al., 1999a; Deng and Huang, 2006) would be expected to result in a reduced cholinergic activity. This, in turn, should lead to increased levels of post-synaptic cholinergic receptors in an attempt to compensate for transmission deficit as well as potentially causing a decrease in pre-synaptic receptors to reduce the feedback regulation of the cholinergic system. These outcomes are not in keeping with the alterations in the cholinergic system commonly reported in schizophrenia [see "The Central Cholinergic System in Schizophrenia and Mood Disorders" and (Scarr and Dean, 2009)]. However, given the modulation of the GABAergic system by nicotinic receptors, the decreased expression of some nicotinic α7 receptor variants (Severance and Yolken, 2008), may reduce GABA release (Lena et al., 1993; Wonnacott et al., 2006), resulting in increased levels of postsynaptic GABAergic receptors, an effect widely reported in schizophrenia (Benes et al., 1996b; Dean et al., 1999a; Deng and Huang, 2006). Whilst this concept appears to have face validity, it will depend on whether the α7 receptor does indeed modulate GABA and should also result in changes in $GABA_B$ receptors, which have been reported to be decreased in the hippocampus (Mizukami et al., 2000) and the entorhinal cortex (Mizukami et al., 2002) as have cortical $GABA_{B1a}$ subunits (Ishikawa et al., 2005). Since $GABA_B$ receptors have been shown to be both pre- and post-synaptic (Bettler et al., 2012), it is possible these decreases reflect an attempt to reduce the feedback on the pre-synaptic neuron. However, until the localization of the reduced $GABA_B$ receptors is known, this association between nicotinic and GABAergic systems in schizophrenia remains speculative.

INTERACTIONS WITH OTHER PROJECTION SYSTEMS

The systems considered in this section are neurotransmitter systems whose neurons arise from discreet brain structures and project to distal regions of the brain, affecting the activity of the intrinsic neurotransmitters in those regions. The choice of projection systems to be included in this review was driven, in part, by the known pathophysiologies of schizophrenia, and therefore focuses on the dopaminergic and serotonergic systems.

DOPAMINE

Central dopaminergic systems

Dopaminergic cells are found almost exclusively in the substantia nigra (SN) and ventral tegmental area (VTA), forming four major dopaminergic pathways in the mammalian brain, these are the (i) mesolimbic, (ii) mesocortical, (iii) nigrostriatal, and (iv) tuberoinfundibular pathways (Albanese et al., 1986) (see **Figure 3**). In brief, the mesolimbic pathway consists of dopamine-containing cell bodies in the VTA, which project to limbic structures such as the nucleus acumbens, hippocampus, and amygdale as well as the medial prefrontal cortex (Albanese and Minciacchi, 1983). This pathway is thought to be important for the acquisition of behaviors reinforceable by the inappropriate stimuli of addictive drugs (Le Moal and Simon, 1991; Lester et al., 2010). The mesocortical system is closely associated with the mesolimbic system, connecting the VTA to the cerebral cortex, particularly the frontal cortex. It is considered essential for cognitive functions involving the dorsolateral prefrontal cortex and is thought to play a major role in memory, motivation, and emotional response (Noback et al., 2005). Dopamine-containing cell bodies originating in substantia nigra pars compacta (SNpc) of the midbrain and projecting predominantly to the caudate-putamen constitute the nigrostriatal pathway (Albanese et al., 1986), which is thought to play a major role in motor coordination and has been implicated in Parkinson's disease and chorea. Finally, the tuberoinfundibular pathway originates in the arcuate and periventricular nuclei of the hypothalamus and projects to the median eminence, the infundibular and the pituitary (Albanese et al., 1986); where it inhibits prolactin secretion.

There are two types of G-protein coupled dopamine receptors, which are widely distributed centrally; D1-like receptors (D1 and D5), which couple to G_s proteins and stimulate cAMP production and D2-like receptors (D2,3, and 4), which couple to $G_{i/o}$ proteins and either have no effect on or inhibit cAMP (Schetz, 2009). D1and D2 receptors are widespread throughout the central nervous system and are generally present at higher levels than the D3, 4, and 5 receptors; such a distribution is in keeping with the diverse functions these receptors are implicated in mediating (Mansour and Watson, 1995).

In both Lewy Body dementia and Alzheimer's disease, where there is a loss of cholinergic neurons, patients have a loss of cognitive function and neuropsychiatric symptoms. Although both groups have similar levels of delusions, anxiety, and depression, patients with mild Lewy Body dementia have more visual and auditory hallucinations than patients with Alzheimer's disease (Auning et al., 2011; Bjoerke-Bertheussen et al., 2012). This

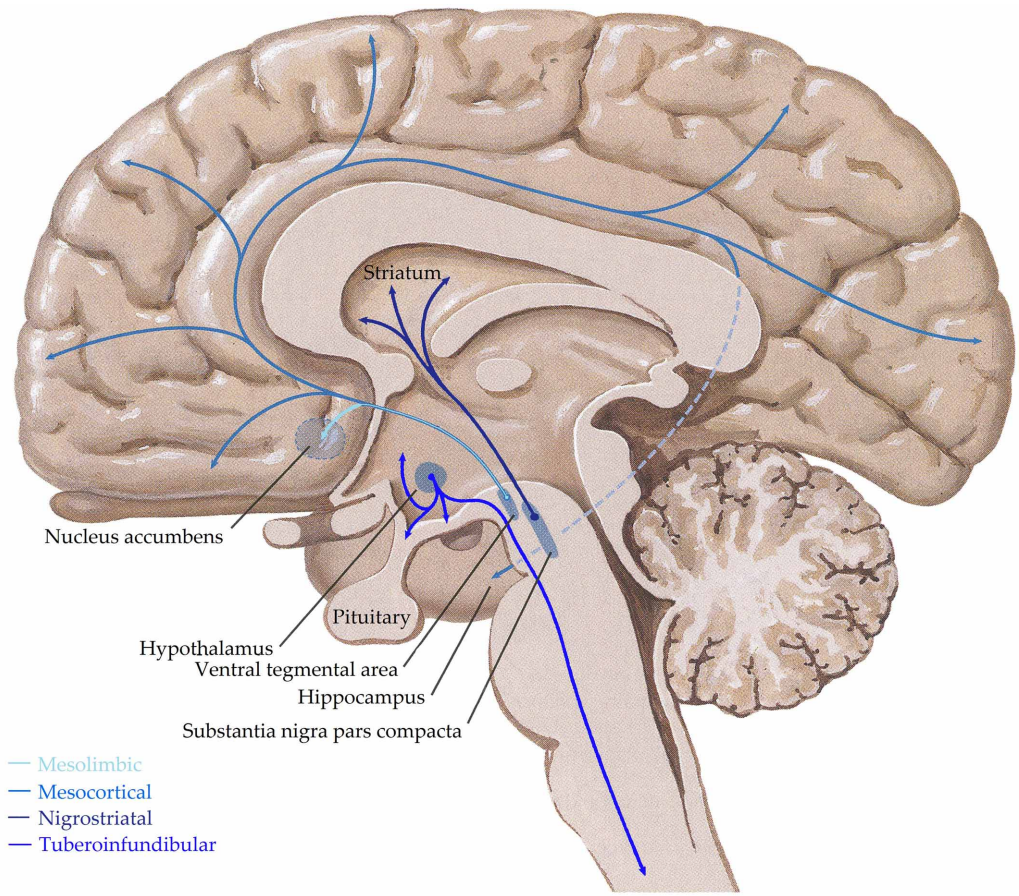

FIGURE 3 | Schematic representation of the human central dopaminergic systems. Adapted from (Felten and Shetty, 2010).

difference in clinical presentation may be due to the increased severity in cholinergic degeneration seen in Lewy Body dementia (Francis and Perry, 2007) or to the dopaminergic degeneration that also occurs in this disorder (Klein et al., 2010). Thus, the benefits of understanding the interactions between the cholinergic and dopaminergic systems will be beneficial for disorders other than schizophrenia and mood disorders.

Dopamine in schizophrenia

The dopaminergic system has long been considered a major component of schizophrenia pathophysiology (Carlsson, 1988). The dopamine hypothesis of schizophrenia is based on the observation that stimulation of the dopaminergic system with drugs such as amphetamine often leads to transient psychotic symptoms, and that a large number of antipsychotics used to treat the disorder block the activity of dopamine receptors (see Carlsson et al., 1997; Emilien et al., 1999). Although it has long been accepted that glutamate and GABA modulate activity of dopamine neurons, the discovery that acetylcholine may be as important in controlling dopamine release was made more recently. It is now postulated that an imbalance between dopaminergic and cholinergic systems contribute to disorders of the central nervous system (Tandon and Greden, 1989). Therefore, restoring the balance between the two systems is considered a practical treatment strategy (Knable and Weinberger, 1997).

The classic hypothesis for schizophrenia proposed that hyperactivity of dopaminergic transmission was responsible for the positive symptoms, however, the awareness of enduring negative symptoms and cognitive deficits, with their resistance to D2 antagonism, led to a reformulation of this hypothesis. Functional imaging studies suggested that altered functionality of the prefrontal cortex [PFC; see (Knable and Weinberger, 1997)] may contribute to the symptomatology of schizophrenia. Numerous pre-clinical studies have demonstrated the importance of prefrontal activation of D1 receptors for optimal PFC performance, [see (Goldman-Rakic et al., 2000) for example]. These findings led to the current view that an imbalance between subcortical and cortical dopaminergic systems is responsible for the symptoms of schizophrenia; a hyperactivity of the dopaminergic system in the subcortical regions (resulting in hyperstimulation of D2 receptors) causes the positive symptoms while hypoactivity of the mesocortical dopamine projections (resulting in hypostimulation of D1 receptors) is responsible for both negative symptoms and cognitive impairment (Guillin et al., 2007). In support of this hypothesis, imaging studies have consistently demonstrated that schizophrenia is associated with increased presynaptic activity of dopaminergic neurons projecting to the striatum, and a decrease in D1 receptor-like binding, as measured with positron emission tomography, was reported in the PFC of patients with schizophrenia, correlating with cognitive dysfunction and negative symptoms (Okubo et al., 1997). This correlation with symptoms has consistently been reported, even though the decrease in binding was not always replicated, with reports of increased (Abi-Dargham et al., 2002) and unchanged (Karlsson et al., 2002) levels of D1 receptors.

Blocking the D2 receptor reduces positive symptoms in people with schizophrenia (Carlsson, 1974; Creese et al., 1976; Seeman et al., 1976; Kapur and Remington, 2001). However, the data from studies on the levels of D2-like receptors are highly variable, with reports of increases (Lee et al., 1978; Mackay et al., 1982), decreases (Dean et al., 2004) and no change (Reynolds et al., 1981). To further complicate matters, the changes appear to be region specific (Dean et al., 2004) and it is possible that antipsychotic drugs may affect the outcomes (Mackay et al., 1980), although there is debate about this point (Mita et al., 1986). D4 receptors have consistently been reported to be increased (Seeman et al., 1993; Sumiyoshi et al., 1995; Marzella et al., 1997), whilst there is little data available for D3 receptors many ligands see D2/D3 receptors, hence the reporting of D2-like receptors.

The apparent inconsistencies between dopaminergic systems has been resolved by studies showing reciprocal and opposite regulation between the cortical and subcortical systems (Pycock et al., 1980) [for review see (Tzschentke, 2001)], with prefrontal dopaminergic activity exerting an inhibitory influence on subcortical dopaminergic activity (Deutch et al., 1990; Kolachana et al., 1995; Karreman and Moghaddam, 1996; Wilkinson, 1997). Significantly, chronic blockade of D2 receptors leads to a decrease in D1 receptors in the PFC region, along with impairments in working memory in non-human primates (Castner et al., 2000). Thus, there is evidence that a dopaminergic imbalance may be involved in schizophrenia, contributing to some of the key symptom domains associated with the disorder.

Cholinergic regulation of dopaminergic function

The striatum is densely innervated by tonically active cholinergic interneurons (Butcher and Woolf, 1984; Woolf, 1991; Aosaki et al., 1995; Bennett and Wilson, 1999), which interact closely with dopaminergic neurons to modulate their activity. Given the heterogeneity of muscarinic receptors and their signaling cascades, it is not surprising that activating muscarinic receptors results in both excitation and inhibition of dopaminergic activity in the basal ganglia. There is considerable evidence that interactions between cholinergic and dopaminergic systems are critical for the proper regulation of motor control, a function strongly attributed to the striatum. For example, an imbalance between striatal muscarinic and dopaminergic tone is thought to contribute to the severe motor deficits experienced by people with Parkinson's disease and other extrapyramidal motor disorders (Hornykiewicz, 1981; Brown and Taylor, 1996). Indeed, dopamine agonists and muscarinic antagonists are useful in the treatment of Parkinson's disease (Hornykiewicz, 1981; Fahn et al., 1990; Brown and Taylor, 1996), where degeneration of dopaminergic neurons in the SNpc causes reduced striatal dopaminergic function (Hornykiewicz, 1981; Graybiel, 1990).

All muscarinic receptors are expressed in the striatum, suggesting all have the potential to modulate dopamine release (Weiner et al., 1990; Bernard et al., 1992; Yasuda et al., 1993; Hersch et al., 1994). Almost all D1 receptor-expressing striatonigral neurons also express both M1 (Weiner et al., 1990; Bernard et al., 1992) and M4, whereas the D2 receptor expressing striatalpalladial neurons express M1 but less than half express M4. Pharmacological determinations of which muscarinic receptors modulated cholinergic and dopaminergic interactions were hindered by a lack of specific ligands, resulting in disparate findings

(Raiteri et al., 1984; Schoffelmeer et al., 1986; De Klippel et al., 1993; Smolders et al., 1997). The development of more specific ligands revealed that stimulating M1/M4 receptors causes potent dopamine release in the striatum and cortex (Bymaster et al., 1994; Ichikawa et al., 2002; Goldman-Rakic et al., 2004). Furthermore, the cognitive deficits produced by scopolamine, a muscarinic antagonist, could be reversed by D1 blockade (McGurk et al., 1988).

A more direct approach to delineating the muscarinic-dopaminergic interactions, came from studies on M1-5 receptor deficient mice (Hamilton et al., 1997; Gomeza et al., 1999a,b; Matsui et al., 2000; Miyakawa et al., 2001; Yamada et al., 2001a,b; Fisahn et al., 2002). In striatal slices, a lack of M1 or M2 receptors did not affect oxotremorine-mediated dopamine release (Zhang et al., 2002). However, *in vivo* microdialysis showed that M1-deficient mice had elevated striatal extracellular dopamine (Gerber et al., 2001), possibly due to extrastriatal receptors exerting an inhibitory striato-nigral feedback. Further studies found that M2 were required for muscarinic regulation of dopamine release in dorsal but not limbic striatal regions (Threlfell et al., 2010) and that oxotremorine-mediated dopamine release was enhanced in M3 KO mice and abolished in M4 KO mice (Zhang et al., 2002), suggesting that M3 receptors inhibit and M4 receptors promote striatal dopamine output. Furthermore, blockade of M3 receptors increased striatal but not nucleus accumbens dopamine efflux, suggesting that muscarinic modulation of dopaminergic transmission is region specific (Miller and Blaha, 2005). In addition, M4 receptors appear to inhibit dopamine D1 receptor-stimulated adenylyl cyclase activity (Olianas and Onali, 1996; Olianas et al., 1996), which would account for the hypersensitivity of mice lacking M4 receptors to the stimulatory locomotor effects of D1 receptor activation (Gomeza et al., 1999b), possibly due to a lack of striatal inhibition. Finally, M5 are the only muscarinic receptors expressed on dopaminergic neurons in the substantia nigra pars compacta (Weiner et al., 1990), where they regulate dopamine release (Forster et al., 2002; Yamada et al., 2003; Bendor et al., 2010; Steidl et al., 2011). Deletion of theM5 results in impaired dopamine release (Yamada et al., 2001a), improved latent inhibition (Wang et al., 2004) and increased D2 expression in the striatum, hypothalamus, hindbrain, and tectum (Zhang et al., 2002), possibly reflecting a compensatory mechanism. This is of interest because striatal D2 receptors have been shown to be upregulated in schizophrenia (Laruelle et al., 1996; Abi-Dargham et al., 1998) and unmedicated patients with acute schizophrenia display poor latent inhibition (Gray et al., 1995), thus M5 dysfunction might occur in schizophrenia.

The initial association between nicotine addiction and dopaminergic striatal signaling suggested the existence of a nicotinic-dopaminergic interaction (see Corrigall, 1999, for a review). Studies showed that dopaminergic antagonists, lesions of dopaminergic neurons or of the nucleus accumbens (Corrigall et al., 1992) could reduce nicotine self-administration. Nicotinic receptors are commonly expressed pre-synaptically, with activation resulting in rapid increases in neurotransmission. This, coupled with the overlap of the striatal cholinergic and dopaminergic systems, suggests that frequent, rapid regulation occurs between the two (Zhou et al., 2001).

Systemic nicotine has been shown to increase dopamine release in the mesolimbic (Imperato et al., 1986; Damsma et al., 1989; Benwell and Balfour, 1994; Nisell et al., 1994a; Pontieri et al., 1996), nigrostriatal (Benwell and Balfour, 1994; Imperato et al., 1986; Toth et al., 1992), and mesocortical (Toth et al., 1992; Nisell et al., 1994a) systems. Microdialysis experiments showed nicotine, applied to cortical terminal regions, evokes an increase in extracellular dopamine levels, albeit to a lesser extent than in the striatum and accumbens (Mifsud et al., 1989; Nakamura et al., 1992; Toth et al., 1992; Nisell et al., 1994b; Marshall et al., 1997), possibly due to fewer nicotinic receptors on cortical dopaminergic terminals. Blockade of nicotinic receptors in the VTA abolished the nicotine-induced increase in dopamine and its metabolites, however blockade in the nucleus acumbens had no effect (Nisell et al., 1994b), suggesting nicotine was acting via somatodendritic receptors on dopamine neurons, i.e., pre-synaptic.

Subsequent experiments demonstrated that striatal nicotinic control of dopamine release is mediated predominantly by receptors containing the β2 subunit (Zhou et al., 2001), a finding supported by a report that α4β2 agonists stimulate dopamine and acetylcholine release in the hippocampus and frontal cortex in rats (Bontempi et al., 2001). There is also evidence that the roles of α7 and α4β2 receptors in the cognitive impairments associated with schizophrenia are mediated through the dopaminergic system. For example, haloperidol potentiated the memory deficits induced by a nicotinic antagonist. The deficit was potentiated by the D2 antagonist raclopride, but not the D1 antagonist SCH 23390 (McGurk et al., 1989), and reversed by D2 but not D1 agonists (Levin et al., 1989), suggesting this effect is due to D2 blockade. Furthermore, a combination of an acetylcholinesterase inhibitor and risperidone produced synergistic improvements of cognitive impairment and increased extracellular dopamine in the mouse prefrontal cortex. These effects were blocked by D1 and nicotinic antagonists but not a muscarinic antagonist (Wang et al., 2007), indicating the effect is independent of the muscarinic system, and that a combination of nicotinic and D1 agonism may improve cognition in people with schizophrenia, possibly via activation-dependent effects on the D1 receptor in the prefrontal cortex. Nicotine can improve attention and some aspects of positive symptoms in schizophrenia (Nisell et al., 1995), thus, it has been postulated that the higher rate of smoking observed in patients with schizophrenia may be a form of self-medication; enhancing cortical dopamine release.

Whilst it is apparent that nicotine can stimulate dopamine release (Marshall et al., 1997), the mechanism is complex, with glutamatergic transmission involved in nicotine-induced dopamine release from the striatum (Toth et al., 1992; Marshall et al., 1997). Local applications of NMDA antagonists significantly reduced the effect of nicotine (Toth et al., 1992). It was also demonstrated that nicotine elevated striatal glutamate, an effect blocked by a nicotinic antagonist (Toth et al., 1992). Together, these data suggest that presynaptic nicotinic receptors, on glutamatergic terminals, stimulate glutamate release which in turn acts on NMDA receptors on dopaminergic terminals to increase dopamine release (Toth et al., 1992; Marshall et al., 1997).

Dopaminergic regulation of cholinergic function

Early studies proposed that dopamine inhibits acetylcholine transmission, with the development of more specific ligands and newer techniques, it is now apparent that blockade of D1 receptors reduces acetylcholine release whilst activation stimulates release (Bertorelli and Consolo, 1990; Damsma et al., 1990; Consolo et al., 1992; Di Chiara et al., 1993). Conversely, activation of D2 receptors reduces acetylcholine release while inhibition of these receptors stimulates the release (Damsma et al., 1991). Studies have revealed a polymorphism in the untranslated region of the D1 receptor to be associated with nicotine dependence (Huang et al., 2008), alcohol dependence (Batel et al., 2008) and autism spectrum disorder (Hettinger et al., 2008). Interestingly, differential expression of the alleles was affected by a microRNA, miR-504 (Huang and Li, 2009), suggesting that as we discover more about the processes involved in the regulation of gene product expression our understanding of the mechanisms contributing to the pathophysiology of psychiatric disorders will also be expanded.

Acetylcholine and dopamine in schizophrenia

The lack of consensus regarding the status of dopaminergic receptors in schizophrenia makes it difficult to speculate as to whether they may impact on cholinergic function. Conversely, there is strong evidence to suggest that nicotine stimulates dopamine release, with receptors containing a β2 subunit playing a significant role (Zhou et al., 2001). Thus, given the reported increase of such receptors in schizophrenia (Martin-Ruiz et al., 2003), it is possible that one consequence is a facilitation of dopamine release, either directly or indirectly (Toth et al., 1992; Marshall et al., 1997). With respect to the muscarinic system, M1 receptors are consistently reported to be decreased in the brains from people with schizophrenia. Since M1 null mice have increased levels of striatal dopamine, it is possible that the low levels of M1 also contribute to an increased dopamine release. Finally, there is a single report of hippocampal M4 receptors being decreased in tissue from people with schizophrenia (Scarr et al., 2007), mice that lack M4 receptors appear to be hypersensitive to D1 stimulation. Unfortunately, given the disparity of data related to D1 receptors in schizophrenia, it is not clear whether the decrease in M4 receptors contributes to the imbalance of dopaminergic systems postulated to exist in schizophrenia. Therefore, it is possible, at this stage, to suggest that the changes in nicotinic and M1 receptors may play a role in the dopaminergic dysregulation. However, given the possibility that the glutamatergic system acts as an intermediary for some, if not all of the cholinergic regulation of dopamine function, we need to consider schizophrenia as a disorder of central neurotransmission, rather than focusing on particular combinations of neurotransmitters.

CHOLINERGIC INTERACTIONS WITH SEROTONIN

Central serotonergic systems

The central serotonergic system is widespread, innervating nearly all brain regions [see (Hornung, 2003) for review; **Figure 4**]

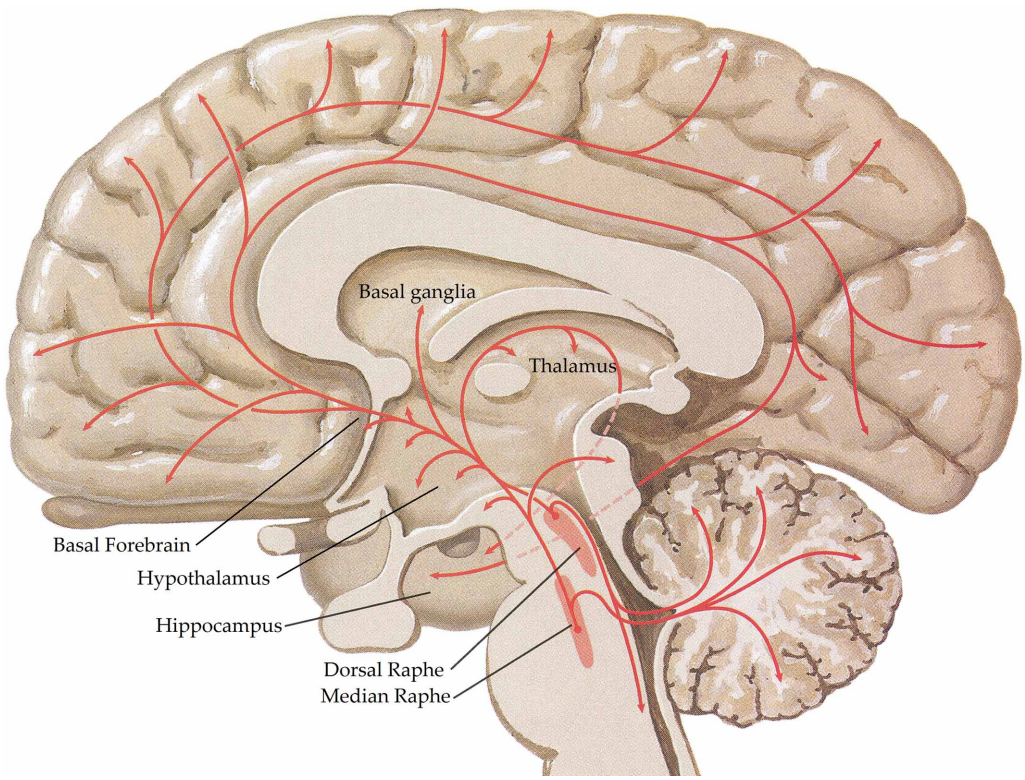

FIGURE 4 | Schematic representation of the human central serotonergic systems. Adapted from (Felten and Shetty, 2010).

and exerting its actions via 11 functional serotonergic receptors (Sharman et al., 2011). The majority of projections arise from the dorsal and median raphe nuclei, in the brainstem (Olszewski and Baxter, 1954), which innervate the amygdala, basal forebrain, hypothalamus, thalamus, caudate-putamen, cerebral cortex, and part of the hippocampus (Azmitia and Segal, 1978; Steinbusch, 1981). Axons of the dorsal raphe innervate all of the cerebral cortex with more dense innervation in the primary sensory areas (Wilson and Molliver, 1991) and, due to the lack of classic synapses, are thought to be involved in diffuse volume transmission rather than the targeted transmission associated with the axons from the median raphe, terminals of which are most abundant in the frontal cortex and hippocampus (Hornung, 2003). Whilst many structures are innervated by both dorsal and median raphe nuclei, the hippocampus receives predominantly median inputs whilst the thalamus, caudate, and putamen are heavily innervated by the dorsal raphe (Geyer et al., 1976).

The large family of serotonergic receptors gives the neurotransmitter an even greater functional capacity than is conferred by the diffuse serotonergic projections. In this review 5-ht_{5a}, and 5-ht_{1e} receptors are not considered because of their lack of a robust signal in native tissue. Of the remaining 11 receptors, most are metabotropic; 5-$HT_{4,6\&7}$ receptors canonically couple to G_s, increasing levels of cAMP; the 5-HT_1 receptors canonically couple with $G_{i/o}$ and reduce levels of cAMP whilst the 5-HT_2 receptors canonically couple to $G_{q/11}$ and increase inositol phosphate hydrolysis. The 5-HT_3 receptor is a pentameric ligand-gated cation channel; 5-HT_{3A} subunits can form functional homomeric receptors whilst the 5-$HT_{3B,C,D\&E}$ subunits form functional heteromeric receptors with 5-HT_{3A} subunits (Barnes et al., 2012). The diversity of the central serotonergic system means it regulates a range of processes including cognition and emotion (Buhot et al., 2000), as well as being implicated in the pathophysiologies of central nervous system disorders, particularly schizophrenia [see (Maris, 2002; Ohtsuki et al., 2002; Tanaka et al., 2003) for example].

Serotonin and schizophrenia

The first suggestion that serotonin might play a role in the pathophysiology of schizophrenia arose from the observation that lysergic acid diethylamide (LSD), a serotonergic agonist, caused psychoses which were proposed to have similarities to the positive symptoms of schizophrenia (Wooley and Shaw, 1954). After initial interest, the role of serotonin was largely unexplored until the advent of the second generation of antipsychotic drugs, with their high affinities for various serotonergic receptors, in particular as antagonists at the 5-HT_{2A} receptor (Meltzer, 1995). Although M-100907, a selective 5-HT_{2A} antagonist, failed to show an antipsychotic effect in phase III clinical trials (de Paulis, 2001) interest in the role of central serotonin in schizophrenia continues.

A number of studies looked at the major serotonin metabolite, 5-hydroxyindoleacetic acid, in cerebrospinal fluid from people with schizophrenia; the results are inconclusive, with reports of increases, decreases, and no change (see Abi-Dargham et al., 1997). Due to the rapid degradation of neurotransmitters and their metabolites, most studies have focused on markers of the serotonergic system as indirect indices of serotonergic function. In brief, the strongest indication that serotonin plays a role in the pathophysiology of schizophrenia is the widespread decreases reported in the 5-HT_{2A} receptor by multiple studies [see (Dean, 2003) for a comprehensive review]. Although a recent study reported increased cortical levels of 5-HT_{2A} receptors in the cortex of people with schizophrenia (Gonzalez-Maeso et al., 2008), a confound that was not adequately discussed was that 21 of the 25 subjects with schizophrenia had died by suicide compared to a 0% suicide rate in the control subjects. This is important because a number of studies have reported increased levels of central 5-HT_{2A} receptors in people who died as a result of suicide (see Stanley and Mann, 1983; Hrdina and Du, 2001; Pandey et al., 2002; Garbett et al., 2008; Klempan et al., 2009; for example). Therefore, it is not possible to determine whether the findings in this study relate to mode of death or the pathophysiology of schizophrenia. Given that the cholinergic and serotonergic systems are both implicated in the pathophysiology of schizophrenia and the considerable overlap between the two systems, it remains to be determined whether the changes in these systems are linked or whether they occur independently of each other.

Cholinergic regulation of serotonin

Projections from the medial septal nucleus and the diagonal band of Broca innervate the raphe nuclei (Kalén and Wiklund, 1989) in the rat, suggesting that the cholinergic system exerts a regulatory influence over the serotonergic network. However, a lack of [3H]choline in the basal forebrain nuclei following loading of the raphe nuclei and a lack of colocalization of the retrograde tracer with acetylcholinesterase immune reactivity in the basal forebrain lead the authors to suggest that few of the neurons projecting to the raphe were cholinergic. Later studies mapping afferents of the raphe nuclei did not identify the phenotypes of cells in the basal forebrain (Peyron et al., 1997). Conversely, a study mapping the projections of the pedunculopontine and laterodorsal tegmental nuclei of the brainstem found that cells positive for choline acetyltransferase projected to all of the raphe nuclei (Woolf and Butcher, 1989). These projections, coupled with the presence of nicotinic receptors on serotonergic neurons in the dorsal raphe (Cucchiaro et al., 2005; Galindo-Charles et al., 2008), suggest acetylcholine modulates the serotonergic system. Particularly since nicotine has been shown to increase serotonin release (Ma et al., 2005) and the firing rate of some serotonergic neurons in the dorsal raphe (Chang et al., 2011). However, it was previously shown that acetylcholine inhibits dorsal raphe neurons (Koyama and Kayama, 1993), suggesting that there may be a muscarinic component to the cholinergic modulation. An autoradiographic study, using a pan-muscarinic ligand, reported the presence of muscarinic receptors in the raphe (Cortes et al., 1984), supporting the concept that the cholinergic system may exert opposing effects on the serotoninergic system. Further complexity is added by the finding that approximately 90% of cholinergic neurons in the pedunculopontine and laterodorsal tegmental nuclei express 5-HT_{2A} receptors (Morilak and Ciaranello, 1993) and that serotonin has been shown to inhibit laterodorsal tegmental neurons (Koyama and Kayama, 1993); suggesting a feedback exists between the two systems.

Acetylcholine has been reported to stimulate serotonin release in the caudate via nicotinic (Becquet et al., 1988; Reuben and Clarke, 2000) but not muscarinic (Becquet et al., 1988) receptors. This effect was blocked by a GABA antagonist, indicating this might be an indirect effect, modulated by the GABAergic interneurons (File et al., 2000). Nicotine was shown to stimulate serotonin release in the hippocampus (Kenny et al., 2000) and frontal cortex (Ribeiro et al., 1993), an effect that was inhibited by methyllycaconitine in the hippocampus (Tucci et al., 2003), implicating the α7 nicotinic receptors. Interestingly, the muscarinic antagonist pirenzepine also stimulated hippocampal serotonin release (Kenny et al., 2000), suggesting that M1 or M4 may tonically inhibit hippocampal serotonin release. Together these data support the concept that the cholinergic system can enhance serotonergic activity via nicotinic receptors. Although there are indications that muscarinic receptors have an inhibitory role in serotonergic regulation, more research is required to address this hypothesis.

Serotonergic regulation of acetylcholine

The basal forebrain receives afferents from numerous systems, including serotonergic fibers from the dorsal raphe (Semba et al., 1988). An autoradiographic study revealed the presence of predominantly 5-HT_1 with fewer 5-HT_2 receptors in the basal forebrain (Zilles et al., 1991), an immunohistochemical study later identified 5-HT_{1A} receptors on the cholinergic neurons (Kia et al., 1996). Serotonin and 5-HT_{1A} agonists have been shown to cause hyperpolarisation of cholinergic cells (Khateb et al., 1993), suggesting that serotonin can regulate basal forebrain cholinergic neurons.

5-HT_{1A} agonists were also shown to facilitate acetylcholine release in the cortex (Bianchi et al., 1990; Katsu, 2001; Millan et al., 2004) and hippocampus (Lazaris et al., 2003; Millan et al., 2004). However, a similar effect is also seen with 5-HT_{1A} antagonists in both cortex (Kehr et al., 2010) and hippocampus (Schechter et al., 2005; Kehr et al., 2010), suggesting that the opposing actions may be mediated by direct and indirect mechanisms, possibly involving interneurons. In addition to the complexity of the 5-HT_{1A} receptor, the autoreceptor, the 5-$HT_{1B,1D}$ in guinea pigs and humans (Hoyer and Middlemiss, 1989), is proposed to tonically inhibit cholinergic neurons (Maura et al., 1989; Rutz et al., 2006) and stimulation of the 5-HT_3 receptors decreased acetylcholine release (Bianchi et al., 1990). Furthermore, activation of the 5-HT_{2A} (Nair and Gudelsky, 2004) and 5-HT_4 (Johnson et al., 2012) receptors increase cortical acetylcholine release, whilst stimulation of 5-HT_{2C} and 5-HT_7 receptors were shown to activate striatal cholinergic interneurons (Bonsi et al., 2007). Blockade of the 5-HT_6 receptors causes increases in acetylcholine release (West et al., 2009), suggesting they may be involved in the tonic inhibition of the cholinergic system. Since cholinergic neurons do not appear to express 5-HT_6 receptors (Marcos et al., 2006), the mechanism is probably an indirect one, possibly involving glutamatergic neurons. Although serotonin does appear to be capable of modulating the cholinergic system, the effect depends both upon the receptor stimulated and its localization, making the regulation extremely complex.

Acetylcholine and serotonin in schizophrenia

The most reproduced finding for the cholinergic system in schizophrenia is a decrease in central muscarinic receptors, in particular the M1, in people with the disorder [see (Scarr and Dean, 2008) for a comprehensive review]. There are indications that M1 and/or M4 may tonically inhibit serotonin release (Cortes et al., 1984; Kenny et al., 2000), thus it is possible the decreases in muscarinic receptors seen in schizophrenia reduce the tonic inhibition of serotonin, resulting in an over activation of the system with the potential to cause decreases in post-synaptic receptors, such as the 5-HT_{2A}, which have been reported in schizophrenia (see Dean, 2003) and increases in the pre-synaptic receptors, for example the 5-HT_{1D}, which have not been reported (Scarr et al., 2004; Dean et al., 2006).

Another reproducible finding in schizophrenia is decreased expression of nicotinic receptors (Martin-Ruiz et al., 2003; Severance and Yolken, 2008), since these receptors regulate serotonin release in the hippocampus and possibly cortex, a loss of these receptors may result in decreased serotonergic function, resulting in increased post-synaptic receptors and decreased pre-synaptic receptors. Thus, it is possible that the small overall increases reported in cortical 5-HT_{1A} receptors (Tauscher et al., 2002; Gray et al., 2006) are part of the serotonergic response to decreased nicotinic receptors.

The most widely reproduced finding for the serotonergic system in schizophrenia is the decrease in cortical 5-HT_{2A} receptors (see Dean, 2003). Since 5-HT_{2A} receptors modulate acetylcholine release in the cortex, it is possible that a loss would result in decreased cholinergic efflux. Such an event would be expected to cause an increase in postsynaptic receptors such as the muscarinic M1,3,4 and the α7 nicotinic receptors, which is not in keeping with the data on the pathophysiology of schizophrenia (see Scarr and Dean, 2008, 2009). Of the other serotonergic receptors implicated in the regulation of the cholinergic system, the 5-HT_{1A} receptor has been reported to be either increased (Hashimoto et al., 1991; Joyce et al., 1993; Burnet et al., 1997) or unchanged (Dean et al., 1999b; Cruz et al., 2004; Scarr et al., 2004), making it difficult to interpret the data. However, two studies have reported small global increases in cortical 5-HT_{1A} receptors (Tauscher et al., 2002; Gray et al., 2006), which may result in increased acetylcholine efflux and the subsequent down-regulation of postsynaptic receptors—an outcome consistent with the pathophysiology of schizophrenia (see Scarr and Dean, 2008, 2009). Cortical 5-HT_7 receptors have been reported to be decreased in schizophrenia (Dean et al., 2006), if their role in the cortex is similar to that in the striatum, this would be expected to result in a similar pattern to that described for decreased 5-HT_{2A} receptors, increased post-synaptic and decreased pre-synaptic cholinergic receptors, which does not fit with the pathophysiology of schizophrenia.

In summary, although the data that best fits with the current knowledge regarding the pathophysiology of schizophrenia is that reduced levels of muscarinic receptors contribute to a reduced inhibition of the serotonergic system and a subsequent decrease in post-synaptic serotonergic receptors, as seen with 5-HT_{2A} and 5-HT_7 receptors, this is overly simplistic given that these are not the only post-synaptic serotonergic receptors; the data regarding

5-HT_{1A} receptors in schizophrenia is inconclusive but there are no reports of decreased levels in schizophrenia. Studies that look at all components of both systems in a single cohort are required to reach definitive conclusions about the interactions of the two systems in schizophrenia.

Historically, the focus of the pathophysiology of psychiatric disorders has been on markers of neurotransmission. There is, however, growing data suggesting that molecules traditionally associated with a response to inflammation or infection are abnormally expressed in people with psychiatric disorders (see Potvin et al., 2008, for example). Thus, we finish the exploration of the interactions of the central cholinergic system and their relevance to biological psychiatry with a consideration of these pathways.

INTERACTIONS OF THE CHOLINERGIC SYSTEM WITH INFLAMMATORY AND IMMUNE PATHWAYS

A significant body of literature suggests there are physiologically relevant interactions between the cholinergic system (neuronal and non-neuronal) and inflammatory/immune pathways in the periphery (Bencherif et al., 2011; Pena et al., 2011; Verbout and Jacoby, 2012). Thus, it seems reasonable to hypothesize that similar interactions may be important in modulating central inflammatory-pathways. This portion of this review therefore focuses on the evidence to support such a hypothesis. However, in considering this data it is important to acknowledge there is a growing body of evidence to suggest that proteins involved in peripheral inflammatory/or immune processes may have more diverse roles in the central nervous system (Dean, 2011). This means that changes in individual proteins, linked to inflammatory or immune processes in the periphery, may not be indicative of a derangement of the same processes centrally.

CHOLINERGIC MODULATION OF CENTRAL INFLAMMATORY/IMMUNE SYSTEM

The hypothesis that the cholinergic system is involved in modulating central inflammatory and/or immune system is perhaps best tested at the systems level. With this regard, it is significant that microglial cells, which are widely viewed as resident macrophages in the central nervous system, express α7 nicotinic receptors, activation of which attenuates the pro-inflammatory responses in cultured microglial cells (Carnevale et al., 2007). These data appear to be relevant centrally because it has been shown that an α7 agonist, 3-(2,4-dimethoxybenzylidene) anabaseine, reduces tumor necrosis factor (TNF)-α release *in vivo* (Giebelen et al., 2007). Moreover, the relationship between the cholinergic system and, at least cytokines, seems to be a "whole of body" association since the same outcome has been observed in blood after treatment with an α7 agonist (Li et al., 2011). Interestingly, the activity of the α7 nicotinic receptor is reduced by kynurenic acid (Hilmas et al., 2001) via an as yet unknown mechanism. This is significant because kynurenines are components of pro-inflammatory pathways which have been suggested to be involved in the pathophysiology of a number of psychiatric disorders (Schwarcz et al., 2012). Thus, it is possible that α7 nicotinic receptors may be a convergence point for interactions between disparate inflammatory related pathways, providing a common route to inducing some of the symptoms of a number of psychiatric disorders. Whether or not this is proven to be the case, current evidence clearly supports a potential interaction between the central cholinergic system and an inflammatory/immune response as a mechanism involved in maintaining homeostasis within the central nervous system.

Additional evidence suggests that the ability of the cholinergic system to modulate the activity of microglia may be multifaceted; donepezil, a reversible, non-competitive cholinesterase inhibitor, has been shown to attenuate microglial production of nitric oxide and TNF, possibly by inhibiting the canonical inflammatory NF-κB signaling (Hwang et al., 2010). Whilst these data are difficult to interpret because the doses of donepezil used are higher than the therapeutic dose, they do reinforce a functional interaction between acetylcholine and the inflammatory and/or immune system. This interaction appears to be functional because rivastigmine, another cholinesterase inhibitor, has been shown to ameliorate the inflammation induced in experimental autoimmune encephalomyelitis (EAE) (Kawamata and Shimohama, 2011). In this study, it was shown that rivastigmine reduced demyelination, microglia activation, and axonal damage as well as the production of pro-inflammatory cytokines TNF, interferon γ (IFN) and interleukin (IL) −17. A third cholinesterase inhibitor, neostigmine, has similar effects on inflammatory/immune pathways (Tyagi et al., 2010), suggesting that this outcome is a drug class effect. Moreover, two studies showed these effects were abolished by an α7 antagonist (Kawamata and Shimohama, 2011; Tyagi et al., 2010) reinforcing the concept that the primary interaction between the central cholinergic system and microglia revolve around this receptor.

INFLAMMATORY/IMMUNE SYSTEM CONTROL OF CENTRAL CHOLINERGIC SYSTEM

As with all biological systems, there seems to be the potential for a two-way interaction between the inflammatory/immune systems and the nicotinic system. For example, it has been shown that IL-1β and TNF can alter nicotinic receptor sub-unit assembly (Gahring et al., 2005). In particular, these cytokines affect the way in which α4, β2, and β4 sub-units are incorporated into functional receptors; IL-1β enhancing α4/β2 and decreasing α4/β4 containing receptors, whereas TNF promotes α4/β2/β4 sub-unit containing receptors. It has long been known that changes in nicotinic receptor sub-unit assembly has functional consequences (Dingledine et al., 1999), therefore the effects of these cytokines would be expected to have an impact on central cholinergic neurotransmission. This hypothesis has some support as it has been shown in pre-clinical models that targeting nicotinic receptors with drugs that favor receptors of specific sub-unit composition have different therapeutic outcomes (Nirogi et al., 2011).

The physiological outcomes of the cholinergic system are usually determined by the balance between nicotinic and muscarinic receptors (Decker and McGaugh, 1991). At present there seems to be little evidence from central studies that such interactions are required in the regulation of inflammatory/immune systems but some data implicates muscarinic receptors as modulators of peripheral interactions (Sales, 2010). Focusing on the muscarinic

receptors, the M2 receptor seems to be an important modulator of inflammation/immune pathways in the lung (Costello et al., 1998; Fryer et al., 1999). This proposal is reinforced by the observation that mice which lack M2 receptors have problems with controlling infections and abnormal inflammatory responses (Turner et al., 2010). Significantly, similar to the data for nicotinic receptors, it seems that interactions between M2 and TNF are involved in the cross-talk between cholinergic and inflammatory/immune pathways in the lung (Nie et al., 2011). However, interactions between components of the inflammation/immune pathway and muscarinic receptors appear complex (see **Figure 5**). For instance, it has been shown that a synergistic action involving TNF and IL-1β reduces M2 expression (Haddad et al., 1996). The interactions between cytokines and muscarinic receptors seem to be quite extensive given the demonstration that IL-6 can reduce the amnesic effects of the muscarinic receptor antagonist, scopolamine (Bianchi et al., 1997). A recent study suggests that interactions between the cholinergic and inflammation/immune system may also involve the M3 receptor (Xu et al., 2012). However, mice lacking M3 have been reported to not have changes in inflammatory/immune responses (Matsui et al., 2000), suggesting a more tenuous link for the receptor in modulating inflammation/immune pathways. Moreover, some of these studies suggest that nicotinic and muscarinic receptors have opposing modulatory roles on the inflammation/immune systems (Razani-Boroujerdi et al., 2008).

INTERACTIONS BETWEEN THE TWO SYSTEMS IN PSYCHIATRIC DISORDERS

Somewhat surprisingly, it has been shown that activating central, but not peripheral, M2 receptors modulates levels of TNF in serum (Pavlov et al., 2006), suggesting that these molecules could regulate the interactions between central cholinergic and inflammation/immune systems. This is significant because our laboratory has reported cortical decreases in M2 (Gibbons et al., 2009) and increases in TNF-regulated pathways (Dean et al., 2010, 2012) in people with mood disorders. It is therefore intriguing as to whether these changes are independent of each other or reflect changes in the central cholinergic/inflammation/immune systems. Pursuing this hypothesis would be worthwhile given data showing that muscarinic agonists can reduce TNF levels in rodents (Pavlov and Tracey, 2006) and act as antidepressants (Drevets and Furey, 2010).

Given the clear relationship between the cholinergic and the inflammation/immune systems it remains to conceptualize a mechanism by which this can occur centrally. There are a number of options, one of which is that these changes are the result of interactions between the M2 and α7 receptors and inflammation/immune pathways within microglia. Significantly, it has been shown that carbachol, a pan-muscarinic receptor agonist, caused a rapid influx of calcium into microglia (Zhang et al., 1998), suggesting that they do express functional muscarinic receptors. Although a recent microarray study suggests microglia express both M2 and M3 receptors, it is not known

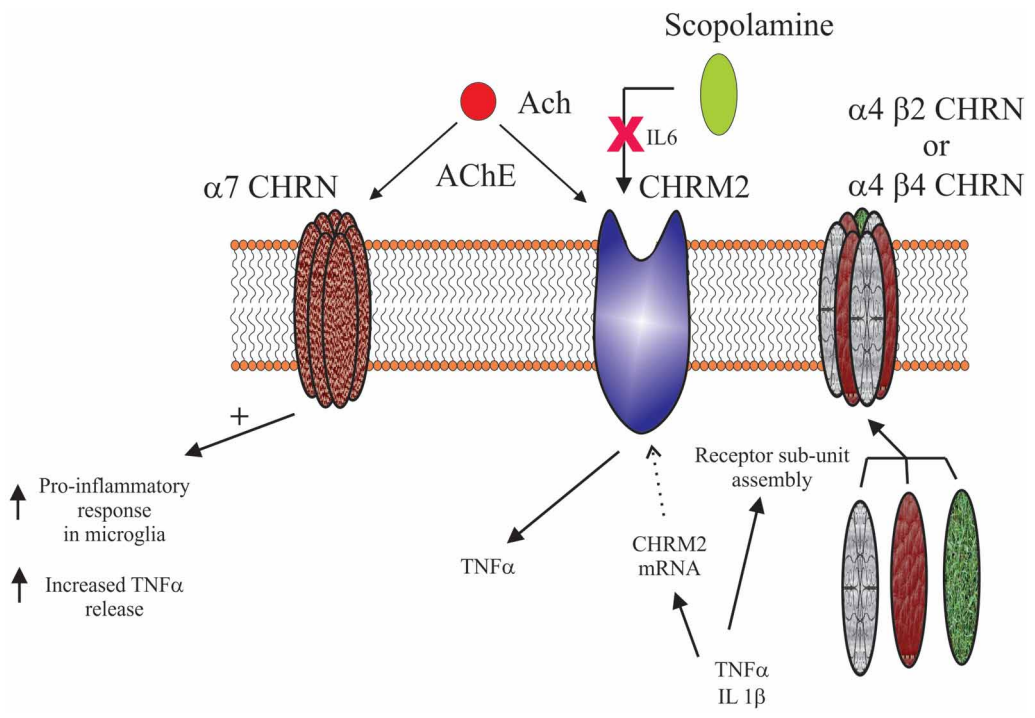

FIGURE 5 | Schematic showing the complex interactions between cholinergic receptors (α7 nicotinic receptor (α7 CHRN), muscarinic M2 receptor (CHRM2), α4 β2 nicotinic receptor (α4 β2 CHRN) and the α4 β4 nicotinic receptor (α4 β4 CHRN) and the cytokines; tumor necrosis factor α (TNFα), interleukin 1β (IL1β) and interleukin 6 (IL6). Current data suggests some of these interactions may involve cholinesterase inhibitors (AChE) and their ability to regulate acetylcholine (Ach) and the interactions of the muscarinic receptor antagonist scopolamine with CHRM2.

if this is the totality of their muscarinic component (Myers et al., 2009). It has also been reported that microglia, at least in culture, express α7 receptors (Shytle et al., 2004), providing further support for this hypothesis. Given that microglia also produce cytokines (Hanisch, 2002), it is not unreasonable to suggest that the cholinergic/inflammation/immune interactions occur within these cells. However, as cholinesterase inhibitors can also mediate the interaction between the cholinergic and inflammation/immune system (Hwang et al., 2010; Kawamata and Shimohama, 2011) this process appears to be activated by acetylcholine which, in the brain, is likely to be of neuronal origin. Furthermore, M2 and α7 receptors are expressed by neurons (Baghdoyan et al., 1998; Zarei et al., 1999), specifically GABAergic neurons (Azam et al., 2003) and astrocytes (Duffy et al., 2011; Roda et al., 2008). Thus, it is presumptive to assume microglia are the only cells modulating central cholinergic and inflammation/immune interactions. Given the growing recognition that the interactions between these systems may be important in the pathophysiologies of mood disorders, obtaining a better understanding of the mechanisms by which these interactions occur should be a priority.

CONCLUSION

To briefly summarise the potential interactions that might occur in psychiatric disorders, in schizophrenia where decreases in M1 are widely reported, these could result in reduced kainate function, which in turn could contribute to a glutamatergic hypofunction. The reduced α7 nicotinic capacity reported to exist in schizophrenia would result in reduced GABA efflux, with the potential to cause increased levels of postsynaptic GABAergic, such as $GABA_A$ receptors. An expected consequence of the increased levels of β2 containing nicotinic receptors and the decreased levels of M1 and/or M4 receptors is an increase in dopamine release, potentially contributing to the imbalance in dopaminergic systems proposed to exist in schizophrenia. With regards to the serotonergic system, the decreases in M1/M4 receptors seen in schizophrenia could cause an increase in serotonin release, which would cause the downregulation of postsynaptic receptors, including $5\text{-}HT_{2A}$. Conversely, if the small global increase in the $5\text{-}HT_{1A}$ receptors is substantiated, this could affect the cholinergic system causing increased cholinergic release, a consequence of which might be the downregulation of postsynaptic cholinergic receptors, including the M1 and M4. Finally, it is possible that the dysregulation of molecules traditionally associated with inflammation/immune responses in psychiatric disorders centers around disrupted interactions between the central cholinergic system, mediated by M2 and α7 receptors and microglia.

It is evident from these brief overviews that a dysfunctional central cholinergic system can have far reaching consequences. A common theme in considering these interactions is that the regulatory mechanisms are two-way systems, often with a third implicated as an intermediary. Thus, even considering the interactions between two systems is overly simplistic, suggesting that a whole systems approach is necessary to fully understand the relationships between central systems that become unstable in psychiatric disorders.

FUTURE DIRECTIONS

In this review, we identified the most commonly replicated changes in neurochemical markers associated with psychiatric disorders and interpreted them in the light of basic research elucidating interactions between the cholinergic and other central neurotransmitter systems. Acetylcholine was chosen as the pivotal transmitter system because of the extent of its innervations and because it is a target of choice for many drug development strategies aimed at novel therapies for psychiatric disorders. For example, acetylcholinesterase inhibitor use has expanded from their initial role of improving cognitive impairment in dementias (Hollander et al., 1986) to their specific use as adjuncts for the treatment of visual hallucinations (Patel et al., 2010; Abad et al., 2011). Efforts to target specific cholinergic receptors to provoke therapeutic outcomes are ongoing, with particular emphasis on the α7 nicotinic (Lieberman et al., 2013) and M1 muscarinic (Patel et al., 2010) receptors to improve cognitive performance. Meanwhile, attempts to develop new antipsychotic agents are focusing on the M4 muscarinic receptor (Leach et al., 2010). In mood disorders, the ability of scopolamine to ameliorate depressive symptoms, in people with major depressive and bipolar disorders (Drevets et al., 2012) has rejuvenated research into new targets for anti-depressant drugs. These developments, combined with the cholinergic regulation of the inflammation/immune system, which appears to play a role in the pathophysiology of psychiatric disorders, made the cholinergic system an obvious choice for the central factor in our review. Increasing our understanding of the interactions between the central neurotransmitter systems will provide alternative means of modulating systems rather than trying to target specific components of the system of interest—which may prove to be undruggable for various reasons. Such an approach has already been used in Parkinson's disease, where anti-cholinergic drugs were employed to ameliorate the tremor associated with the disorder.

One caveat of this review is that most of the data related to the neurochemical changes in psychiatric disorders has arisen from postmortem studies. Therefore, we cannot ascertain which of the chemical changes occurred first, hindering our attempts to construct a theory around these changes. Even if we could look at all of the markers detailed in this review in the same cohort of living people, whilst we would be able to confirm or disprove some of the proposed interactions, we still would not be able to determine the cause and effect relationship.

Furthermore, given the emphasis on the neurodevelopmental aspect of many of these disorders (Sigurdsson et al., 1999; Piper et al., 2012), we do not know at which stage in development such changes occurred. In order to gain a better understanding of the impact disruptions one neurochemical has on others, it would be necessary to model such changes in animals. Ideally, this approach would involve the sophisticated gene knockout techniques that are capable of targeting specific genes in a select group of neurons or tissue (Wess, 2012). Such a course would enable the dysregulation of individual components of neurotransmitter systems at any selected time point during development and allow researchers to assess the effects of disrupting specific interactions at these times. This, in turn would enable the identification of the specific components involved in the interactions between central systems and

provide an insight into the long term consequences of specific neurotransmitter system dysfunctions during development.

The development of new technologies and our increasing understanding of the processes involved in the translation from gene sequence to active product also offer a number of new approaches that can be utilized to improve our knowledge regarding the interactions between central transmitter systems. For example, the relatively new field of optogenetics—where light can be used to activate specific neurons—offers great scope to activate specific receptors in tissue of interest and identify the consequences of that activation. This approach will be of particular use in determining which receptors are involved in the cross-talk between transmitter systems, thereby circumventing the problems associated with using drugs that, although they have a high affinity for a particular receptor often have the capacity to stimulate or inhibit the actions of other receptors.

What was once a "simple" process of a gene being transcribed into RNA which was then translated into the corresponding protein is gradually being unraveled to reveal a far more complex series of events than previously imagined. We now know that factors such as gene methylation and histone modification (epigenetics) can determine whether or not a gene can be transcribed. Assuming the RNA is generated, the next step in the process can also be regulated, this time by microRNAs (miRNAs) which have the ability to block the translation of mRNA into proteins. Therefore, these factors also have to be taken into account when considering the interactions between central neurotransmitters, particularly since both epigenetics and miRNAs have been implicated in psychiatric disorders. For example, does the activation of one system affect the prevalence or type of epigenetic markers on the genes that encode components other systems? Will such changes in turn affect the fundamental regulation of expression for that gene? Does transmitter X affect the expression of particular miRNAs? If so, which of the myriad of theoretical interactions ascribed to each miRNA actually occur physiologically and of those, which are relevant to the process under investigation? The involvement of both miRNAs (Dwivedi, 2011; Banigan et al., 2013) and epigenetic markers (Zhao et al., 2012; Sun et al., 2013) in psychiatric disorders mean that a great deal of progress is being made in understanding the consequences of such factors. As screening protocols, such as the miRNA microarray, are developed, they can be applied to the study of interactions between central neurotransmitters, which in turn will feed into our understanding of the neurochemical changes associated with psychiatric disorders, paving the way for the development of targeted therapeutic approaches.

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REFERENCES

Abad, N. H., Doulatabad, N. S., Mohammadi, A., and Srazi, H. R. (2011). Treatment of Visual hallucinations in schizophrenia by acetylcholinesterase inhibitors: a case report. *Iran J. Psychiatry* 6, 161–163.

Abi-Dargham, A., Gil, R., Krystal, J., Baldwin, R. M., Seibyl, J. P., Bowers, M., et al. (1998). Increased striatal dopamine transmission in schizophrenia: confirmation in a second cohort. *Am. J. Psychiatry* 155, 761–767.

Abi-Dargham, A., Laruelle, M., Aghajanian, G. K., Charney, D., and Krystal, J. (1997). The role of serotonin in the pathophysiology and treatment of schizophrenia. *J. Neuropsychiatry Clin. Neurosci.* 9, 1–17.

Abi-Dargham, A., Mawlawi, O., Lombardo, I., Gil, R., Martinez, D., Huang, Y., et al. (2002). Prefrontal dopamine D1 receptors and working memory in schizophrenia. *J. Neurosci.* 22, 3708–3719.

Adler, L. E., Hoffer, L. J., Griffith, J., Waldo, M. C., and Freedman, R. (1992). Normalization by nicotine of deficient auditory sensory gating in the relatives of schizophrenics. *Biol. Psychiatry* 32, 607–616.

Akbarian, S., Sucher, N. J., Bradley, D., Tafazzoli, A., Trinh, D., Hetrick, W. P., et al. (1996). Selective alterations in gene expression for NMDA receptor subunits in prefrontal cortex of schizophrenics. *J. Neurosci.* 16, 19–30.

Albanese, A., Altavista, M. C., and Rossi, P. (1986). Organization of central nervous system dopaminergic pathways. *J. Neural Transm. Suppl.* 22, 3–17.

Albanese, A., and Minciacchi, D. (1983). Organization of the ascending projections from the ventral tegmental area: a multiple fluorescent retrograde tracer study in the rat. *J. Comp. Neurol.* 216, 406–420.

Allen, T. G. J., Abogadie, F. C., and Brown, D. A. (2006). Simultaneous release of glutamate and acetylcholine from single magnocellular "cholinergic" basal forebrain neurons. *J. Neurosci.* 26, 1588–1595.

Anderson, J. J., Kuo, S., and Chase, T. N. (1994). Endogenous excitatory amino acids tonically stimulate striatal acetlycholine release through NMDA but not AMPA receptors. *Neurosci. Lett.* 176, 264–268.

Aosaki, T., Kimura, M., and Graybiel, A. M. (1995). Temporal and spatial characteristics of tonically active neurons of the primate's striatum. *J. Neurophysiol.* 73, 1234–1252.

Atzori, M., Kanold, P. O., Pineda, J. C., Flores-Hernandez, J., and Paz, R. D. (2005). Dopamine prevents muscarinic-induced decrease of glutamate release in the auditory cortex. *Neuroscience* 134, 1153–1165.

Auning, E., Rongve, A., Fladby, T., Booij, J., Hortobágyi, T., Siepel, F. J., et al. (2011). Early and presenting symptoms of dementia with lewy bodies. *Dement. Geriatr. Cogn. Disord.* 32, 202–208.

Azam, L., Winzer-Serhan, U., and Leslie, F. M. (2003). Co-expression of alpha7 and beta2 nicotinic acetylcholine receptor subunit mRNAs within rat brain cholinergic neurons. *Neuroscience* 119, 965–977.

Azmitia, E. C., and Segal, M. (1978). An autoradiographic analysis of the differential ascending projections of the dorsal and median raphe nuclei in the rat. *J. Comp. Neurol.* 179, 641–667.

Baghdoyan, H. A., Lydic, R., and Fleegal, M. A. (1998). M2 muscarinic autoreceptors modulate acetylcholine release in the medial pontine reticular formation. *J. Pharmacol. Exp. Ther.* 286, 1446–1452.

Banigan, M. G., Kao, P. F., Kozubek, J. A., Winslow, A. R., Medina, J., Costa, J., et al. (2013). Differential expression of exosomal microRNAs in prefrontal cortices of schizophrenia and bipolar disorder patients. *PLoS ONE* 8:e48814. doi: 10.1371/journal.pone.0048814

Barnard, E. A., Skolnick, P., Olsen, R. W., Mohler, H., Sieghart, W., Biggio, G., et al. (1998). International union of pharmacology. XV. Subtypes of gamma-aminobutyric acid A receptors: classification on the basis of subunit structure and receptor function. *Pharmacol. Rev.* 50, 291–313.

Barnes, N. M., Andrade, R., Bockaert, J., Butler, A., Hamon, M., Hensler, J., et al. (2012). 5-Hydroxytryptamine receptors, introductory chapter. *IUPHAR-DB.* Available online at: http://www.iuphar-db.org/DATABASE/FamilyIntroductionForward?familyId=1. (Accessed November 20, 2012).

Batel, P., Houchi, H., Daoust, M., Ramoz, N., Naassila, M., and Gorwood, P. (2008). A haplotype of the DRD1 gene is associated with alcohol dependence. *Alcohol Clin. Exp. Res.* 32, 567–572.

Becquet, D., Faudon, M., and Hery, F. (1988). *In vivo* evidence for

acetylcholine control of serotonin release in the cat caudate nucleus: influence of halothane anaesthesia. *Neuroscience* 27, 819–826.

Bencherif, M., Lippiello, P. M., Lucas, R., and Marrero, M. B. (2011). Alpha7 nicotinic receptors as novel therapeutic targets for inflammation-based diseases. *Cell Mol. Life Sci.* 68, 931–949.

Bendor, J., Lizardi-Ortiz, J. E., Westphalen, R. I., Brandstetter, M., Hemmings, H. C., Jr., Sulzer, D., et al. (2010). AGAP1/AP-3-dependent endocytic recycling of M5 muscarinic receptors promotes dopamine release. *EMBO J.* 29, 2813–2826.

Benes, F. M., Khan, Y., Vincent, S. L., and Wickramasinghe, R. (1996a). Differences in the subregional and cellular distribution of GABAA receptor binding in the hippocampal formation of schizophrenic brain. *Synapse* 22, 338–349.

Benes, F. M., Vincent, S. L., Marie, A., and Khan, Y. (1996b). Up-regulation of GABAA receptor binding on neurons of the prefrontal cortex in schizophrenic subjects. *Neuroscience* 75, 1021–1031.

Benes, F. M., Lim, B., Matzilevich, D., Walsh, J. P., Subburaju, S., and Minns, M. (2007). Regulation of the GABA cell phenotype in hippocampus of schizophrenics and bipolars. *Proc. Natl. Acad. Sci. U.S.A.* 104, 10164–10169.

Benes, F. M., McSparren, J., Bird, E. D., SanGiovanni, J. P., and Vincent, S. L. (1991). Deficits in small interneurons in prefrontal and cingulate cortices of schizophrenic and schizoaffective patients. *Arch. Gen. Psychiatry* 48, 996–1001.

Benes, F. M., Vincent, S. L., Alsterberg, G., Bird, E. D., and SanGiovanni, J. P. (1992). Increased GABAA receptor binding in superficial layers of cingulate cortex in schizophrenics. *J. Neurosci.* 12, 924–929.

Beneyto, M., Kristiansen, L. V., Oni-Orisan, A., McCullumsmith, R. E., and Meador-Woodruff, J. H. (2007). Abnormal glutamate receptor expression in the medial temporal lobe in schizophrenia and mood disorders. *Neuropsychopharmacology* 32, 1888–1902.

Bennett, B. D., and Wilson, C. J. (1999). Spontaneous activity of neostriatal cholinergic interneurons *in vitro*. *J. Neurosci.* 19, 5586–5596.

Benueniste, M., Wilhelm, J., Dingledine, R. J., and Mott, D. D. (2010). Subunit-dependent modulation of kainate receptors by muscarinic acetylcholine receptors. *Brain Res.* 1352, 61–69.

Benwell, M. E. M., and Balfour, D. J. (1994). "Comparison of the effects of constant nicotine infusion on nucleus accumbens and stratal dopamine responses to acute nicotine," in *The Effects of Nicotine on Biological Systems II*. eds P. B. S. Clarke, M. Quik, K. Thurau, and F. Aldkofer (Basel: Birkauser Verlag), 66.

Bernard, V., Normand, E., and Bloch, B. (1992). Phenotypical characterization of the rat striatal neurons expressing muscarinic receptor genes. *J. Neurosci.* 12, 3591–3600.

Bertorelli, R., and Consolo, S. (1990). D1 and D2 dopaminergic regulation of acetylcholine release from striata of freely moving rats. *J. Neurochem.* 56, 2145–2148.

Bettler, B., Bowery, N. G., Cryan, J. F., Enna, S. J., Farb, D. H., Foestl, W., et al. (2012). GABAB receptors, introductory chapter. *IUPHAR-DB*. Available online at: http://www.iuphar-db.org/DATABASE/FamilyIntroductionForward?familyId=26. (Accessed December 4, 2012).

Bianchi, C., Ferraro, L., Tanganelli, S., Morari, M., Spalluto, G., Simonato, M., et al. (1995). 5-Hydroxytryptamine-mediated effects of nicotine on endogenous GABA efflux from guinea-pig cortical slices. *Br. J. Pharmacol.* 116, 2724–2728.

Bianchi, C., Siniscalchi, A., and Beani, L. (1990). 5-HT1A agonists increase and 5-HT3 agonists decrease acetylcholine efflux from the cerebral cortex of freely-moving guinea-pigs. *Br. J. Pharmacol.* 101, 448–452.

Bianchi, M., Ferrario, P., Clavenna, A., and Panerai, A. E. (1997). Interleukin-6 affects scopolamine-induced amnesia, but not brain amino acid levels in mice. *Neuroreport* 8, 1775–1778.

Bjoerke-Bertheussen, J., Ehrt, U., Rongve, A., Ballard, C., and Aarsland, D. (2012). Neuropsychiatric symptoms in mild dementia with lewy bodies and Alzheimer's disease. *Dement. Geriatr. Cogn. Disord.* 34, 1–6.

Blum, B. P., and Mann, J. J. (2002). The GABAergic system in schizophrenia. *Int. J. Neuropsychopharmacol.* 5, 159–179.

Bonsi, P., Cuomo, D., Ding, J., Sciamanna, G., Ulrich, S., Tscherter, A., et al. (2007). Endogenous serotonin excites striatal cholinergic interneurons via the activation of 5-HT 2C, 5-HT6, and 5-HT7 serotonin receptors: implications for extra pyramidal side effects of serotonin reuptake inhibitors. *Neuropsychopharmacology* 32, 1840–1854.

Bontempi, B., Whelan, K. T., Risbrough, V. B., Rao, T. S., Buccafusco, J. J., Lloyd, G. K., et al. (2001). SIB-1553A, (+/-)-4-[[2-(1-methyl-2-pyrrolidinyl)ethyl] thio]phenol hydrochloride, a subtype-selective ligand for nicotinic acetylcholine receptors with putative cognitive-enhancing properties: effects on working and reference memory performances in aged rodents and nonhuman primates. *J. Pharmacol. Exp. Ther.* 299, 297–306.

Brown, J. H., and Taylor, P. (1996). "Muscarinic receptor agonists and antagonists" in *Goodman & Gilman's The Pharmacological Basis of Therapeutics*. eds J. G. Hardman and L. E. Limbird (New York, NY: McGraw-Hill), 141–160.

Buchanan, R. W., Conley, R. R., Dickinson, D., Ball, M. P., Feldman, S., Gold, J. M., et al. (2008). Galantamine for the treatment of cognitive impairments in people with schizophrenia. *Am. J. Psychiatry* 165, 82–89.

Buchanan, R. W., Summerfelt, A., Tek, C., and Gold, J. (2003). An open-labeled trial of adjunctive donepezil for cognitive impairments in patients with schizophrenia. *Schizophr. Res.* 59, 29–33.

Buhot, M. C., Martin, S., and Segu, L. (2000). Role of serotonin in memory impairment. *Ann. Med.* 32, 210–221.

Burnet, P. W., Eastwood, S. L., and Harrison, P. J. (1997). [3H]WAY-100635 for 5-HT1A receptor autoradiography in human brain: a comparison with [3H]8-OH-DPAT and demonstration of increased binding in the frontal cortex in schizophrenia. *Neurochem. Int.* 30, 565–574.

Butcher, A. J., Torrecilla, I., Young, K. W., Kong, K. C., Mistry, S. C., Bottrill, A. R., et al. (2009). N-Methyl-D-aspartate receptors mediate the phosphorylation and desensitization of muscarinic receptors in cerebellar granule neurons. *J. Biol. Chem.* 284, 17147–17156.

Butcher, L. L., and Woolf, N. J. (1984). "Histochemical distribution of acetylcholinesterase in the central nervous system: clues to the localization of cholinergic neurons," in *Handbook of Chemical Neuroanatomy, Volume 3: Classical Transmitters and Transmitter Receptors in the CNS, Part II*, eds A. Björkland, T. Hökfelt, and M. J. Kuhar (Amsterdam: Elsevier), 1–50.

Bymaster, F. P., Wong, D. T., Mitch, C. H., Ward, J. S., Calligaro, D. O., Schoepp, D. D., et al. (1994). Neurochemical effects of the M1 muscarinic agonist xanomeline (LY246708/NNC11-0232). *J. Pharmacol. Exp. Ther.* 269, 282–289.

Cannon, D. M., Carson, R. E., Nugent, A. C., Eckelman, W. C., Kiesewetter, D. O., Williams, J., et al. (2006). Reduced muscarinic type 2 receptor binding in subjects with bipolar disorder. *Arch. Gen. Psychiatry* 63, 741–747.

Carlsson, A. (1974). Antipsychotic drugs and catecholamine synapses. *J. Psychiatr. Res.* 11, 57–64.

Carlsson, A. (1988). The current status of the dopamine hypothesis of schizophrenia. *Neuropsychopharmacology* 1, 179–186.

Carlsson, A., Hansson, L. O., Waters, N., and Carlsson, M. L. (1997). Neurotransmitter aberrations in schizophrenia: new perspectives and therapeutic implications. *Life Sci.* 61, 75–94.

Carnevale, D., De, S. R., and Minghetti, L. (2007). Microglia-neuron interaction in inflammatory and degenerative diseases: role of cholinergic and noradrenergic systems. *CNS Neurol. Disord. Drug Targets.* 6, 388–397.

Castner, S. A., Williams, G. V., and Goldman-Rakic, P. S. (2000). Reversal of antipsychotic-induced working memory deficits by short-term dopamine D1 receptor stimulation. *Science* 287, 2020–2022.

Challiss, R. A. J., and Tobin, A. B. (2009). Acetylcholine receptors (muscarinic), introductory chapter. *IUPHAR-DB*

Chang, B., Daniele, C. A., Gallagher, K., Madonia, M., Mitchum, R. D., Barrett, L., et al. (2011). Nicotinic excitation of serotonergic projections from dorsal raphe to the nucleus accumbens. *J. Neurophysiol.* 106, 801–808.

Consolo, S., Girotti, P., Russi, G., and Di Chiara, G. (1992). Endogenous dopamine facilitates striatal *in vivo* acetylcholine release by acting on D1 receptors localized in the striatum. *J. Neurochem.* 59, 1555–1557.

Corrigall, W. A. (1999). Nicotine self-administration in animals as a dependence model. *Nicotine Tob. Res.* 1, 11–20.

Corrigall, W. A., Franklin, K. B., Coen, K. M., and Clarke, P. B. (1992). The mesolimbic dopaminergic system is implicated in the reinforcing effects

of nicotine. *Psychopharmacology* 107, 285–289.

Cortes, R., Probst, A., and Palacios, J. M. (1984). Quantitative light microscopic autoradiographic localization of cholinergic muscarinic receptors in the human brain: brainstem. *Neuroscience* 12, 1003–1026.

Costello, R. W., Jacoby, D. B., and Fryer, A. D. (1998). Pulmonary neuronal M2 muscarinic receptor function in asthma and animal models of hyperreactivity. *Thorax* 53, 613–616.

Couturier, S., Bertrand, D., Matter, J. M., Hernandez, M. C., Bertrand, S., Millar, N., et al. (1990). A neuronal nicotinic acetylcholine receptor subunit (α7) is developmentally regulated and forms a homo-oligomeric channel blocked by aBTX. *Neuron* 5, 847–856.

Creese, I., Burt, D. R., and Snyder, S. H. (1976). Dopamine receptor binding predicts clinical and pharmacological potencies of antischizophrenic drugs. *Science* 192, 481–483.

Cruz, D. A., Eggan, S. M., Azmitia, E. C., and Lewis, D. A. (2004). Serotonin1A receptors at the axon initial segment of prefrontal pyramidal neurons in schizophrenia. *Am. J. Psychiatry* 161, 739–742.

Cucchiaro, G., Chaijale, N., and Commons, K. G. (2005). The dorsal raphe nucleus as a site of action of the antinociceptive and behavioral effects of the β4 nicotinic receptor agonist epibatidine. *J. Pharmacol. Exp. Ther.* 313, 389–394.

Curley, A. A., Arion, D., Volk, D. W., Asafu-Adjei, J. K., Sampson, A. R., Fish, K. N., et al. (2011). Cortical deficits of glutamic acid decarboxylase 67 expression in schizophrenia: clinical, protein, and cell type-specific features. *Am. J. Psychiatry* 168, 921–929.

Dale, H. H. (1914). The action of certain esters and ethers of choline and their relation to muscarine. *J. Pharmacol. Exp. Ther.* 6, 147–190.

Damsma, G., Day, J., and Fibiger, H. C. (1989). Lack of tolerance to nicotine-induced dopamine release in the nucleus accumbens. *Eur. J. Pharmacol.* 186, 363–368.

Damsma, G., Robertson, G. S., Tham, C. S., and Fibiger, H. C. (1991). Dopaminergic regulation of striatal acetylcholine release: importance of D1 and N-methyl-D-aspartate receptors. *J. Pharmacol. Exp. Ther.* 259, 1064–1072.

Damsma, G., Tham, C. S., Robertson, G. S., and Fibiger, H. C. (1990). Dopamine D1 receptor stimulation increases striatal acetylcholine release in the rat. *Eur. J. Pharmacol.* 186, 335–338.

Dasari, S., and Gulledge, A. T. (2011). M1 and M4 receptors modulate hippocampal pyramidal neurons. *J. Neurophysiol.* 105, 779–792.

De Klippel, N., Sarre, S., Ebinger, G., and Michotte, Y. (1993). Effect of M1- and M2-muscarinic drugs on striatal dopamine release and metabolism: an *in vivo* microdialysis study comparing normal and 6-hydroxydopamine-lesioned rats. *Brain Res.* 630, 57–64.

de Paulis, T. (2001). M-100907 (Aventis). *Curr. Opin. Investig. Drugs* 2, 123–132.

Dean, B. (2003). The cortical serotonin2A receptor and the pathology of schizophrenia: a likely accomplice. *J. Neurochem.* 85, 1–13.

Dean, B. (2011). Understanding the role of inflammatory-related pathways in the pathophysiology and treatment of psychiatric disorders: evidence from human peripheral studies and CNS studies. *Int. J. Neuropsychopharmacol.* 14, 997–1012.

Dean, B., Gibbons A. S., Tawadros, N., Brooks, L., Everall, I. P., and Scarr, E. (2012). Different changes in cortical tumour necrosis factor-related pathways in schizophrenia and mood disorders. *Mol. Psychiatry.* doi: 10.1038/mp.2012.95. [Epub ahead of print].

Dean, B., Hussain, T., Hayes, W., Scarr, E., Kitsoulis, S., Hill, C., et al. (1999a). Changes in serotonin2A and GABA(A) receptors in schizophrenia: studies on the human dorsolateral prefrontal cortex. *J. Neurochem.* 72, 1593–1599.

Dean, B., Tomaskovic-Crook, E., Opeskin, K., Keks, N., and Copolov, D. (1999b). No change in the density of the serotonin1A receptor, the serotonin4 receptor or the serotonin transporter in the dorsolateral prefrontal cortex from subjects with schizophrenia. *Neurochem. Int.* 34, 109–115.

Dean, B., Pavey, G., McLeod, M., Opeskin, K., Keks, N., and Copolov, D. (2001). A change in the density of [H-3]flumazenil, but not [H-3]muscimol binding, in Brodmann's Area 9 from subjects with bipolar disorder. *J. Affect. Disord.* 66, 147–158.

Dean, B., Pavey, G., Scarr, E., Goeringer, K., and Copolov, D. L. (2004). Measurement of dopamine D2-like receptors in postmortem CNS and pituitary: differential regional changes in schizophrenia. *Life Sci.* 74, 3115–3131.

Dean, B., Pavey, G., Thomas, D., and Scarr, E. (2006). Cortical serotonin7, 1D and 1F receptors: effects of schizophrenia, suicide and antipsychotic drug treatment. *Schizophr. Res.* 88, 265–274.

Dean, B., Tawadros, N., Scarr, E., and Gibbons, A. S. (2010). Regionally-specific changes in levels of tumour necrosis factor in the dorsolateral prefrontal cortex obtained post-mortem from subjects with major depressive disorder. *J. Affect. Disord.* 120, 245–248.

Decker, M. W., and McGaugh, J. L. (1991). The role of interactions between the cholinergic system and other neuromodulatory systems in learning and memory. *Synapse* 7, 151–168.

Del Arco, A., Segovia, G., and Mora, F. (2008). Blockade of NMDA receptors in the prefrontal cortex increases dopamine and acetylcholine release in the nucleus accumbens and motor activity. *Psychopharmacology* 201, 325–338.

Deng, C., and Huang, X. F. (2006). Increased density of GABAA receptors in the superior temporal gyrus in schizophrenia. *Exp. Brain Res.* 168, 587–590.

Deutch, A. Y., Clark, W. A., and Roth, R. H. (1990). Prefrontal cortical dopamine depletion enhances the responsiveness of mesolimbic dopamine neurons to stress. *Brain Res.* 521, 311–315.

Di Chiara, G., Carboni, E., Morelli, M., Cozzolino, A., Tanda, G. L., Pinna, A., et al. (1993). Stimulation of dopamine transmission in the dorsal caudate nucleus by pargyline as demonstrated by dopamine and acetylcholine microdialysis and Fos immunohistochemistry. *Neuroscience* 55, 451–456.

DiFiglia, M., Pasik, P., and Pasik, T. (1976). A Golgi study of neuronal types in the neostriatum of monkeys. *Brain Res.* 114, 245–256.

Dilsaver, S. C. (1986). Cholinergic mechanisms in depression. *Brain Res.* 396, 285–316.

Dingledine, R., Borges, K., Bowie, D., and Traynelis, S. F. (1999). The glutamate receptor ion channels. *Pharmacol. Rev.* 51, 7–61.

Dracheva, S., Elhakem, S. L., McGurk, S. R., Davis, K. L., and Haroutunian, V. (2004). GAD67 and GAD65 mRNA and protein expression in cerebrocortical regions of elderly patients with schizophrenia. *J. Neurosci. Res.* 76, 581–592.

Dracheva, S., Marras, S. A., Elhakem, S. L., Kramer, F. R., Davis, K. L., and Haroutunian, V. (2001). N-methyl-D-aspartic acid receptor expression in the dorsolateral prefrontal cortex of elderly patients with schizophrenia. *Am. J. Psychiatry* 158, 1400–1410.

Drevets, W. C., and Furey, M. L. (2010). Replication of scopolamine's antidepressant efficacy in major depressive disorder: a randomized, placebo-controlled clinical trial. *Biol. Psychiatry* 67, 432–438.

Drevets, W. C., Zarate, J., and Furey, M. L. (2012). Antidepressant effects of the muscarinic cholinergic receptor antagonist scopolamine: a review. *Biol. Psychiatry.* doi: 10.1016/j.biopsych.2012.09.031. [Epub ahead of print].

Duffy, A. M., Fitzgerald, M. L., Chan, J., Robinson, D. C., Milner, T. A., Mackie, K., et al. (2011). Acetylcholine alpha7 nicotinic and dopamine D2 receptors are targeted to many of the same postsynaptic dendrites and astrocytes in the rodent prefrontal cortex. *Synapse* 65, 1350–1367.

Dwivedi, Y. (2011). Evidence demonstrating role of microRNAs in the etiopathology of major depression. *J. Chem. Neuroanat.* 42, 142–156.

Dyer, M. A., Freudenreich, O., Culhane, M. A., Pachas, G. N., Deckersbach, T., Murphy, E., et al. (2008). High-dose galantamine augmentation inferior to placebo on attention, inhibitory control and working memory performance in nonsmokers with schizophrenia. *Schizophr. Res.* 102, 88–95.

Emilien, G., Maloteaux, J. M., Geurts, M., Hoogenberg, K., and Cragg, S. (1999). Dopamine receptors–physiological understanding to therapeutic intervention potential. *Pharmacol. Ther.* 84, 133–156.

Everitt, B. J., and Robbins, T. W. (1997). Central cholinergic systems and cognition. *Ann. Rev. Psychol.* 48, 649–684.

Fahn, S., Burke, R., and Stern, Y. (1990). Antimuscarinic drugs in the treatment of movement disorders. *Prog. Brain Res.* 84, 389–397.

Fatemi, S. H., Stary, J. M., Earle, J. A., Araghi-Niknam, M., and Eagan, E. (2005). GABAergic dysfunction in schizophrenia and mood disorders as reflected by decreased levels of glutamic acid decarboxylase 65 and 67 kDa and Reelin proteins in cerebellum. *Schizophr. Res.* 72, 109–122.

Felten, D. L., and Shetty, A. N. (2010). *Netter's Atlas of Neuroscience*. 2 Edn. Philapdelphia: Saunders; Elsevier, 438.

File, S. E., Kenny, P. J., and Cheeta, S. (2000). The role of the dorsal hippocampal serotonergic and cholinergic systems in the modulation of

anxiety. *Pharmacol. Biochem. Behav.* 66, 65–72.

Fisahn, A., Yamada, M., Duttaroy, A., Gan, J. W., Deng, C. X., McBain, C. J., et al. (2002). Muscarinic induction of hippocampal gamma oscillations requires coupling of the M1 receptor to two mixed cation currents. *Neuron* 33, 615–624.

Fisher, J. L., Pidoplichko, V. I., and Dani, J. A. (1998). Nicotine modifies the activity of ventral tegmental area dopaminergic neurons and hippocampal GABAergic neurons. *J. Physiol. Paris* 92, 209–213.

Forster, G. L., Yeomans, J. S., Takeuchi, J., and Blaha, C. D. (2002). M5 muscarinic receptors are required for prolonged accumbal dopamine release after electrical stimulation of the pons in mice. *J. Neurosci.* 22, RC190.

Fournier, G. N., Materi, L. M., Semba, K., and Rasmusson, D. D. (2004). Cortical acetylcholine release and electroencephalogram activation evoked by ionotropic glutamate receptor agonists in the rat basal forebrain. *Neuroscience* 123, 785–792.

Francis, P. T., and Perry, E. K. (2007). Cholinergic and other neurotransmitter mechanisms in Parkinson's disease, Parkinson's disease dementia, and dementia with Lewy bodies. *Mov. Disord.* 22, S351–S357.

Frank, E., Newell, K. A., and Huang, X. F. (2011). Density of metabotropic glutamate receptors 2 and 3 (mGluR2/3) in the dorsolateral prefrontal cortex does not differ with schizophrenia diagnosis but decreases with age. *Schizophr. Res.* 128, 56–60.

Freedman, R., Hall, M., Adler, L. E., and Leonard, S. (1995). Evidence in postmortem brain tissue for decreased numbers of hippocampal nicotinic receptors in schizophrenia. *Biol. Psychiatry* 38, 22–33.

Freedman, R., Olincy, A., Buchanan, R. W., Harris, J. G., Gold, J. M., Johnson, L., et al. (2008). Initial phase 2 trial of a nicotinic agonist in schizophrenia. *Am. J. Psychiatry* 165, 1040–1047.

Friedman, J. I. (2004). Cholinergic targets for cognitive enhancement in schizophrenia: focus on cholinesterase inhibitors and muscarinic agonists. *Psychopharmacology (Berl.)* 174, 45–53.

Fryer, A. D., Adamko, D. J., Yost, B. L., and Jacoby, D. B. (1999). Effects of inflammatory cells on neuronal M2 muscarinic receptor function in the lung. *Life Sci.* 64, 449–455.

Furey, M. L., and Drevets, W. C. (2006). Antidepressant efficacy of the antimuscarinic drug scopolamine: a randomized, placebo-controlled clinical trial. *Arch. Gen. Psychiatry* 63, 1121–1129.

Gahring, L. C., Days, E. L., Kaasch, T., Gonzalez de, M. M., Owen, L., Persiyanov, K., et al. (2005). Pro-inflammatory cytokines modify neuronal nicotinic acetylcholine receptor assembly. *J. Neuroimmunol.* 166, 88–101.

Galindo-Charles, L., Hernandez-Lopez, S., Galarraga, E., Tapia, D., Bargas, J., Garduño, J., et al. (2008). Serotoninergic dorsal raphe neurons possess functional postsynaptic nicotinic acetylcholine receptors. *Synapse* 62, 601–615.

Gao, X. M., Sakai, K., Roberts, R. C., Conley, R. R., Dean, B., and Tamminga, C. A. (2000). Ionotropic glutamate receptors and expression of N-methyl-D-aspartate receptor subunits in subregions of human hippocampus: effects of schizophrenia. *Am. J. Psychiatry* 157, 1141–1149.

Garbett, K., Gal-Chis, R., Gaszner, G., Lewis, D. A., and Mirnics, K. (2008). Transcriptome alterations in the prefrontal cortex of subjects with schizophrenia who committed suicide. *Neuropsychopharmacol. Hung.* 10, 9–14.

Gerber, D. J., Sotnikova, T. D., Gainetdinov, R. R., Huang, S. Y., Caron, M. G., and Tonegawa, S. (2001). Hyperactivity, elevated dopaminergic transmission, and response to amphetamine in M1 muscarinic acetylcholine receptor-deficient mice. *Proc. Natl. Acad. Sci. U.S.A.* 98, 15312–15317.

Gerfen, C. R., and Surmeier, D. J. (2011). Modulation of striatal projection systems by dopamine. *Annu. Rev. Neurosci.* 34, 441–466.

Gershon, S., and Shaw, F. H. (1961). Psychiatric sequelae of chronic exposure to organophosphorus insecticides. *Lancet* 1, 1371–1374.

Geyer, M. A., Puerto, A., Dawsey, W. J., Knapp, S., Bullard, W. P., and Mandell, A. J. (1976). Histologic and enzymatic studies of the mesolimbic and mesostriatal serotonergic pathways. *Brain Res.* 106, 241–256.

Gibbons, A. S., Scarr, E., Boer, S., Money, T., Jeon, W. J., Felder, C., et al. (2013). Widespread decreases in cortical muscarinic receptors in a subset of people with schizophrenia. *Int. J. Neuropsychopharmacol.* 16, 37–46.

Gibbons, A. S., Scarr, E., McLean, C., Sundram, S., and Dean, B. (2009). Decreased muscarinic receptor binding in the frontal cortex of bipolar disorder and major depressive disorder subjects. *J. Affect. Disord.* 116, 184–191.

Giebelen, I. A., van Westerloo, D. J., Larosa, G. J., de Vos, A. F., and van der Poll, T. (2007). Stimulation of alpha 7 cholinergic receptors inhibits lipopolysaccharide-induced neutrophil recruitment by a tumor necrosis factor alpha-independent mechanism. *Shock* 27, 443–447.

Goldman-Rakic, P. S., Castner, S. A., Svensson, T. H., Siever, L. J., and Williams, G. V. (2004). Targeting the dopamine D1 receptor in schizophrenia: insights for cognitive dysfunction. *Psychopharmacology (Berl.)* 174, 3–16.

Goldman-Rakic, P. S., Muly, E. C. III, and Williams, G. V. (2000). D(1) receptors in prefrontal cells and circuits. *Brain Res. Rev.* 31, 295–301.

Gomeza, J., Shannon, H., Kostenis, E., Felder, C., Zhang, L., Brodkin, J., et al. (1999a). Pronounced pharmacologic deficits in M2 muscarinic acetylcholine receptor knockout mice. *Proc. Natl. Acad. Sci. U.S.A.* 96, 1692–1697.

Gomeza, J., Zhang, L., Kostenis, E., Felder, C., Bymaster, F., Brodkin, J., et al. (1999b). Enhancement of D1 dopamine receptor-mediated locomotor stimulation in M(4) muscarinic acetylcholine receptor knockout mice. *Proc. Natl. Acad. Sci. U.S.A.* 96, 10483–10488.

Gonzalez-Maeso, J., Ang, R. L., Yuen, T., Chan, P., Weisstaub, N. V., Lopez-Gimenez, J. F., et al. (2008). Identification of a serotonin/glutamate receptor complex implicated in psychosis. *Nature* 452, 93–97.

Graveland, G. A., Williams, R. S., and DiFiglia, M. (1985). A Golgi study of the human neostriatum: neurons and afferent fibers. *J. Comp. Neurol.* 234, 317–333.

Gray, L., Scarr, E., and Dean, B. (2006). Serotonin 1a receptor and associated G-protein activation in schizophrenia and bipolar disorder. *Psychiatry Res.* 143, 111–120.

Gray, N. S., Pilowsky, L. S., Gray, J. A., and Kerwin, R. W. (1995). Latent inhibition in drug naive schizophrenics: relationship to duration of illness and dopamine D2 binding using SPET. *Schizophr. Res.* 17, 95–107.

Graybiel, A. M. (1990). Neurotransmitters and neuromodulators in the basal ganglia. *Trends Neurosci.* 13, 244–254.

Grilli, M., Summa, M., Salamone, A., Olivero, G., Zappettini, S., Di Prisco, S., et al. (2012). *In vitro* exposure to nicotine induces endocytosis of presynaptic AMPA receptors modulating dopamine release in rat nucleus accumbens nerve terminals. *Neuropharmacology* 63, 916–926.

Grilli, M., Zappettini, S., Raiteri, L., and Marchi, M. (2009). Nicotinic and muscarinic cholinergic receptors coexist on GABAergic nerve endings in the mouse striatum and interact in modulating GABA release. *Neuropharmacology* 56, 610–614.

Grishin, A. A., Benquet, P., and Gerber, U. (2005). Muscarinic receptor stimulation reduces NMDA responses in CA3 hippocampal pyramidal cells via Ca^{2+}-dependent activation of tyrosine phosphatase. *Neuropharmacology* 49, 328–337.

Grishin, A. A., Gee, C. E., Gerber, U., and Benquet, P. (2004). Differential calcium-dependent modulation of NMDA currents in CA1 and CA3 hippocampal pyramidal cells. *J. Neurosci.* 24, 350–355.

Gu, Z., and Yakel, J. (2011). Timing-dependent septal cholinergic induction of dynamic hippocampal synaptic plasticity. *Neuron* 71, 155–165.

Guidotti, A., Auta, J., Davis, J. M., Di-Giorgi-Gerevini, V., Dwivedi, Y., Grayson, D. R., et al. (2000). Decrease in reelin and glutamic acid decarboxylase67 (GAD67) expression in schizophrenia and bipolar disorder: a postmortem brain study. *Arch. Gen. Psychiatry* 57, 1061–1069.

Guillin, O., Abi-Dargham, A., and Laruelle, M. (2007). Neurobiology of dopamine in schizophrenia. *Int. Rev. Neurobiol.* 78, 1–39.

Guo, J. D., Hazra, R., Dabrowska, J., Muly, E. C., Wess, J., and Rainnie, D. G. (2012). Presynaptic muscarinic M-2 receptors modulate glutamatergic transmission in the bed nucleus of the stria terminalis. *Neuropharmacology* 62, 1671–1683.

Haddad, E. B., Rousell, J., Lindsay, M. A., and Barnes, P. J. (1996). Synergy between tumor necrosis factor alpha and interleukin 1beta in inducing transcriptional down-regulation of muscarinic M2 receptor gene expression. Involvement of protein kinase A and ceramide pathways. *J. Biol. Chem.* 271, 32586–32592.

Hakak, Y., Walker, J. R., Li, C., Wong, W. H., Davis, K. L., Buxbaum, J. D., et al. (2001). Genome-wide expression analysis reveals dysregulation of myelination-related genes

in chronic schizophrenia. *Proc. Natl. Acad. Sci. U.S.A.* 98, 4746–4751.

Hamam, B. N., Sinai, M., Poirier, G., and Chapman, C. A. (2007). Cholinergic suppression of excitatory synaptic responses in layer II of the medial entorhinal cortex. *Hippocampus* 17, 103–113.

Hamilton, S. E., Loose, M. D., Qi, M., Levey, A. I., Hille, B., McKnight, G. S., et al. (1997). Disruption of the m1 receptor gene ablates muscarinic receptor-dependent M current regulation and seizure activity in mice. *Proc. Natl. Acad. Sci. U.S.A.* 94, 13311–13316.

Hanada, S., Mita, T., Nishino, N., and Tanaka, C. (1987). [3H]muscimol binding sites increased in autopsied brains of chronic schizophrenics. *Life Sci.* 40, 259–266.

Hanisch, U. K. (2002). Microglia as a source and target of cytokines. *Glia* 40, 140–155.

Hashimoto, T., Nishino, N., Nakai, H., and Tanaka, C. (1991). Increase in serotonin 5-HT1A receptors in prefrontal and temporal cortices of brains from patients with chronic schizophrenia. *Life Sci.* 48, 355–363.

Healy, D. J., Haroutunian, V., Powchik, P., Davidson, M., Davis, K. L., Watson, S. J., et al. (1998). AMPA receptor binding and subunit mRNA expression in prefrontal cortex and striatum of elderly schizophrenics. *Neuropsychopharmacology* 19, 278–286.

Heckers, S., and Konradi, C. (2010). Hippocampal pathology in schizophrenia. *Curr. Top. Behav. Neurosci.* 4, 529–553.

Hersch, S. M., Gutekunst, C. A., Rees, H. D., Heilman, C. J., and Levey, A. I. (1994). Distribution of m1-m4 muscarinic receptor proteins in the rat striatum: light and electron microscopic immunocytochemistry using subtype-specific antibodies. *J. Neurosci.* 14, 3351–3363.

Hettinger, J. A., Liu, X., Schwartz, C. E., Michaelis, R. C., and Holden, J. J. (2008). A DRD1 haplotype is associated with risk for autism spectrum disorders in male-only affected sib-pair families. *Am. J. Med. Genet. B Neuropsychiatr. Genet.* 147B, 628–636.

Hilmas, C., Pereira, E. F., Alkondon, M., Rassoulpour, A., Schwarcz, R., and Albuquerque, E. X. (2001). The brain metabolite kynurenic acid inhibits alpha7 nicotinic receptor activity and increases non-alpha7 nicotinic receptor expression: physiopathological implications. *J. Neurosci.* 21, 7463–7473.

Hollander, E., Mohs, R. C., and Davis, K. L. (1986). Cholinergic approaches to the treatment of Alzheimer's disease. *Br. Med. Bull.* 42, 97–100.

Holt, D. J., Herman, M. M., Hyde, T. M., Kleinman, J. E., Sinton, C. M., German, D. C., et al. (1999). Evidence for a deficit in cholinergic interneurons in the striatum in schizophrenia. *Neuroscience* 94, 21–31.

Hornung, J. P. (2003). The human raphe nuclei and the serotonergic system. *J. Chem. Neuroanat.* 26, 331–343.

Hornykiewicz, O. (1981). "Brain neurotransmitter changes in Parkinson's disease" in *Movement Disorders* eds. Marsden, D. C. and S. Fahn (London, UK: Butterworth), 41–58.

Hoyer, D., and Middlemiss, D. N. (1989). Species differences in the pharmacology of terminal 5-HT autoreceptors in mammalian brain. *Trends Pharmacol. Sci.* 10, 130–132.

Hrdina, P. D., and Du, L. (2001). Levels of serotonin receptor 2A higher in suicide victims? *Am. J. Psychiatry* 158, 147–148.

Huang, W., and Li, M. D. (2009). Differential allelic expression of dopamine D1 receptor gene (DRD1) is modulated by microRNA miR-504. *Biol. Psychiatry* 65, 702–705.

Huang, W., Ma, J. Z., Payne, T. J., Beuten, J., Dupont, R. T., and Li, M. D. (2008). Significant association of DRD1 with nicotine dependence. *Hum. Genet.* 123, 133–140.

Hwang, J., Hwang, H., Lee, H. W., and Suk, K. (2010). Microglia signaling as a target of donepezil. *Neuropharmacology* 58, 1122–1129.

Hyland, N. P., and Cryan, J. F. (2010). A gut feeling about GABA: focus on GABA(B) receptors. *Front. Pharmacol.* 1:124. doi: 10.3389/fphar.2010.00124

Ichikawa, J., Chung, Y. C., Li, Z., Dai, J., and Meltzer, H. Y. (2002). Cholinergic modulation of basal and amphetamine-induced dopamine release in rat medial prefrontal cortex and nucleus accumbens. *Brain Res.* 958, 176–184.

Impagnatiello, F., Guidotti, A. R., Pesold, C., Dwivedi, Y., Caruncho, H., Pisu, M. G., et al. (1998). A decrease of reelin expression as a putative vulnerability factor in schizophrenia. *Proc Natl. Acad. Sci. U.S.A.* 95, 15718–15723.

Imperato, A., Mulas, A., and Di Chiara, G. (1986). Nicotine preferentially stimulates dopamine release in the limbic system of freely moving rats. *Eur. J. Pharmacol.* 132, 337–338.

Ishida, K., Murata, M., Kato, M., Utsunomiya, I., Hoshi, K., and Taguchi, K. (2005). Beta-phenylethylamine stimulates striatal acetylcholine release through activation of the AMPA glutamatergic pathway. *Biol. Pharm. Bull.* 28, 1626–1629.

Ishikawa, M., Mizukami, K., Iwakiri, M., and Asada, T. (2005). Immunohistochemical and immunoblot analysis of gamma-aminobutyric acid B receptor in the prefrontal cortex of subjects with schizophrenia and bipolar disorder. *Neurosci. Lett.* 383, 272–277.

Ishikawa, M., Mizukami, K., Iwakiri, M., Hidaka, S., and Asada, T. (2004). GABAA receptor gamma subunits in the prefrontal cortex of patients with schizophrenia and bipolar disorder. *Neuroreport* 15, 1809–1812.

Izzo, P. N., and Bolam, J. P. (1988). Cholinergic synaptic input to different parts of spiny striatonigral neurons in the rat. *J. Comp. Neurol.* 269, 219–234.

Javoy, F., Euvrard, C., Herbet, A., and Glowinski, J. (1977). Involvement of the dopamine nigrostriatal system in the picrotoxin effect on striatal acetylcholine levels. *Brain Res.* 126, 382–386.

Johnson, D. E., Drummond, E., Grimwood, S., Sawant-Basak, A., Miller, E., Tseng, E., et al. (2012). The 5-hydroxytryptamine4 receptor agonists prucalopride and PRX-03140 increase acetylcholineand histamine levels in the rat prefrontal cortex and the power of stimulated hippocampal θ oscillations. *J. Pharmacol. Exp. Ther.* 341, 681–691.

Johnston, G. A. (2005). GABA(A) receptor channel pharmacology. *Curr. Pharm. Des.* 11, 1867–1885.

Jones, C. K., Byun, N., and Bubser, M. (2012). Muscarinic and nicotinic acetylcholine receptor agonists and allosteric modulators for the treatment of schizophrenia. *Neuropsychopharmacology* 37, 16–42.

Joyce, J. N., Shane, A., Lexow, N., Winokur, A., Casanova, M. F., and Kleinman, J. E. (1993). Serotonin uptake sites and serotonin receptors are altered in the limbic system of schizophrenics [see comments]. *Neuropsychopharmacology* 8, 315–336.

Kalén, P., and Wiklund, L. (1989). Projections from the medial septum and diagonal band of Broca to the dorsal and central superior raphe nuclei: a non-cholinergic pathway. *Exp. Brain Res.* 75, 401–416.

Kamsler, A., McHugh, T. J., Gerber, D., Huang, S. Y., and Tonegawa, S. (2010). Presynaptic m1 muscarinic receptors are necessary for mGluR long-term depression in the hippocampus. *Proc. Natl. Acad. Sci. U.S.A.* 107, 1618–1623.

Kapur, S., and Remington, G. (2001). Dopamine D(2) receptors and their role in atypical antipsychotic action: still necessary and may even be sufficient. *Biol. Psychiatry* 50, 873–883.

Karlsson, P., Farde, L., Halldin, C., and Sedvall, G. (2002). PET study of D(1) dopamine receptor binding in neuroleptic-naive patients with schizophrenia. *Am. J. Psychiatry* 159, 761–767.

Karreman, M., and Moghaddam, B. (1996). The prefrontal cortex regulates the basal release of dopamine in the limbic striatum: an effect mediated by ventral tegmental area. *J. Neurochem.* 66, 589–598.

Katsu, H. (2001). Selective 5-HT1A receptor agonist, 8-OH-DPAT, locally administered into the dorsal raphe nucleus increased extracellular acetylcholine concentrations in the medial prefrontal cortex of conscious rats. *Nihon Shinkei Seishin Yakurigaku Zasshi* 21, 121–123.

Kawamata, J., and Shimohama, S. (2011). Stimulating nicotinic receptors trigger multiple pathways attenuating cytotoxicity in models of Alzheimer's and Parkinson's diseases. *J. Alzheimers. Dis.* 24(Suppl. 2), 95–109.

Kayadjanian, N., Retaux, S., Menetrey, A., and Besson, M. J. (1994). Stimulation by nicotine of the spontaneous release of [3H]gamma-aminobutyric acid in the substantia nigra and in the globus pallidus of the rat. *Brain Res.* 649, 129–135.

Keefe, R. S., Malhotra, A. K., Meltzer, H. Y., Kane, J. M., Buchanan, R. W., Murthy, A., et al. (2008). Efficacy and safety of donepezil in patients with schizophrenia or schizoaffective disorder: significant placebo/practice effects in a 12-week, randomized, double-blind, placebo-controlled trial. *Neuropsychopharmacology* 33, 1217–1228.

Kehr, J., Hu, X. J., Yoshitake, T., Wang, F. H., Osborne, P., Stenfors, C., et al. (2010). The selective 5-HT(1A) receptor antagonist NAD-299 increases acetylcholine release but not extracellular glutamate levels in the frontal cortex and hippocampus of awake rat. *Eur. Neuropsychopharmacol.* 20, 487–500.

Kenny, P. J., File, S. E., and Neal, M. J. (2000). Evidence for a complex influence of nicotinic acetylcholine receptors on hippocampal serotonin release. *J. Neurochem.* 75, 2409–2414.

Khateb, A., Fort, P., Alonso, A., Jones, B. E., and Mülethaler, M. (1993). Pharmacological and immunohistochemical evidence for serotonergic modulation of cholinergic nucleus basalis neurons. *Eur. J. Neurosci.* 5, 541–547.

Kia, H. K., Brisorgueil, M. J., Daval, G., Langlois, X., Hamon, M., and Verge, D. (1996). Serotonin1A receptors are expressed by a subpopulation of cholinergic neurons in the rat medial septum and diagonal band of Broca–a double immunocytochemical study. *Neuroscience* 74, 143–154.

Klein, J. C., Eggers, C., Kalbe, E., Weisenbach, S., Hohmann, C., Vollmar, S., et al. (2010). Neurotransmitter changes in dementia with Lewy bodies and Parkinson disease dementia *in vivo*. *Neurology* 74, 885–892.

Klempan, T. A., Sequeira, A., Canetti, L., Lalovic, A., Ernst, C., ffrench-Mullen, J., et al. (2009). Altered expression of genes involved in ATP biosynthesis and GABAergic neurotransmission in the ventral prefrontal cortex of suicides with and without major depression. *Mol. Psychiatry* 14, 175–189.

Knable, M. B., and Weinberger, D. R. (1997). Dopamine, the prefrontal cortex and schizophrenia. *J. Psychopharmacol.* 11, 123–131.

Koike, H., Fukumoto, K., Iijima, M., and Chaki, S. (2013). Role of BDNF/TrkB signaling in antidepressant-like effects of a group II metabotropic glutamate receptor antagonist in animal models of depression. *Behav. Brain Res.* 238, 48–52.

Kolachana, B. S., Saunders, R. C., and Weinberger, D. R. (1995). Augmentation of prefrontal cortical monoaminergic activity inhibits dopamine release in the caudate nucleus: an *in vivo* neurochemical assessment in the rhesus monkey. *Neuroscience* 69, 859–868.

Koos, T., and Tepper, J. M. (2002). Dual cholinergic control of fast-spiking interneurons in the neostriatum. *J. Neurosci.* 22, 529–535.

Koyama, Y., and Kayama, Y. (1993). Mutual interactions among cholinergic, noradrenergic and serotonergic neurons studied by ionophoresis of these transmitters in rat brainstem nuclei. *Neuroscience* 55, 1117–1126.

Ladinsky, H., Consolo, S., Bianchi, S., and Jori, A. (1976). Increase in striatal acetylcholine by picrotoxin in the rat: evidence for a gabergic-dopaminergic-cholinergic link. *Brain Res.* 108, 351–361.

Lahti, A. C., Weiler, M. A., Michaelidis, T., Parwani, A., and Tamminga, C. A. (2001). Effects of ketamine in normal and schizophrenic volunteers. *Neuropsychopharmacology* 25, 455–467.

Laruelle, M., Abi-Dargham, A., van Dyck, C. H., Gil, R., D'Souza, C. D., Erdos, J., et al. (1996). Single photon emission computerized tomography imaging of amphetamine-induced dopamine release in drug-free schizophrenic subjects. *Proc. Natl. Acad. Sci. U.S.A.* 93, 9235–9240.

Lazaris, A., Bertrand, F., Lazarus, C., Galani, R., Stemmelin, J., Poirier, R., et al. (2003). Baseline and 8-OH-DPAT-induced release of acetylcholine in the hippocampus of aged rats with different levels of cognitive dysfunction. *Brain Res.* 967, 181–190.

Le Moal, M., and Simon, H. (1991). Mesocorticolimbic dopaminergic network: functional and regulatory roles. *Physiol. Rev.* 71, 155–234.

Leach, K., Loiacono, R. E., Felder, C. C., McKinzie, D. L., Mogg, A., Shaw, D. B., et al. (2010). Molecular mechanisms of action and *in vivo* validation of an m(4) muscarinic acetylcholine receptor allosteric modulator with potential antipsychotic properties. *Neuropsychopharmacology* 35, 855–869.

Lee, T., Seeman, P., Tourtellotte, W. W., Farley, I. J., and Hornykeiwicz, O. (1978). Binding of 3H-neuroleptics and 3H-apomorphine in schizophrenic brains. *Nature* 274, 897–900.

Lena, C., Changeux, J. P., and Mulle, C. (1993). Evidence for "preterminal" nicotinic receptors on GABAergic axons in the rat interpeduncular nucleus. *J. Neurosci.* 13, 2680–2688.

Lester, D. B., Rogers, T. D., and Blaha, C. D. (2010). Acetylcholine-dopamine interactions in the pathophysiology and treatment of CNS disorders. *CNS Neurosci. Ther.* 16, 137–162.

Levin, E. D., McGurk, S. R., Rose, J. E., and Butcher, L. L. (1989). Reversal of a mecamylamine-induced cognitive deficit with the D2 agonist, LY 171555. *Pharmacol. Biochem. Behav.* 33, 919–922.

Lewis, D. A., Cho, R. Y., Carter, C. S., Eklund, K., Forster, S., Kelly, M. A., et al. (2008). Subunit-selective modulation of GABA type A receptor neurotransmission and cognition in schizophrenia. *Am. J. Psychiatry* 165, 1585–1593.

Li, D. P., and Pan, H. L. (2001). Potentiation of glutamatergic synaptic input to supraoptic neurons by presynaptic nicotinic receptors. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 281, R1105–R1113.

Li, J., Mathieu, S. L., Harris, R., Ji, J., Anderson, D. J., Malysz, J., et al. (2011). Role of alpha7 nicotinic acetylcholine receptors in regulating tumor necrosis factor-alpha (TNF-alpha) as revealed by subtype selective agonists. *J. Neuroimmunol.* 239, 37–43.

Lieberman, J. A., Dunbar, G., Segreti, A. C., Girgis, R. R., Seoane, F., Beaver, J. S., et al. (2013). A randomized exploratory trial of an alpha-7 nicotinic receptor agonist (TC-5619) for cognitive enhancement in schizophrenia. *Neuropsychopharmacology* 38, 968–975.

Lu, W. Y., Xiong, Z. G., Lei, S., Orser, B. A., Dudek, E., Browning, M. D., et al. (1999). G-protein-coupled receptors act via protein kinase C and Src to regulate NMDA receptors. *Nat. Neurosci.* 2, 331–338.

Lucas-Meunier, E., Fossier, P., Baux, G., and Amar, M. (2003). Cholinergic modulation of the cortical neuronal network. *Pflugers Arch. Eur. J. Physiol.* 446, 17–29.

Ma, Z., Strecker, R. E., McKenna, J. T., Thakkar, M. M., McCarley, R. W., and Tao, R. (2005). Effects on serotonin of (-)nicotine and dimethylphenylpiperazinium in the dorsal raphe and nucleus accumbens of freely behaving rats. *Neuroscience* 135, 949–958.

Mackay, A. V., Bird, E. D., Spokes, E. G., Rossor, M., Iversen, L. L., Creese, I., et al. (1980). Dopamine receptors and schizophrenia: drug effect or illness? *Lancet* 2, 915–916.

Mackay, A. V., Iversen, L. L., Rossor, M., Spokes, E., Bird, E., Arregui, A., et al. (1982). Increased brain dopamine and dopamine receptors in schizophrenia. *Arch. Gen. Psychiatry* 39, 991–997.

Maeng, S., Zarate, C. A., Du, J., Schloesser, R. J., McCammon, J., Chen, G., et al. (2008). Cellular mechanisms underlying the antidepressant effects of ketamine: Role of alpha-amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptors. *Biol. Psychiatry* 63, 349–352.

Mahmoodi, G., Ahmadi, S., Pourmotabbed, A., Oryan, S., and Zarrindast, M. R. (2010). Inhibitory avoidance memory deficit induced by scopolamine: interaction of cholinergic and glutamatergic systems in the ventral tegmental area. *Neurobiol. Learn. Mem.* 94, 83–90.

Mancama, D., Arranz, M. J., Landau, S., and Kerwin, R. (2003). Reduced expression of the muscarinic 1 receptor cortical subtype in schizophrenia. *Am. J. Med. Genet. B Neuropsychiatr. Genet.* 119, 2–6.

Mansour, A., and Watson, S. J. (1995)."Dopamine receptor expression in the central nervous system" in *Psychopharmacology – The Fourth Generation of Progress*. eds F. E. Bloom and D. J. Kupfer (New York, NY; Raven Press), 19.

Marchi, M., Bocchieri, P., Garbarino, L., and Raiteri, M. (1989). Muscarinic inhibition of endogenous glutamate release from rat hippocapmus synaptosomes. *Neurosci. Lett.* 96, 229–234.

Marchi, M., Sanguineti, P., and Raiteri, M. (1990). Muscarinic receptors mediate direct inhibition of GABA release from rat striatal nerve terminals. *Neurosci. Lett.* 116, 347–351.

Marcos, B., Gil-Bea, F. J., Hirst, W. D., García-Alloza, M., and Ramírez, M. J. (2006). Lack of localization of 5-HT6 receptors on cholinergic neurons: implication of multiple neurotransmitter systems in 5-HT6 receptor-mediated acetylcholine release. *Eur. J. Neurosci.* 24, 1299–1306.

Maris, R. W. (2002). Suicide. *Lancet* 360, 319–326.

Marshall, D. L., Redfern, P. H., and Wonnacott, S. (1997). Presynaptic nicotinic modulation of dopamine release in the three ascending pathways studied by *in vivo* microdialysis: comparison of naive and chronic nicotine-treated rats. *J. Neurochem.* 68, 1511–1519.

Martin-Ruiz, C. M., Haroutunian, V. H., Long, P., Young, A. H., Davis, K. L., Perry, E. K., et al. (2003). Dementia rating and nicotinic receptor expression in the prefrontal cortex in schizophrenia. *Biol. Psychiatry* 54, 1222–1233.

Marzella, P. L., Hill, C., Keks, N., Singh, B., and Copolov, D. (1997). The binding of both [3H]nemonapride and [3H]raclopride is increased in schizophrenia. *Biol. Psychiatry* 42, 648–654.

Matsui, M., Motomura, D., Karasawa, H., Fujikawa, T., Jiang, J., Komiya, Y., et al. (2000). Multiple functional defects in peripheral autonomic organs in mice lacking muscarinic acetylcholine receptor gene

for the M3 subtype. *Proc. Natl. Acad. Sci. U.S.A.* 97, 9579–9584.

Maura, G., Fedele, E., and Raiteri, M. (1989). Acetylcholine release from rat hippocampal slices is modulated by 5-hydroxytryptamine. *Eur. J. Pharmacol.* 165, 173–179.

McCullumsmith, R. E., Kristiansen, L. V., Beneyto, M., Scarr, E., Dean, B., and Meador-Woodruff, J. H. (2007). Decreased NR1, NR2A, and SAP102 transcript expression in the hippocampus in bipolar disorder. *Brain Res.* 1127, 108–118.

McGurk, S. R., Levin, E. D., and Butcher, L. L. (1988). Cholinergic-dopaminergic interactions in radial-arm maze performance. *Behav. Neural Biol.* 49, 234–239.

McGurk, S. R., Levin, E. D., and Butcher, L. L. (1989). Radial-arm maze performance in rats is impaired by a combination of nicotinic-cholinergic and D2 dopaminergic antagonist drugs. 99, *Psychopharmacology (Berl.)* 371–373.

Meltzer, H. Y. (1995). Role of serotonin in the action of atypical antipsychotic drugs. *J. Clin. Neurosci.* 3, 64–75.

Mifsud, J. C., Hernandez, L., and Hoebel, B. G. (1989). Nicotine infused into the nucleus accumbens increases synaptic dopamine as measured by *in vivo* microdialysis. *Brain Res.* 478, 365–367.

Millan, M. J., Gobert, A., Roux, S., Porsolt, R., Meneses, A., Carli, M., et al. (2004). The serotonin1A receptor partial agonist S15535 [4-(benzodioxan-5-yl)1-(indan-2-yl) piperazine] enhances cholinergic transmission and cognitive function in rodents: a combined neurochemical and behavioral analysis. *J. Pharmacol. Exp. Ther.* 311, 190–203.

Millar, N. S., Gotti, C., Marks, M. J., and Wonnacott, S. (2011). Nicotinic acetylcholine receptors, introductory chapter. *IUPHAR-DB.* Available online at: http://www.iuphar-db.org/DATABASE/FamilyIntroductionForward?familyId=76. (Accessed November 23, 2012).

Miller, A. D., and Blaha, C. D. (2005). Midbrain muscarinic receptor mechanisms underlying regulation of mesoaccumbens and nigrostriatal dopaminergic transmission in the rat. *Eur. J. Neurosci.* 21, 1837–1846.

Mita, T., Hanada, S., Nishino, N., Kuno, T., Nakai, H., Yamadori, T., et al. (1986). Decreased serotonin S2 and increased dopamine D2 receptors in chronic schizophrenics. *Biol. Psychiatry* 21, 1407–1414.

Miyakawa, T., Yamada, M., Duttaroy, A., and Wess, J. (2001). Hyperactivity and intact hippocampus-dependent learning in mice lacking the M1 muscarinic acetylcholine receptor. *J. Neurosci.* 21, 5239–5250.

Mizukami, K., Ishikawa, M., Hidaka, S., Iwakiri, M., Sasaki, M., and Iritani, S. (2002). Immunohistochemical localization of GABAB receptor in the entorhinal cortex and inferior temporal cortex of schizophrenic brain. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 26, 393–396.

Mizukami, K., Sasaki, M., Ishikawa, M., Iwakiri, M., Hidaka, S., Shiraishi, H., et al. (2000). Immunohistochemical localization of gamma-aminobutyric acid(B) receptor in the hippocampus of subjects with schizophrenia. *Neurosci. Lett.* 283, 101–104.

Morilak, D. A., and Ciaranello, R. D. (1993).5-HT2 receptor immunoreactivity on cholinergic neurons of the pontomesencephalic tegmentum shown by double immunofluorescence. *Brain Res.* 627, 49–54.

Myers, T. A., Kaushal, D., and Philipp, M. T. (2009). Microglia are mediators of Borrelia burgdorferi-induced apoptosis in SH-SY5Y neuronal cells. *PLoS Pathog.* 5:e1000659. doi: 10.1371/journal.ppat.1000659

Nair, S. G., and Gudelsky, G. A. (2004). Activation of 5-HT2 receptors enhances the release of acetylcholine in the prefrontal cortex and hippocampus of the rat. *Synapse* 53, 202–207.

Nakamura, M., and Jang, I. S. (2012). Muscarinic M4 receptors regulate GABAergic transmission in rat tuberomammillary nucleus neurons. *Neuropharmacology* 63, 936–944.

Nakamura, S., Goshima, Y., Yue, J. L., Miyamae, T., and Misu, Y. (1992). Transmitter-like 3,4-dihydroxyphenylalanine is tonically released by nicotine in striata of conscious rats. *Eur. J. Pharmacol.* 222, 75–80.

Navakkode, S., and Korte, M. (2012). Cooperation between cholinergic and glutamatergic receptors are essential to induce BDNF-dependent long-lasting memory storage. *Hippocampus* 22, 335–346.

Nelson, C. L., Sarter, M., and Bruno, J. P. (2005). Prefrontal cortical modulation of acetylcholine release in posterior parietal cortex. *Neuroscience* 132, 347–359.

Newell, K. A., Zavitsanou, K., Jew, S. K., and Huang, X. F. (2007). Alterations of muscarinic and GABA receptor binding in the posterior cingulate cortex in schizophrenia. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 31, 225–233.

Nie, Z., Scott, G. D., Weis, P. D., Itakura, A., Fryer, A. D., and Jacoby, D. B. (2011). Role of TNF-alpha in virus-induced airway hyperresponsiveness and neuronal M(2) muscarinic receptor dysfunction. *Br. J. Pharmacol.* 164, 444–452.

Nirogi, R., Jabaris, S. L., Jayarajan, P., Abraham, R., Shanmuganathan, D., Rasheed, M. A., et al. (2011). Antinociceptive activity of alpha4beta2 neuronal nicotinic receptor agonist A-366833 in experimental models of neuropathic and inflammatory pain. *Eur. J. Pharmacol.* 668, 155–162.

Nisell, M., Nomikos, G. G., Hertel, P., and Svensson, T. H. (1994a). "Acute nicotine administration differentially affects mesolimbic and mesocortical dopamine activity," in *The Effects of Nicotine on Biological Systems II*. eds P. B. S. Clarke, M. Quik, K. Thurau and F. Adkolfer, (Basel; Birkauser Verlag), 74.

Nisell, M., Nomikos, G. G., and Svensson, T. H. (1994b). Infusion of nicotine in the ventral tegmental area or the nucleus accumbens of the rat differentially affects accumbal dopamine release. *Pharmacol. Toxicol.* 75, 348–352.

Nisell, M., Nomikos, G. G., and Svensson, T. H. (1995). Nicotine dependence, midbrain dopamine systems and psychiatric disorders. *Pharmacol. Toxicol.* 76, 157–162.

Niswender, C. M., and Conn, P. J. (2010). Metabotropic glutamate receptors: physiology, pharmacology, and disease. *Ann. Rev. Pharmacol. Toxicol.* 50, 295–322.

Noback, C. R., Ruggerio, D. A., Demarest, R. J., and Strominger, N. L. (2005). "Reticular formation and limbic system" in *The Human Nervous System: Structure and Function*. eds C. R. Noback, D. A. Ruggerio, R. J. Demarest, and N. L. Strominger (New Jersey, NJ: Humana Press), 387–405.

Noga, J. T., Hyde, T. M., Bachus, S. E., Herman, M. M., and Kleinman, J. E. (2001). AMPA receptor binding in the dorsolateral prefrontal cortex of schizophrenics and controls. *Schizophr. Res.* 48, 361–363.

Noga, J. T., and Wang, H. (2002). Further postmortem autoradiographic studies of AMPA receptor binding in schizophrenia. *Synapse* 45, 250–258.

Ohtsuki, T., Ishiguro, H., Detera-Wadleigh, S. D., Toyota, T., Shimizu, H., Yamada, K., et al. (2002). Association between serotonin 4 receptor gene polymorphisms and bipolar disorder in Japanese case-control samples and the NIMH Genetics Initiative Bipolar Pedigrees. *Mol. Psychiatry* 7, 954–961.

Okubo, Y., Suhara, T., Suzuki, K., Kobayashi, K., Inoue, O., Terasaki, O., et al. (1997). Decreased prefrontal dopamine D1 receptors in schizophrenia revealed by PET. *Nature* 385, 634–636.

Olianas, M. C., Adem, A., Karlsson, E., and Onali, P. (1996). Rat striatal muscarinic receptors coupled to the inhibition of adenylyl cyclase activity: potent block by the selective m4 ligand muscarinic toxin 3 (MT3). *Br. J. Pharmacol.* 118, 283–288.

Olianas, M. C., and Onali, P. (1996). Antagonism of striatal muscarinic receptors inhibiting dopamine D1 receptor-stimulated adenylyl cyclase activity by cholinoceptor antagonist used to treat Parkinson's disease. *Br. J. Pharmacol.* 118, 827–828.

Olszewski, J., and Baxter, D. (1954). Cytoarchitecture of the human brainstem. By Jerzy Olszewski and Donald Baxter. *J. Comp. Neurol.* 101, 825–825.

Pandey, G. N., Dwivedi, Y., Rizavi, H. S., Ren, X., Pandey, S. C., Pesold, C., et al. (2002). Higher expression of serotonin 5-HT(2A) receptors in the postmortem brains of teenage suicide victims. *Am. J. Psychiatry* 159, 419–429.

Park, J. Y., and Spruston, N. (2012). Synergistic actions of metabotropic acetylcholine and glutamate receptors on the excitability of hippocampal CA1 pyramidal neurons. *J. Neurosci.* 32, 6081–6091.

Patel, S. S., Attard, A., Jacobsen, P., and Shergill, S. (2010). Acetylcholinesterase Inhibitors (AChEI's) for the treatment of visual hallucinations in schizophrenia: a case report. *BMC Psychiatry* 10:68. doi: 10.1186/1471-244X-10-68

Pavlov, V. A., Ochani, M., Gallowitsch-Puerta, M., Ochani, K., Huston, J. M., Czura, C. J., et al. (2006). Central muscarinic cholinergic regulation of the systemic inflammatory response during endotoxemia. *Proc. Natl. Acad. Sci. U.S.A.* 103, 5219–5223.

Pavlov, V. A., and Tracey, K. J. (2006). Controlling inflammation: the cholinergic anti-inflammatory pathway. *Biochem. Soc. Trans.* 34, 1037–1040.

Paylor, R., Nguyen, M., Crawley, J. N., Patrick, J., Beaudet, A., and Orr-Urtreger, A. (1998). α7 Nicotinic receptor subunits are not necessary

for hippocampal-dependent learning or sensorimotor gating: a behavioral characterization of acra7-deficient mice. *Learn. Mem.* 5, 302–316.

Pena, G., Cai, B., Ramos, L., Vida, G., Deitch, E. A., and Ulloa, L. (2011). Cholinergic regulatory lymphocytes re-establish neuromodulation of innate immune responses in sepsis. *J. Immunol.* 187, 718–725.

Perl, O., Ilani, T., Strous, R. D., Lapidus, R., and Fuchs, S. (2003). The alpha7 nicotinic acetylcholine receptor in schizophrenia: decreased mRNA levels in peripheral blood lymphocytes. *FASEB J.* 17, 1948–1950.

Perry, E., Walker, M., Grace, J., and Perry, R. (1999). Acetylcholine in mind: a neurotransmitter correlate of consciousness? *Trends Neurosci.* 22, 273–280.

Petroff, O. A., and Rothman, D. L. (1998). Measuring human brain GABA *in vivo*: effects of GABA-transaminase inhibition with vigabatrin. *Mol. Neurobiol.* 16, 97–121.

Peyron, C., Petit, J. M., Rampon, C., Jouvet, M., and Luppi, P. H. (1997). Forebrain afferents to the rat dorsal raphe nucleus demonstrated by retrograde and anterograde tracing methods. *Neuroscience* 82, 443–468.

Piper, M., Beneyto, M., Burne, T. H. J., Eyles, D. W., Lewis, D. A., and McGrath, J. J. (2012). The neurodevelopmental hypothesis of schizophrenia: convergent clues from epidemiology and neuropathology. *Psychiatr. Clin. North Am.* 35, 571–584.

Pontieri, F. E., Tanda, G., Orzi, F., and Di Chiara, G. (1996). Effects of nicotine on the nucleus accumbens and similarity to those of addictive drugs. *Nature* 382, 255–257.

Porter, R. H. P., Eastwood, S. L., and Harrison, P. J. (1997). Distribution of kainate receptor subunit mRNAs in human hippocampus, neocortex and cerebellum, and bilateral reduction of hippocampal GluR6 and KA2 transcripts in schizophrenia. *Brain Res.* 751, 217–231.

Potvin, S., Stip, E., Sepehry, A. A., Gendron, A., Bah, R., and Kouassi, E. (2008). Inflammatory cytokine alterations in schizophrenia: a systematic quantitative review. *Biol. Psychiatry* 63, 801–808.

Pycock, C. J., Kerwin, R. W., and Carter, C. J. (1980). Effect of lesion of cortical dopamine terminals on subcortical dopamine receptors in rats. *Nature* 286, 74–76.

Radcliffe, K. A., Fisher, J. L., Gray, R., and Dani, J. A. (1999). Nicotinic modulation of glutamate and GABA synaptic transmission of hippocampal neurons. *Ann. N. Y. Acad. Sci.* 868, 591–610.

Raedler, T. J., Bymaster, F. P., Tandon, R., Copolov, D., and Dean, B. (2006). Towards a muscarinic hypothesis of schizophrenia. *Mol. Psychiatry* 12, 232–246.

Raedler, T. J., Knable, M. B., Jones, D. W., Urbina, R. A., Gorey, J. G., Lee, K. S., et al. (2003). *In vivo* determination of muscarinic acetylcholine receptor availability in schizophrenia. *Am. J. Psychiatry* 160, 118–127.

Raiteri, M., Leardi, R., and Marchi, M. (1984). Heterogeneity of presynaptic muscarinic receptors regulating neurotransmitter release in the rat brain. *J. Pharmacol. Exp. Ther.* 228, 209–214.

Razani-Boroujerdi, S., Behl, M., Hahn, F. F., Pena-Philippides, J. C., Hutt, J., and Sopori, M. L. (2008). Role of muscarinic receptors in the regulation of immune and inflammatory responses. *J. Neuroimmunol.* 194, 83–88.

Reuben, M., and Clarke, P. B. S. (2000). Nicotine-evoked [3H]5-hydroxytryptamine release from rat striatal synaptosomes. *Neuropharmacology* 39, 290–299.

Reynolds, G. P., Riederer, P., Jellinger, K., and Gabriel, E. (1981). Dopamine receptors and schizophrenia: the neuroleptic drug problem. *Neuropharmacology* 20, 1319–1320.

Reynolds, L. M., and Reynolds, G. P. (2011). Differential regional N-acetylaspartate deficits in postmortem brain in schizophrenia, bipolar disorder and major depressive disorder. *J. Psychiatr. Res.* 45, 54–59.

Ribeiro, E. B., Bettiker, R. L., Bogdanov, M., and Wurtman, R. J. (1993). Effects of systemic nicotine on serotonin release in rat brain. *Brain Res.* 621, 311–318.

Roda, E., Coccini, T., Acerbi, D., Castoldi, A., Bernocchi, G., and Manzo, L. (2008). Cerebellum cholinergic muscarinic receptor (subtype-2 and -3) and cytoarchitecture after developmental exposure to methylmercury: an immunohistochemical study in rat. *J. Chem. Neuroanat.* 35, 285–294.

Rowntree, D. W., Nevin, S., and Wilson, A. (1950). The effects of diisopropylfluorophosphonate in schizophrenia and manic depressive psychosis. *J Neurol. Neurosurg. Psychiatry* 13, 47–62.

Rutz, S., Riegert, C., Rothmaier, A. K., Buhot, M. C., Cassel, J. C., and Jackisch, R. (2006). Presynaptic serotonergic modulation of 5-HT and acetylcholine release in the hippocampus and the cortex of 5-HT1B-receptor knockout mice. *Brain Res. Bull.* 70, 81–93.

Sales, M. E. (2010). Muscarinic receptors as targets for anti-inflammatory therapy. *Curr. Opin. Investig. Drugs* 11, 1239–1245.

Scarr, E. (2012). Muscarinic receptors: their roles in disorders of the central nervous system and potential as therapeutic targets. *CNS. Neurosci. Ther.* 18, 369–379.

Scarr, E., Beneyto, M., Meador-Woodruff, J. H., and Dean, B. (2005). Cortical glutamatergic markers in schizophrenia. *Neuropsychopharmacology* 30, 1521–1531.

Scarr, E., Cowie, T. F., Kanellakis, S., Sundram, S., Pantelis, C., and Dean, B. (2009). Decreased cortical muscarinic receptors define a subgroup of subjects with schizophrenia. *Mol. Psychiatry* 14, 1017–1023.

Scarr, E., and Dean, B. (2008). Muscarinic receptors: Do they have a role in the pathology of schizophrenia? *J. Neurochem.* 107, 1188–1195.

Scarr, E., and Dean, B. (2009). Role of the cholinergic system in the pathology and treatment of schizophrenia. *Expert. Rev. Neurother.* 9, 73–86.

Scarr, E., Pavey, G., Copolov, D., and Dean, B. (2004). Hippocampal 5-hydroxytryptamine receptors: abnormalities in postmortem brain from schizophrenic subjects. *Schizophr. Res.* 71, 383–392.

Scarr, E., Sundram, S., Keriakous, D., and Dean, B. (2007). Altered hippocampal muscarinic M4, but not M1, receptor expression from subjects with schizophrenia. *Biol. Psychiatry* 61, 1161–1170.

Scatton, B., and Bartholini, G. (1982). gamma-Aminobutyric acid (GABA) receptor stimulation. IV. Effect of progabide (SL 76002) and other GABAergic agents on acetylcholine turnover in rat brain areas. *J. Pharmacol. Exp. Ther.* 220, 689–695.

Schechter, L. E., Smith, D. L., Rosenzweig-Lipson, S., Sukoff, S. J., Dawson, L. A., Marquis, K., Jones, D., et al. (2005). Lecozotan (SRA-333): a selective serotonin 1A receptor antagonist that enhances the stimulated release of glutamate and acetylcholine in the hippocampus and possesses cognitive-enhancing properties. *J. Pharmacol. Exp. Ther.* 314, 1274–1289.

Schetz, J. A. (2009). Dopamine receptors, introductory chapter. *IUPHAR-DB.* Available online at: http://www.iuphar-db.org/DATABASE/FamilyIntroductionForward?familyId=20. (Accessed December 5, 2012).

Schoffelmeer, A. N., Van Vliet, B. J., Wardeh, G., and Mulder, A. H. (1986). Muscarine receptor-mediated modulation of [3H]dopamine and [14C]acetylcholine release from rat neostriatal slices: selective antagonism by gallamine but not pirenzepine. *Eur. J. Pharmacol.* 128, 291–294.

Schwarcz, R., Bruno, J. P., Muchowski, P. J., and Wu, H. Q. (2012). Kynurenines in the mammalian brain: when physiology meets pathology. *Nat. Rev. Neurosci.* 13, 465–477.

Seeman, P., Guan, H. C., and Van Tol, H. H. (1993). Dopamine D4 receptors elevated in schizophrenia. *Nature* 365, 441–445.

Seeman, P., Lee, T., Chau-Wong, M., and Wong, K. (1976). Antipsychotic drug doses and neuroleptic/dopamine receptors. *Nature* 261, 717–719.

Semba, K., Reiner, P. B., McGeer, E. G., and Fibiger, H. C. (1988). Brainstem afferents to the magnocellular basal forebrain studied by axonal transport, immunohistochemistry, and electrophysiology in the rat. *J. Comp. Neurol.* 267, 433–453.

Severance, E. G., and Yolken, R. H. (2008). Novel alpha7 nicotinic receptor isoforms and deficient cholinergic transcription in schizophrenia. *Genes Brain Behav.* 7, 37–45.

Sharman, J. L., Mpamhanga, C. P., Spedding, M., Germain, P., Staels, B., Dacquet, C., et al. (2011). IUPHAR-DB: new receptors and tools for easy searching and visualization of pharmacological data. *Nucleic Acids Res.* 39, D534–D538.

Shytle, R. D., Mori, T., Townsend, K., Vendrame, M., Sun, N., Zeng, J., et al. (2004). Cholinergic modulation of microglial activation by alpha 7 nicotinic receptors. *J. Neurochem.* 89, 337–343.

Sigurdsson, E., Fombonne, E., Sayal, K., and Checkley, S. (1999). Neurodevelopmental antecedents of early-onset bipolar affective disorder. *Br. J. Psychiatry* 174, 121–127.

Smolders, I., Bogaert, L., Ebinger, G., and Michotte, Y. (1997). Muscarinic modulation of striatal dopamine, glutamate, and GABA release, as measured with *in vivo* microdialysis. *J. Neurochem.* 68, 1942–1948.

Stanley, M., and Mann, J. J. (1983). Increased serotonin-2 binding sites in frontal cortex of suicide victims. *Lancet* 1, 214–216.

Steidl, S., Miller, A. D., Blaha, C. D., and Yeomans, J. S. (2011). M(5) muscarinic receptors mediate striatal dopamine activation by ventral tegmental morphine and pedunculopontine stimulation in mice. *PLoS ONE*, e27538. doi: 10.1371/journal.pone.0027538

Steiger, J. L., and Russek, S. J. (2004). GABAA receptors: building the bridge between subunit mRNAs, their promoters, and cognate transcription factors. *Pharmacol. Ther.* 101, 259–281.

Steinbusch, H. W. (1981). Distribution of serotonin-immunoreactivity in the central nervous system of the rat-cell bodies and terminals. *Neuroscience* 6, 557–618.

Sugita, S., Uchimura, N., Jiang, Z. G., and North, R. A. (1991). Distinct muscarinic receptors inhibit release of gamma-aminobutyric acid and excitatory amino acids in mammalian brain. *Proc. Natl. Acad. Sci. U.S.A.* 88, 2608–2611.

Sullivan, M. A., Chen, H., and Morikawa, H. (2008). Recurrent inhibitory network among striatal cholinergic interneurons. *J. Neurosci.* 28, 8682–8690.

Sumiyoshi, T., Stockmeier, C. A., Overholser, J. C., Thompson, P. A., and Meltzer, H. Y. (1995). Dopamine D4 receptors and effects of guanine nucleotides on [3H]raclopride binding in postmortem caudate nucleus of subjects with schizophrenia or major depression. *Brain Res.* 681, 109–116.

Sun, H., Kennedy, P. J., and Nestler, E. J. (2013). Epigenetics of the depressed brain: role of histone acetylation and methylation. *Neuropsychopharmacology* 38, 124–137.

Tanaka, S., Matsunaga, H., Kimura, M., Tatsumi, K., Hidaka, Y., Takano, T., et al. (2003). Autoantibodies against four kinds of neurotransmitter receptors in psychiatric disorders. *J. Neuroimmunol.* 141, 155–164.

Tandon, R., and Greden, J. F. (1989). Cholinergic hyperactivity and negative schizophrenic symptoms. A model of cholinergic/dopaminergic interactions in schizophrenia. *Arch Gen. Psychiatry* 46, 745–753.

Tauscher, J., Kapur, S., Verhoeff, N. P., Hussey, D. F., Daskalakis, Z. J., Tauscher-Wisniewski, S., et al. (2002). Brain serotonin 5-HT(1A) receptor binding in schizophrenia measured by positron emission tomography and [11C]WAY-100635. *Arch Gen. Psychiatry* 59, 514–520.

Tepper, J. M., Tecuapetla, F., Koos, T., and Ibanez-Sandoval, O. (2010). Heterogeneity and diversity of striatal GABAergic interneurons. *Front. Neuroanat.* 4:150. doi: 10.3389/fnana.2010.00150

Thompson, M., Weickert, C. S., Wyatt, E., and Webster, M. J. (2009). Decreased glutamic acid decarboxylase(67) mRNA expression in multiple brain areas of patients with schizophrenia and mood disorders. *J. Psychiatr. Res.* 43, 970–977.

Thomsen, M. S., Weyn, A., and Mikkelsen, J. D. (2011). Hippocampal +¦7 nicotinic acetylcholine receptor levels in patients with schizophrenia, bipolar disorder, or major depressive disorder. *Bipol. Disord.* 13, 701–707.

Threlfell, S., Clements, M. A., Khodai, T., Pienaar, I. S., Exley, R., Wess, J., et al. (2010). Striatal muscarinic receptors promote activity dependence of dopamine transmission via distinct receptor subtypes on cholinergic interneurons in ventral versus dorsal striatum. *J. Neurosci.* 30, 3398–3408.

Toro, C., and Deakin, J. F. (2005). NMDA receptor subunit NRI and postsynaptic protein PSD-95 in hippocampus and orbitofrontal cortex in schizophrenia and mood disorder. *Schizophr Res.* 80, 323–330.

Toth, E., Sershen, H., Hashim, A., Vizi, E. S., and Lajtha, A. (1992). Effect of nicotine on extracellular levels of neurotransmitters assessed by microdialysis in various brain regions: role of glutamic acid. *Neurochem. Res.* 17, 265–271.

Traynelis, S. F., Wollmuth, L. P., McBain, C. J., Menniti, F. S., Vance, K. M., Ogden, K. K., et al. (2010). Glutamate receptor ion channels: structure, regulation, and function. *Pharmacol. Rev.* 62, 405–496.

Tucci, S. A., Genn, R. F., and File, S. E. (2003). Methyllycaconitine (MLA) blocks the nicotine evoked anxiogenic effect and 5-HT release in the dorsal hippocampus: possible role of +¦7 receptors. *Neuropharmacology* 44, 367–373.

Turner, J., Hughes, L. F., and Toth, L. A. (2010). Sleep, activity, temperature and arousal responses of mice deficient for muscarinic receptor M2 or M4. *Life Sci.* 86, 158–169.

Tyagi, E., Agrawal, R., Nath, C., and Shukla, R. (2010). Cholinergic protection via alpha7 nicotinic acetylcholine receptors and PI3K-Akt pathway in LPS-induced neuroinflammation. *Neurochem. Int.* 56, 135–142.

Tzschentke, T. M. (2001). Pharmacology and behavioral pharmacology of the mesocortical dopamine system. *Prog. Neurobiol.* 63, 241–320.

van Elst, L. T., Valerius, G., Buchert, M., Thiel, T., Rusch, N., Bubl, E., et al. (2005). Increased prefrontal and hippocampal glutamate concentration in schizophrenia: evidence from a magnetic resonance spectroscopy study. *Biol. Psychiatry* 58, 724–730.

Verbout, N. G., and Jacoby, D. B. (2012). Muscarinic receptor agonists and antagonists: effects on inflammation and immunity. *Handb. Exp. Pharmacol.* 208, 403–427.

Vinson, P. N., and Conn, P. J. (2012). Metabotropic glutamate receptors as therapeutic targets for schizophrenia. *Neuropharmacology* 62, 1461–1472.

Volk, D., Austin, M., Pierri, J., Sampson, A., and Lewis, D. (2001). GABA transporter-1 mRNA in the prefrontal cortex in schizophrenia: decreased expression in a subset of neurons. *Am. J. Psychiatry* 158, 256–265.

Volk, D. W., Austin, M. C., Pierri, J. N., Sampson, A. R., and Lewis, D. A. (2000). Decreased glutamic acid decarboxylase67 messenger RNA expression in a subset of prefrontal cortical gamma-aminobutyric acid neurons in subjects with schizophrenia. *Arch Gen. Psychiatry* 57, 237–245.

Volk, D. W., Eggan, S. M., and Lewis, D. A. (2010). Alterations in Metabotropic Glutamate Receptor 1 alpha and Regulator of G Protein Signaling 4 in the Prefrontal Cortex in Schizophrenia. *Am. J. Psychiatry* 167, 1489–1498.

Volk, D. W., Pierri, J. N., Fritschy, J. M., Auh, S., Sampson, A. R., and Lewis, D. A. (2002). Reciprocal alterations in pre- and postsynaptic inhibitory markers at chandelier cell inputs to pyramidal neurons in schizophrenia. *Cereb. Cortex* 12, 1063–1070.

Wang, D., Noda, Y., Zhou, Y., Nitta, A., Furukawa, H., and Nabeshima, T. (2007). Synergistic effect of combined treatment with risperidone and galantamine on phencyclidine-induced impairment of latent visuospatial learning and memory: Role of nAChR activation-dependent increase of dopamine D1 receptor-mediated neurotransmission. *Neuropharmacology* 53, 379–389.

Wang, H., Ng, K., Hayes, D., Gao, X., Forster, G., Blaha, C., et al. (2004). Decreased amphetamine-induced locomotion and improved latent inhibition in mice mutant for the M5 muscarinic receptor gene found in the human 15q schizophrenia region. *Neuropsychopharmacology* 29, 2126–2139.

Weiner, D. M., Levey, A. I., and Brann, M. R. (1990). Expression of muscarinic acetylcholine and dopamine receptor mRNAs in rat basal ganglia. *Proc. Natl. Acad. Sci. U.S.A.* 87, 7050–7054.

Wess, J. (2012). "Novel Muscarinic Receptor Mutant Mouse Models". in *Muscarinic receptors.* eds A. D. Fryer, A. Christopoulos, and N. M. Nathanson (Heidleberg; Berlin: Springer), 95–117.

West, P. J., Marcy, V. R., Marino, M. J., and Schaffhauser, H. (2009). Activation of the 5-HT6 receptor attenuates long-term potentiation and facilitates GABAergic neurotransmission in rat hippocampus. *Neuroscience* 164, 692–701.

Whiting, P. J. (2003). GABA-A receptor subtypes in the brain: a paradigm for CNS drug discovery? *Drug Discov. Today* 8, 445–450.

Wilkinson, L. S. (1997). The nature of interactions involving prefrontal and striatal dopamine systems. *J. Psychopharmacol.* 11, 143–150.

Wilson, M. A., and Molliver, M. E. (1991). The organization of serotonergic projections to cerebral cortex in primates: regional distribution of axon terminals. *Neuroscience* 44, 537–553.

Witten, I. B., Lin, S. C., Brodsky, M., Prakash, R., Diester, I., Anikeeva, P., Gradinaru, V., Ramakrishnan, C., and Deisseroth, K. (2010). Cholinergic Interneurons Control Local Circuit Activity and Cocaine Conditioning. *Science* 330, 1677–1681.

Wonnacott, S., Barik, J., Dickinson, J., and Jones, I. W. (2006). Nicotinic receptors modulate transmitter cross talk in the CNS: nicotinic modulation of transmitters. *J. Mol. Neurosci.* 30, 137–140.

Woo, T. U., Shrestha, K., Amstrong, C., Minns, M. M., Walsh, J. P., and Benes, F. M. (2007). Differential alterations of kainate receptor subunits in inhibitory interneurons in the anterior cingulate cortex in schizophrenia and bipolar disorder. *Schizophr. Res.* 96, 46–61.

Woo, T. U., Walsh, J. P., and Benes, F. M. (2004). Density of glutamic acid decarboxylase 67 messenger RNA-containing neurons that express

the N-methyl-D-aspartate receptor subunit NR2A in the anterior cingulate cortex in schizophrenia and bipolar disorder. *Arch Gen. Psychiatry*, 61, 649–657.

Wooley, D. W., and Shaw, E. (1954). A biological and pharmacological suggestion about certain mental disorders. *Proc. Natl. Acad. Sci. U.S.A.* 40, 228–231.

Woolf, N. J. (1991). Cholinergic systems in mammalian brain and spinal cord. *Prog. Neurobiol.* 37, 475–524.

Woolf, N. J., and Butcher, L. L. (1989). Cholinergic systems in the rat brain: IV. descending projections of the pontomesencephalic tegmentum. *Brain Res. Bull.* 23, 519–540.

Xu, Z. P., Yang, K., Xu, G. N., Zhu, L., Hou, L. N., Zhang, W. H., et al. (2012). Role of M3 mAChR *in vivo* and *in vitro* models of LPS-induced inflammatory response. *Int. Immunopharmacol.* 14, 320–327.

Yamada, M., Basile, A. S., Fedorova, I., Zhang, W., Duttaroy, A., Cui, Y., et al. (2003). Novel insights into M5 muscarinic acetylcholine receptor function by the use of gene targeting technology. *Life Sci.* 74, 345–353.

Yamada, M., Lamping, K. G., Duttaroy, A., Zhang, W., Cui, Y., Bymaster, F. P., et al. (2001a). Cholinergic dilation of cerebral blood vessels is abolished in M(5) muscarinic acetylcholine receptor knockout mice. *Proc. Natl. Acad. Sci. U.S.A.* 98, 14096–14101.

Yamada, M., Miyakawa, T., Duttaroy, A., Yamanaka, A., Moriguchi, T., Makita, R., et al. (2001b). Mice lacking the M3 muscarinic acetylcholine receptor are hypophagic and lean. *Nature* 410, 207–212.

Yasuda, R. P., Ciesla, W., Flores, L. R., Wall, S. J., Li, M., Satkus, S. A., et al. (1993). Development of antisera selective for m4 and m5 muscarinic cholinergic receptors: distribution of m4 and m5 receptors in rat brain. *Mol. Pharmacol.* 43, 149–157.

Yeomans, J. S. (1995). Role of tegmental cholinergic neurons in dopaminergic activation, antimuscarinic psychosis and schizophrenia. *Neuropsychopharmacology* 12, 3–16.

Zarei, M. M., Radcliffe, K. A., Chen, D., Patrick, J. W., and Dani, J. A. (1999). Distributions of nicotinic acetylcholine receptor alpha7 and beta2 subunits on cultured hippocampal neurons. *Neuroscience* 88, 755–764.

Zavitsanou, K., Katsifis, A., Mattner, F., and Xu-Feng, H. (2004). Investigation of m1/m4 muscarinic receptors in the anterior cingulate cortex in schizophrenia, bipolar disorder, and major depression disorder. *Neuropsychopharmacology* 29, 619–625.

Zhang, L., McLarnon, J. G., Goghari, V., Lee, Y. B., Kim, S. U., and Krieger, C. (1998). Cholinergic agonists increase intracellular Ca^{2+} in cultured human microglia. *Neurosci. Lett.* 255, 33–36.

Zhang, L. M., and Warren, R. A. (2002). Muscarinic and nicotinic presynaptic modulation of EPSCs in the nucleus accumbens during postnatal development. *J. Neurophysiol.* 88, 3315–3330.

Zhang, W., Yamada, M., Gomeza, J., Basile, A. S., and Wess, J. (2002). Multiple muscarinic acetylcholine receptor subtypes modulate striatal dopamine release, as studied with M1-M5 muscarinic receptor knock-out mice. *J. Neurosci.* 22, 6347–6352.

Zhao, C., Wang, F., Pun, F. W., Mei, L., Ren, L., Yu, Z., et al. (2012). Epigenetic regulation on GABRB2 isoforms expression: Developmental variations and disruptions in psychotic disorders. *Schizophr. Res.* 134, 260–266.

Zheng, F., and Johnson, S. W. (2003). Metabotropic glutamate and muscarinic cholinergic receptor-mediated preferential inhibition of N-methyl-D-aspartate component of transmissions in rat ventral tegmental area. *Neuroscience* 116, 1013–1020.

Zhou, F. M., Liang, Y., and Dani, J. A. (2001). Endogenous nicotinic cholinergic activity regulates dopamine release in the striatum. *Nat. Neurosci.* 4, 1224–1229.

Zilles, K., Werner, L., Qü, M., Schleicher, A., and Gross, G. (1991). Quantitative auto radiography of 11 different transmitter binding sites in the basal forebrain region of the rat - Evidence of heterogeneity in distribution patterns. *Neuroscience* 42, 473–481.

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Dopamine, cognitive function, and gamma oscillations: role of D4 receptors

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Cognitive deficits in individuals with schizophrenia (SCZ) are considered core symptoms of this disorder, and can manifest at the prodromal stage. Antipsychotics ameliorate positive symptoms but only modestly improve cognitive symptoms. The lack of treatments that improve cognitive abilities currently represents a major obstacle in developing more effective therapeutic strategies for this debilitating disorder. While D4 receptor (D4R)-specific antagonists are ineffective in the treatment of positive symptoms, animal studies suggest that D4R drugs can improve cognitive deficits. Moreover, recent work from our group suggests that D4Rs synergize with the neuregulin/ErbB4 signaling pathway, genetically identified as risk factors for SCZ, in parvalbumin (PV)-expressing interneurons to modulate gamma oscillations. These high-frequency network oscillations correlate with attention and increase during cognitive tasks in healthy subjects, and this correlation is attenuated in affected individuals. This finding, along with other observations indicating impaired GABAergic function, has led to the idea that abnormal neural activity in the prefrontal cortex (PFC) in individuals with SCZ reflects a perturbation in the balance of excitation and inhibition. Here we review the current state of knowledge of D4R functions in the PFC and hippocampus, two major brain areas implicated in SCZ. Special emphasis is given to studies focusing on the potential role of D4Rs in modulating GABAergic transmission and to an emerging concept of a close synergistic relationship between dopamine/D4R and neuregulin/ErbB4 signaling pathways that tunes the activity of PV interneurons to regulate gamma frequency network oscillations and potentially cognitive processes.

Keywords: dopamine, D4 receptor, gamma oscillations, parvalbumin, fast-spiking interneurons, prefrontal cortex, schizophrenia, ADHD

INTRODUCTION

Schizophrenia (SCZ) is characterized by three distinct symptom clusters – positive, negative, and cognitive. Historically, positive symptoms such as hallucinations and delusions have received the most attention, and are often improved by typical and atypical antipsychotics. By contrast, negative symptoms such as social withdrawal, lack of motivation, impaired social interactions, and cognitive impairments including deficits in attention, working memory, and executive function are refractory to antipsychotics. The intractability of cognitive symptoms has been a major obstacle in the development of more effective therapies for SCZ, especially in light of epidemiological findings indicating that the severity of cognitive symptoms is more predictive of long-term prognosis than positive symptoms (Green, 1996), and that cognitive impairments are already present at the prodromal stage (Reichenberg et al., 2002).

Cognitive impairments may arise from altered activity in the prefrontal cortex (PFC). Imaging studies suggest hypofunction of the PFC and reduced signal-to-noise ratios during cognitive tasks in schizophrenic patients (Winterer and Weinberger, 2004; Tost et al., 2005). Local oscillatory network activity in cortical areas, in particular gamma-band rhythms (30–80 Hz), correlate with working memory and selective attention (Womelsdorf and Fries, 2007; Benchenane et al., 2011), and higher cognitive demands correlate with increased gamma power and coherence across different cortical areas (Cho et al., 2006; Basar-Eroglu et al., 2007). Numerous studies have shown that basal, evoked, and induced gamma oscillations are altered in SCZ patients (Herrmann and Demiralp, 2005; Uhlhaas and Singer, 2010). Most of these studies focused on evoked oscillations in sensory cortices, in particular the auditory cortex for its prominent role in auditory hallucinations. Patients with SCZ fail to increase gamma oscillation power in the PFC in response to increased cognitive demands, suggesting reduced signal-to-noise (Cho et al., 2006). Gamma oscillations are an inherent property of recurrent networks of interneurons and pyramidal neurons, and arise by the synchronization of pyramidal neuron output by fast-spiking (FS) basket cell and chandelier interneurons expressing the Ca^{2+} binding protein parvalbumin (PV). The central role of PV-expressing interneurons for the generation of gamma rhythms has recently been demonstrated using optogenetic techniques *in vivo* (Cardin et al., 2009; Sohal et al., 2009). Moreover, numerous postmortem studies in persons

with SCZ have shown changes in PV interneurons, in particular decreases in mRNA and protein expression of GAD67 (glutamic acid decarboxylase 67 kD) and PV itself (Lewis et al., 2011), consistent with the notion of perturbed maturation of inhibitory PFC circuits during adolescence (Benes and Berretta, 2001; Beneyto and Lewis, 2011; Sullivan and O'Donnell, 2012).

It was initially thought that the dopamine (DA) system was generally overactive in SCZ because DA D2-type receptor antagonizing antipsychotic medications mitigated many observed symptoms. However, this original DA hypothesis has evolved over the years to account for evidence from imaging, genetic and metabolic imaging studies more consistent with hypoactivity of frontal cortical circuits in SCZ, hypoactivity of the mesoprefrontal DA system and hyperactivity of mesostriatal and mesonigral DA systems (reviewed in Simpson et al., 2010). While the importance of dopaminergic modulation of the PFC for proper cognitive functions is well supported by experimental evidence from non-human primates and rodents (Goldman-Rakic, 1995; Robbins and Arnsten, 2009), how altered DA activity contributes to PFC hypofunction in SCZ is not understood. Moreover, given its implication in attention and working memory, surprisingly little is known about the possible role of DA signaling in the regulation of oscillatory activity in the PFC and hippocampus, another major allocortical structure implicated in SCZ (Harrison, 2004).

Here we review the current state of research into the role of DA in the modulation of network rhythms in the cortex, particularly the PFC and hippocampus, as new evidence emerges that DA signaling is potently involved in regulating theta and gamma oscillations. A main focus of the review is the D4 receptor (D4R), considered a major D2-type receptor in the forebrain and prominently expressed in PV-expressing interneurons. Recent studies have shown that DA signaling via D4R, in a close and synergistic relationship with the neuregulin 1 (NRG-1)/ErbB4 signaling pathway, itself genetically implicated in SCZ, modulates gamma rhythms in the hippocampus (Andersson et al., 2012b). Taken together with the genetic association of the 7-repeat (7R) variant of the human *DRD4* gene with increased risk for attention deficit hyperactivity disorder (ADHD) and altered gamma-band responses (Demiralp et al., 2007; Gizer et al., 2009), these findings should renew interest in the D4R and its possible contribution to synchronization of PFC networks during cognitive tasks.

FAST-SPIKING INTERNEURONS AND GAMMA OSCILLATIONS

Gamma oscillations are generated through recurrent networks of FS interneurons interconnected by chemical and electrical synapses (for reviews, see Bartos et al., 2007; Colgin, 2011; Whittington et al., 2011; Buzsaki and Wang, 2012). Most FS interneurons are PV-expressing basket cells that form perisomatic connections with multiple pyramidal neurons, faithfully release GABA (gamma-aminobutyric acid) in response to depolarization (Hefft and Jonas, 2005), and discharge action potentials on almost every gamma cycle (Gloveli et al., 2005). Together, these properties make them perfectly suited to entrain pyramidal neurons. In fact, using *in vivo* optogenetic stimulation, depolarizing PV-expressing interneurons increased gamma power, whereas hyperpolarizing PV-expressing interneurons decreased gamma power (Sohal et al., 2009). Moreover, work in the somatosensory cortex suggests that gamma oscillations can be more effectively induced *in vivo* by 40-Hz optogenetic stimulation of PV-expressing interneurons than of pyramidal neurons (Cardin et al., 2009), although optogenetic depolarization of pyramidal neurons has been shown to generate gamma oscillations *in vivo* in the somatosensory cortex (Adesnik and Scanziani, 2010) and in slices from the medial PFC (Yizhar et al., 2011) as well. Notably, optogenetic depolarization of both PV-positive and pyramidal neurons generate gamma synchrony even when stimulated non-rhythmically, supporting the notion that gamma oscillations are an emergent property of coupled networks of excitatory and inhibitory cells (Cardin et al., 2009; Yizhar et al., 2011; for a review, see Tiesinga and Sejnowski, 2009).

EXCITATION/INHIBITION BALANCE

Proper balance of excitatory and inhibitory transmission onto PV interneurons is essential for the generation of normal gamma rhythms. Hippocampal slices prepared from mice with PV interneuron-selective ablation of the GluA1 subunit of the AMPA (alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid) receptor have reduced power of kainate-induced gamma oscillations in area CA3 (Fuchs et al., 2007). PV interneuron-selective ablation of the GluN1 subunit of the NMDA (*N*-methyl-D-aspartate) receptor resulted in elevated spontaneous gamma rhythms in the hippocampus of awake behaving mice (Korotkova et al., 2010), but impaired gamma rhythm induction in the somatosensory cortex in response to optogenetic stimulation (Carlen et al., 2012). Surprisingly, mice lacking GABAergic transmission onto PV interneurons by selective ablation of δ and γ2 subunits of the $GABA_A$ receptor have normal gamma oscillations but severe disruptions in theta oscillations and theta-gamma coupling, suggesting that mutual inhibition of PV interneurons is dispensable for *in vivo* gamma oscillations (Wulff et al., 2009).

N-methyl-D-aspartate receptors on FS interneurons have long been considered critical for normal excitation/inhibition balance in cortical microcircuits and are at the center of the glutamate hypofunction theory of SCZ (Lisman et al., 2008; Nakazawa et al., 2012). This hypothesis is based on findings that dissociative anesthetics that antagonize NMDA receptors such as ketamine and phencyclidine (PCP) elicit psychotic symptoms in normal individuals similar to those found in SCZ subjects and exacerbate psychosis in patients (Kantrowitz and Javitt, 2012). As discussed above, compelling evidence from gene targeting studies implicates NMDA receptors in gamma synchrony. However, a recent study comparing postnatal vs. adult ablation of the GluN1 subunit suggests that NMDA receptors on FS interneurons might be more important for the proper maturation of cortical circuits and the emergence of gamma oscillation during development than for a direct contribution to synaptic processes underlying gamma oscillation induction or propagation (Belforte et al., 2010; Korotkova et al., 2010). Consistent with this idea, electrophysiological studies in wild-type mice show that excitatory postsynaptic potentials (EPSPs) on FS interneurons have large contributions from NMDA receptors in juveniles that diminish during maturation (Wang and Gao, 2009; Rotaru et al., 2011). Notably, since NMDA receptors have slower kinetics than AMPA receptors, the summating properties of excitatory synapses with a large NMDA receptor contribution could make them less suitable to support gamma

rhythms. Therefore, excitatory drive is dominated by AMPA receptors in mature FS interneurons to ensure high temporal precision between excitation and spiking (Rotaru et al., 2011).

PHENOTYPIC ALTERATIONS OF PV INTERNEURONS IN SCZ

Postmortem studies have consistently revealed decreased levels of GAD67 mRNA in the dorsolateral PFC (DLPFC) and hippocampus of SCZ subjects, particularly in PV interneurons (for reviews, see Benes and Berretta, 2001; Gonzalez-Burgos and Lewis, 2012). As GAD67 synthesizes the inhibitory neurotransmitter GABA, it seems reasonable to speculate that diminished GAD67 expression reduces inhibitory drive, perturbs normal excitation/inhibition balance, and thereby impairs neural synchrony. Interestingly, SCZ subjects also have reduced PV expression in the cortex (Hashimoto et al., 2008), a finding that has been suggested to represent a compensatory response to lower GAD67 levels, as PV-deficient mice exhibit increased GABA release and kainate-induced hippocampal gamma oscillations power and frequency (Vreugdenhil et al., 2003). An alternative interpretation, based on the marked dependence of the expression of both GAD67 and PV genes on neural activity, is that reduced levels of GAD67 and PV are a consequence of abnormally low activity of FS interneurons in SCZ subjects.

Work in rodents suggests that GAD67-mediated GABA synthesis has an additional role in shaping GABAergic innervation of cortical networks during adolescence, a period in which PV interneuron networks mature and gamma oscillations become prevalent (Chattopadhyaya et al., 2007). Reduced GAD67 expression during adolescence could therefore conceivably cause persistent changes in GABAergic innervation, resulting in perturbed excitation/inhibition balance and dysfunctional cortical networks in affected individuals. Lastly, it has been proposed that because of their high metabolic demand, FS interneurons are particularly vulnerable to oxidative stress during development. Consistent with this notion, studies have identified abnormal antioxidant systems in SCZ subjects, and work in rodents has demonstrated that exposure to oxidative stress causes loss of PV-immunoreactivity in FS interneurons and reductions in high-frequency network oscillations (Behrens and Sejnowski, 2009; Do et al., 2009).

GAMMA OSCILLATIONS IN SCZ

While abnormalities in neural oscillations have been identified in essentially all frequency bands in patients with SCZ (Moran and Hong, 2011), gamma-band activity is particularly affected and might represent an endophenotypic biomarker of its etiology (Uhlhaas and Singer, 2012). This notion is based on reports of high heritability of altered auditory and visual steady-state evoked potentials (SSEPs) in affected individuals and their first-degree relatives (for example, Hall et al., 2011), and on studies finding that perturbations in gamma-band responses present early in the course of the disorder and are independent of medication status (Gallinat et al., 2004; Symond et al., 2005; Haenschel et al., 2009; Minzenberg et al., 2010).

In general, high-frequency beta and gamma oscillations synchronize local networks, while low-frequency delta, theta, and alpha oscillations establish synchronization over longer distances (von Stein and Sarnthein, 2000). Similarly, gamma oscillations between the PFC and cortical sensory areas mediate attention by enhancing the neuronal representation of attended sensory input, while theta oscillations between the PFC and hippocampus mediate working and long-term memory (Benchenane et al., 2011). A given neuron may participate in several of these frequencies at any given time, and measures of cross-frequency coupling indicate how well one frequency is embedded in another. Evidence of abnormal neural oscillations in SCZ comes from a large number of clinical studies primarily using electroencephalography (EEG) and magnetoencephalography (MEG). These studies revealed changes in sensory-evoked gamma oscillations, oscillations that correlate with propensity for hallucinations, and abnormal modulation of neural oscillations during cognitive tasks.

In "bottom-up" approaches, patients have been shown to have trouble generating, maintaining, and propagating SSEPs entrained by stimuli presented at gamma-band frequency, with impairments in both phase-locking and amplitude. Similarly, "top-down" perceptual processing paradigms such as auditory oddball tasks, sustained tones, speech sounds, and button-press tasks mostly revealed impairments in evoked gamma power (Haig et al., 2000) and synchrony (for an excellent review on both approaches, see Uhlhaas and Singer, 2010). Lastly, evoked oscillations triggered by direct transcranial magnetic stimulation are markedly reduced in patients with SCZ and are confined to the stimulated brain region, whereas gamma oscillations propagate to neighboring cortical regions in healthy controls (Ferrarelli et al., 2008).

Apart from lower stimuli-evoked gamma oscillations, affected individuals also show reduced high-frequency activity at rest (Rutter et al., 2009). Interestingly, they also have a greater number of uncorrelated gamma oscillations at rest, especially between the PFC and other cortical areas, resulting in reduced coordination of cortical activity (Yeragani et al., 2006; Salvador et al., 2010; Kikuchi et al., 2011). The variety of concurrent but uncorrelated oscillations in various areas could be described as "noise" because they potentially mask salient signals evoked from sensory cortices. Gamma oscillation signal-to-noise ratios are reduced in SCZ during cortical information processing, due to elevated spontaneous activity and reduced evoked responses (for example, Winterer et al., 2000; reviewed in Gandal et al., 2012).

Task demands with a strong cognitive component have also been used to reveal gamma deficits in SCZ patients. For example, patients with SCZ have abnormal oscillations during lexical encoding (Xu et al., 2013), and do not display typical increases in gamma activity during mental arithmetic (Kissler et al., 2000). During working memory tasks, healthy controls increase gamma oscillations in frontal cortical areas as the task demands increase. However, SCZ patients show maximal gamma oscillatory activity for simple working memory tasks as well as difficult ones (Cho et al., 2006; Basar-Eroglu et al., 2007). Moreover, gamma power during encoding and maintenance positively correlates with performance in these tasks, and SCZ patients show deficits in producing this delay-period gamma oscillation (Cho et al., 2006; Haenschel et al., 2009; Minzenberg et al., 2010).

Several studies show that increased high-frequency oscillations in primary sensory cortices correlate with the propensity for auditory hallucinations in SCZ patients, even when subjects are not actively hallucinating (Baldeweg et al., 1998; Lee et al., 2006; Mulert et al., 2011). An increased tendency for neural circuits to

enter states of high-frequency synchronization has been suggested to reflect hyperexcitability in sensory cortices (Spencer et al., 2009).

DOPAMINE'S ROLE IN NEURAL OSCILLATIONS AND COGNITION

The importance of DA neurotransmission for attention and normal cognitive function has long been recognized. Deficiencies in mesoprefrontal dopaminergic circuits in humans are associated with major psychiatric disorders such as SCZ, ADHD, and addiction (Winterer and Weinberger, 2004; del Campo et al., 2011; Goldstein and Volkow, 2011). In rodents and non-human primates, DA modulates PFC neuron activity during cognitive tasks and motivational behaviors (Goldman-Rakic, 1995; Grace et al., 2007; Robbins and Arnsten, 2009), and pharmacological lesions of dopaminergic projections to the PFC greatly impair working memory (Brozoski et al., 1979). DA effects on cognitive performance exhibit an inverted U-shaped dose–response curve (Sawaguchi and Goldman-Rakic, 1994; Vijayraghavan et al., 2007). In cortical areas, extracellular DA is cleared mostly by enzymatic inactivation catalyzed by the product of the catechol-*O*-methyltransferase gene (*COMT*). A functional variation exists in the human population (Val158Met) in which the Val allele renders the enzyme significantly more active than the Met allele, thereby inactivating extracellular DA more rapidly. Homozygous carriers of the Val allele are therefore believed to have lower prefrontal DA levels and indeed show reduced performance in working memory and executive function tasks (Goldberg et al., 2003). Moreover, amphetamine improves prefrontal signal-to-noise ratio during a working memory task assayed by fMRI (functional magnetic resonance imaging) in Val homozygotes but decreases signal-to-noise in Met homozygotes, consistent with an inverted U-shaped dose–response curve (Mattay et al., 2003).

DA AND GAMMA OSCILLATIONS

While both DA and network oscillations have long been implicated in cognitive function, the interactions between DA and neural network activity in normal and pathological conditions have remained poorly understood. From a historical perspective this is not entirely surprising, given that DA receptor-targeting first-generation and atypical antipsychotics have proven mostly ineffective in improving cognitive deficits in SCZ patients. However, the need for a better understanding of DA effects on neural oscillations has been acknowledged (Whittington et al., 2011; Gandal et al., 2012), and a number of clinical and preclinical studies have begun to explore these relationships. While there is as yet no unified view of the effects of DA on neural networks owing at least in part to methodological and conceptual differences (*in vitro* vs. *in vivo* recordings, knock-out models vs. acute treatments, general DA signaling vs. selective modulation of distinct DA receptor subtypes), recent studies point to an important role of DA in network dynamics. Available data from human EEG studies suggests that DA increases the power of neural oscillations in the PFC, but has little effect on their induction or frequency. Carriers of polymorphisms in the DA transporter gene *DAT1* that prolong event-related increases in DA, show an increase in evoked gamma power in response to target stimuli (Demiralp et al., 2007). In healthy subjects, the first-generation antipsychotic haloperidol reduces gamma power in response to attended stimuli (Ahveninen et al., 2000), although it is unclear to what extent this effect is attributable to D2-type receptor inhibition or blockade of other haloperidol targets such as NMDA receptors.

Dopamine has been suggested to affect cognitive processes by modulating signal-to-noise ratios in PFC microcircuits (Winterer and Weinberger, 2004). Supporting indirect evidence comes from studies on working memory performance in which D1-type DA receptors optimize signal-to-noise ratio in prefrontal neural representations of non-human primates (Goldman-Rakic, 1995; Williams and Goldman-Rakic, 1995; Vijayraghavan et al., 2007). Interestingly, carriers of the *DRD4.7* allele, a subsensitive variant of the D4R (see Identification of the D4R), exhibit similar increases in auditory cortex gamma power to both standard and target stimuli in an auditory target detection paradigm, suggestive of a reduced signal-to-noise ratio (Demiralp et al., 2007).

Genetic mouse models and acute pharmacological treatments have been employed to study the effects of altered DA transmission on gamma and theta oscillations in the hippocampus and PFC. Hyperdopaminergic *DAT1* knock-out mice show increased hippocampal (but not cortical) gamma power, and enhanced gamma phase synchrony between the hippocampus and the PFC during novelty-induced exploration (Dzirasa et al., 2006, 2009). However, acute elevations of DA levels by amphetamine, apomorphine, or methamphetamine treatments had no significant effect on baseline or auditory-evoked gamma in the rodent cortex and hippocampus (Ma and Leung, 2000; Pinault, 2008; Ehrlichman et al., 2009).

Electrophysiological studies in which gamma oscillations were induced electrically or pharmacologically in rodent brain slices have yielded varying results regarding the involvement of the DA system. Ketamine-induced gamma oscillation power in the rat PFC was reduced by clozapine and haloperidol (Jones et al., 2012). Likewise, in hippocampal area CA1, DA increased the power and duration of stimulation-induced gamma oscillations (Wójtowicz et al., 2009) but decreased the power and duration of carbachol-induced oscillations (Weiss et al., 2003). However, kainate-induced gamma oscillations were found to either be decreased in CA1 and CA3 (Wójtowicz et al., 2009) or unchanged in CA3 (Andersson et al., 2012b). These variances might be partly explained by differences in the mechanisms underlying each type of oscillation.

Pharmacological analysis of the involvement of different types of DA receptors in pharmacologically induced *in vitro* gamma oscillations has revealed an unexpected wealth of targets and effects. The selective D4R agonist PD168077 augmented gamma oscillation power in CA3 (Andersson et al., 2012b); this finding will be discussed in more detail in Section "D4R and Gamma Oscillations." Conversely, clozapine, an atypical antipsychotic with moderate selectivity for D4R and 5-HT_3 receptors, and haloperidol reduced the amplitude and increased the bandwidth of acetylcholine-induced gamma oscillations in CA3. The effects of clozapine were attributed to inhibition of 5-HT_3 serotonergic receptors based on the ability of the 5-HT_3 agonist *m*-chlorophenylbiguanide to augment gamma oscillations; the same study also reported that direct stimulation of the D3R by the preferentially selective agonist PD128907 inhibited gamma oscillations (Schulz et al., 2012). Clozapine also suppressed spontaneous synchronized pyramidal network activity in layer V of the PFC

(Gao, 2007). Lastly, activation of D1-type DA receptors decreased carbachol-induced gamma oscillation power (Weiss et al., 2003). Taken together, the interpretation of DA effects on oscillations are complex and likely depend on multiple factors including the brain area investigated, mechanisms of gamma oscillation induction and DA receptor pharmacology.

There is also evidence for an involvement of DA in theta oscillations. Theta frequencies frequently bind distributed networks oscillating at gamma frequency and predominate in the hippocampus during active behavior. Depleting DA in the hippocampus decreases theta activity (Nakagawa et al., 2000), while injecting DA or apomorphine into the medial septum increases hippocampal theta oscillations (Miura et al., 1987). Moreover, direct infusion of DA into the PFC of anesthetized rats increases theta oscillation coherence between the PFC and the hippocampus (Benchenane et al., 2010).

MODULATION OF FS INTERNEURON PROPERTIES BY DA

Three key determinants of the power and frequency of gamma oscillations are (1) the magnitude and (2) kinetics of synaptic inhibition between interneurons, and (3) the driving excitatory current onto interneurons (Traub et al., 1996). Non-selective stimulation of DA receptors by exogenous DA appears not to change the electrical coupling of interneurons through gap junctions (Towers and Hestrin, 2008). However, selective activation of D1-type or D4 receptors reduces coupling (Hampson et al., 1992; Onn and Grace, 1994; Li et al., 2013) while activation of D2-type receptors increases coupling (Onn and Grace, 1994), suggesting opposing effects of different DA receptor types. Moreover, DA acting through D1-type receptors decreases the amplitude of inhibitory postsynaptic currents (IPSCs) in FS interneurons (Towers and Hestrin, 2008). DA also changes the intrinsic properties of FS interneurons by increasing their evoked firing rate *in vivo* (Tseng et al., 2006), and *in vitro* (Zhou and Hablitz, 1999; Gorelova et al., 2002; Gao et al., 2003; Kröner et al., 2007; Trantham-Davidson et al., 2008; but see Tierney et al., 2008).

By simultaneously reducing spontaneous firing and increasing evoked firing in PV interneurons, DA augments temporal precision and thereby sharpens the spike timing of FS interneurons (Gao et al., 2003; Tierney et al., 2008). Furthermore, D1-type and D2-type receptors increase the amplitude and kinetics of the h-current in layer I PFC interneurons (Wu and Hablitz, 2005). Because blockade of the h-current, an inwardly rectifying hyperpolarization-activated non-selective cation current, causes intrinsic changes in the frequency and power of many cortical oscillation frequencies (Kramer et al., 2008), it seems plausible that increases in the h-current also affect oscillations. Likewise, DA activation reduces potassium currents, thereby increasing the probability of repetitive firing, and potentially changing oscillatory patterns (Gorelova et al., 2002; Schreiber et al., 2004). Computational modeling suggests that D4Rs may modify potassium currents to increase gamma power (Kuznetsova and Deth, 2008), although experimental verification is still pending. We will discuss D4R effects on synaptic and intrinsic properties and on gamma oscillations in more detail in Sections "D4R Signaling Partnerships" and "D4R and Gamma Oscillations."

ROLE OF DA IN THE MATURATION OF FS INTERNEURONS DURING ADOLESCENCE

Prefrontal cortex circuits finish maturing during adolescence and early adulthood, a period of high vulnerability to first psychotic episode in persons with SCZ. Maturation involves concomitant changes in responses to DA within FS interneurons, DA receptor composition in FS interneurons, and gamma-band activity during cognitive performance. From childhood to early adolescence, gamma frequency oscillations dominate most cortical regions, and their power correlates with increases in cognitive performance. Strikingly, gamma oscillations ebb during adolescence then increase dramatically in early adulthood (Uhlhaas et al., 2009). At the end of this period, DA exerts more control over excitation/inhibition balance and thereby aids in the selection of adequate behavioral responses (O'Donnell, 2010).

Dopamine receptor expression and signaling change with age in rodents. D1Rs, D2Rs, and D4Rs increase in density in the PFC through adolescence and then show a reduction in adult animals (Zhang et al., 2004; Brenhouse et al., 2008; Naneix et al., 2012; but see Tarazi and Baldessarini, 2000). Furthermore, pharmacological blockade of D1-type receptors increases the intrinsic excitability of FS interneurons in slices from both adult (PD $>$ 50) and juvenile (PD $<$ 35) rats, while the D2-type receptor agonism increases FS interneuron excitability in post- but not pre-pubertal slices (Tseng and O'Donnell, 2007), thereby enhancing DA's overall effect on intrinsic FS interneuron excitability. Similarly, in slices from pre-pubertal rats, co-administration of a D1-type agonist and NMDA excites pyramidal neurons for only tens of milliseconds, but in adults it creates plateau depolarizations lasting several hundred of milliseconds (Tseng and O'Donnell, 2005). Overall, DA receptor agonists exert more precise control over both excitatory and inhibitory neurons in adult animals, suggesting that DA may modulate oscillatory network behavior more strongly in adults.

Mature FS interneurons have faster membrane oscillations and spikes, less adaptation, and less NMDA contribution (Okaty et al., 2009; Belforte et al., 2010; Wang and Gao, 2010; Goldberg et al., 2011; Rotaru et al., 2011), and DA innervation and signaling might play a role in the maturation of FS interneurons. Treatment with DA or coculture with mesencephalic slices, which increases available DA, accelerates PV interneuron maturation in rat organotypic slices from the frontorbital cortex and increases the density of PV-positive cells in deep cortical layers (Porter et al., 1999; Ross and Porter, 2002). Moreover, elevation of prenatal DA levels by intrauterine cocaine exposure increases the ramification of PV interneurons in the anterior cingulate, a cortical area receiving dense dopaminergic innervation, but not the primary visual cortex, an area that receives little dopaminergic innervation (Wang et al., 1995). Conversely, 6-hydroxydopamine lesions in the medial forebrain bundle cause a reduction of the density of PV-expressing cells in the zona incerta of the diencephalon without reducing the total number of cells, suggesting that DA is required for the maintenance of a mature FS interneuron phenotype (Heise and Mitrofanis, 2005). These findings are consistent with the notion that the DA system is a critical modulator of GABAergic interneurons during postnatal maturation of cortical connectivity (O'Donnell, 2010) and that persons with

SCZ exhibit reduced PV and GAD67 immunoreactivity (Lewis et al., 2011).

PROPERTIES OF THE D4 DOPAMINE RECEPTOR

IDENTIFICATION OF THE D4R

Following the cloning of the genes and cDNAs for the prototypical D2R and D1R, the gene for the human D4R (gene symbol: *DRD4*) was isolated in 1991 by homology cloning in an effort to identify additional D2-related receptors (Van Tol et al., 1991). Similar to the other D2-type receptors, it contains short amino-terminal extracellular and carboxyl-terminal intracellular tail domains, and a fairly long third-intracellular loop. DA has the highest affinity for D4R among all DA receptors (Rondou et al., 2010). Moreover, the other catecholamines epinephrine and norepinephrine bind to and activate the D4R with submicromolar affinities (Lanau et al., 1997). An outstanding feature of the human D4R is its highly polymorphic nature, owing in large part to the presence of a 48-bp variable number tandem repeat in exon III (Van Tol et al., 1992), with four and seven repeats being the most common variants overall but with substantial regional and ethnic differences in frequencies (Chang et al., 1996). By contrast, most non-primate mammal *DRD4* orthologs harbor only two repeats. The repeat encodes a proline-rich sequence located in the third intracellular loop. It conforms to a SH3 (Src Homology 3 Domain)-binding motif and has been implicated in receptor surface expression and interactions with adapter proteins such as Nck and Grb2 (Oldenhof et al., 1998). Using the amplitude of G protein-induced inwardly rectifying (GIRK) potassium currents to determine receptor activation in a heterologous *Xenopus* oocyte expression system, DA was shown to be more potent at the D4.2 and D4.7 receptor variants than the D4.4 receptor variant (Wedemeyer et al., 2007). Functional differences between receptor variants were also reported for clozapine binding, stimulation of GTPγS binding and coupling to adenylyl cyclase (Van Tol et al., 1992; Asghari et al., 1995; Jovanovic et al., 1999; Czermak et al., 2006). Numerous studies have sought to correlate polymorphic *DRD4* variants to psychiatric disorders, including association of the D4.7 variant with increased risk for ADHD (see Pharmacological Properties and Involvement in Psychiatric Disorders), and with the personality trait of novelty seeking (Ebstein et al., 1996), although some failed to replicate the original findings (for example, Hawi et al., 2000). Of particular interest to the topic of this review, D4.7 has been linked with altered cortical auditory-evoked and induced gamma-band responses, thereby implicating polymorphic D4R variants in neural oscillations (Demiralp et al., 2007; see Pharmacological Properties and Involvement in Psychiatric Disorders).

PHARMACOLOGICAL PROPERTIES AND INVOLVEMENT IN PSYCHIATRIC DISORDERS

Initial pharmacological analyses indicated that clozapine has a higher affinity for the D4R than D2R, therefore D4R was proposed to constitute the main pharmacological target of this efficacious atypical antipsychotic (Van Tol et al., 1991). In contrast to typical or first-generation antipsychotics, extrapyramidal effects are observed to a lesser extent, or not at all, in patients treated with clozapine. While these findings generated excitement early on, subsequent studies argued against the possible utility of D4R-targeting drugs to treat the positive symptoms of SCZ. Most importantly, novel, more selective D4R antagonists such as L-745,870 and sonepiprazole were largely ineffective as neuroleptics in clinical trials (Bristow et al., 1997; Kramer et al., 1997; Corrigan et al., 2004). Consistent with this, clozapine is known to target numerous other receptor systems with low nanomolar affinities, including serotonergic (5-HT_2), muscarinic, and β-adrenergic receptors (Baldessarini and Frankenburg, 1991), and it was concluded that its efficacy as a neuroleptic reflected a much more complex multi-target pharmacology with a significant contribution from serotonergic receptors (Meltzer and Huang, 2008). These findings, taken together with genetic association studies that largely failed to identify *DRD4* polymorphic variants as risk factors (Jonsson et al., 2003), and the inability to reproduce earlier radiolabel binding studies that suggested elevated D4R expression in SCZ subjects (Seeman et al., 1993), dampened enthusiasm to pursue the D4R as a promising antipsychotic drug target for SCZ (Tarazi et al., 2004).

However, the D4R soon re-emerged as a genetic risk factor for ADHD, a heterogeneous but highly heritable syndrome characterized by a variable combination of persistent, pervasive and developmentally inappropriate levels of inattention, hyperactivity and impulsiveness that typically lead to poor academic performance (American Psychiatric Association, 2000). Perturbations of catecholaminergic pathways are a leading hypothesis in ADHD, and dopaminergic drugs such as methylphenidate are clinically efficacious. Consistent with this, numerous ADHD candidate genes including *DRD4*, *DAT1*, *COMT*, *MAOA*, and *DBH* are integral parts of the catecholaminergic neurotransmission system. Both case–control and family-based association studies reported increased transmission of the polymorphic *DRD4.7* variant (for example, LaHoste et al., 1996; Rowe et al., 1998; Smalley et al., 1998; Swanson et al., 1998) and other polymorphisms (Barr et al., 2000; Arcos-Burgos et al., 2004) in children diagnosed with the syndrome. Subsequent meta-analyses supported a linkage between the *DRD4.7* allele and ADHD (for example, Faraone et al., 1999; Maher et al., 2002).

Cognitive effects of acute D4R activation

Although D4R antagonists proved ineffective as antipsychotics, D4R-targeting drugs have been examined in cognitive tasks in monkeys and rodents. For the most part, D4R agonists increase working memory performance and fear acquisition according to an inverted U-shaped dose response curve (Bernaerts and Tirelli, 2003; Browman et al., 2005; Woolley et al., 2008; but see Nayak and Cassaday, 2003); interestingly, D4R antagonists have paradoxical promnesic effects at low doses (Zhang et al., 2004). Specifically, low doses of D4R antagonists reverse working memory deficits induced by stress or chronic PCP administration in monkeys (Jentsch and Roth, 1999; Arnsten et al., 2000). In rats, D4R antagonists increase DA release in the PFC (Broderick and Piercey, 1998), possibly explaining these promnesic effects according to the inverted U-shaped dose–response curve for DA and cognition. Further supporting this idea, rats with low baseline memory performance showed the greatest improvements in response to

low doses of the D4R antagonist L-745,870 and impairments at high doses, while rats with high baseline memory performance were impaired by administration of L-745,870 at every concentration (Zhang et al., 2004). In the rat, intra-medial PFC (mPFC) injection of L-741,741, another highly selective D4R inhibitor, blocks the acquisition (but not expression) of fear conditioning and the encoding of emotional memory by mPFC neurons (Laviolette et al., 2005). Conversely, medial PFC injection of the D4R agonist PD168077 increases the salience of sub-threshold foot shocks while it blocks the acquisition of fear conditioning in response to supra-threshold foot shocks (Lauzon et al., 2009; Tye et al., 2009). Additionally, the D4R agonists A-412997 and PD168077 enhance memory for aversive stimuli according to an inverted U-shaped curve (Browman et al., 2005), but have also shown linear increases in working memory performance in a similar procedure and a novel object recognition paradigm (Bernaerts and Tirelli, 2003; Woolley et al., 2008). Taken together, these results suggest that the D4R mediates memory consolidation of both normal and emotionally salient experiences, and that the intensity of the stimulus interacts with DA signaling through D4Rs.

EXPRESSION IN FS PARVALBUMIN INTERNEURONS

A number of studies have found D4R mRNA and protein in PV interneurons of both the PFC and the hippocampus. Expression in these neurons was first observed in the monkey by double immunohistology using a carefully characterized polyclonal antibody against the extracellular amino-terminus of the receptor (Mrzljak et al., 1996). Immunoreactive cells were found in PFC layers III–V and in hippocampal area CA1. In both areas, strongly labeled PV interneurons were accompanied by lightly labeled pyramidal neurons. This study also found intense D4R immunoreactivity in the rodent globus pallidus (homologous to the primate external globus pallidus, or GPe), that is made up entirely of GABAergic projection neurons. Evidence for D4R expression in PV interneurons also comes from a single-cell RT-PCR study of acutely isolated rat PFC neurons (Vysokanov et al., 1998). Moreover, reporter gene expression was detected in PFC pyramidal neurons and interneurons, some of them expressing PV, in mice harboring a BAC transgene in which the green fluorescent protein was expressed under the transcriptional control of the *Drd4* locus (Noain et al., 2006). Two groups additionally investigated D4R expression in GABAergic interneurons using double-*in situ* hybridization for D4R and GAD67, and identified co-expressing cells in layer V of the monkey PFC (de Almeida et al., 2008) and in the mouse hippocampus (Andersson et al., 2012b). The latter study also employed double-immunohistochemistry using an antibody raised against the extracellular amino-terminus of the rat D4R (Ariano et al., 1997) and found that the majority (71%) of D4R-expressing neurons in areas CA1 and CA3 co-express PV and that conversely 21% of PV interneurons co-express D4R. In aggregate, what emerges from all these findings is the notion that in the PFC, both local GABAergic interneurons and pyramidal neurons express D4R transcript and protein, while in the hippocampus the evidence favors D4R expression largely in GABAergic interneurons.

BEHAVIORAL PHENOTYPES OF MICE LACKING D4R FUNCTION

Locomotor effects

An initial behavioral analysis of mixed-background (C57Bl/6J × 129) $Drd4^{-/-}$ mice revealed locomotor hypoactivity and increased sensitivity to locomotor-stimulating effects of acute ethanol, cocaine, and methamphetamine injections, indicative of deficits in nigrostriatal function (Rubinstein et al., 1997). Amphetamine hypersensitivity was confirmed in a subsequent study using $Drd4^{-/-}$ mice congenic on the C57Bl/6J background, and expanded to also include altered behavioral sensitization to repeated amphetamine injections (Kruzich et al., 2004). At the neurochemical level, $Drd4^{-/-}$ mice exhibit altered striatal DA metabolism (Rubinstein et al., 1997), and lower baseline and KCl-evoked extracellular striatal DA levels (Thomas et al., 2007). In contrast, DA synthesis and turnover in the PFC of mutant mice is not significantly different (Rubinstein et al., 2001). Evidence in favor of a role of the D4R in regulating motor activity also comes from studies reporting that D4R antagonism restores normal locomotor activity in periadolescent rats rendered transiently hyperactive by neonatal 6-hydroxydopamine lesions (Zhang et al., 2001). These lesions reduce dopaminergic projections to the forebrain and serve as a neurodevelopmental model of ADHD with good face validity, reproducing a number of its core symptoms. Strikingly, 6-hydroxydopamine-mediated hyperactivity is absent in $Drd4^{-/-}$ mice (Avale et al., 2004). Although these findings strongly suggest a role for the D4R in mediating behavioral responses to perturbed DA function in the striatum, it remains unclear if these effects originate, as suggested, in a hyperexcitable PFC or elsewhere, and what molecular and cellular D4R-dependent processes underlie them.

Cognitive effects

The involvement of the D4R in cognitive functions, in particular avoidance behavior and emotional learning, has been analyzed in both mutant mice as well as in acute pharmacological paradigms (see also Cognitive Effects of Acute D4R Activation). Dulawa et al. (1999) found that $Drd4^{-/-}$ mice exhibited less novelty-seeking behavior compared to wild-type controls without displaying changed anxiety. While this report concluded that D4R deficiency did not affect avoidance behavior, a different study found that $Drd4^{-/-}$ mice exhibited heightened anxiety in the elevated plus maze and light/dark preference exploration tests that was ameliorated by ethanol and the benzodiazepine midazolam (Falzone et al., 2002). The fact that these anxiolytic drugs work by increasing GABAergic transmission, taken together with prominent D4R expression in interneurons in the PFC, striatal DA dysregulation and increased excitability of PFC neurons from mutant mice (Rubinstein et al., 2001), was interpreted as indicative of altered PFC control of striatal DA release in $Drd4^{-/-}$ mice. On the other hand, acute pharmacological inhibition of D4R in the rat mPFC by L-745,870 injection was shown to be anxiolytic in the elevated plus maze and shock-probe burial test (Shah et al., 2004). Taken together, these findings suggest that the D4R is involved in emotional learning, but that the effects of receptor interference depend on the experimental approach utilized (acute/local/pharmacological vs. chronic/global/genetic). Consistent with this notion, studies have found secondary changes in

Drd4$^{-/-}$ mice, including upregulation of D1- and NMDA receptor expression and increased striatal glutamate that might reflect compensatory responses to a lack of D4R function in mutant mice (Gan et al., 2004; Thomas et al., 2009).

Consistent with the notion of altered PFC function in subjects with SCZ, micro-PET (positron emission tomography) imaging experiments in *Drd4*$^{-/-}$ mice indicate reduced baseline PFC glucose metabolism (Michaelides et al., 2010). Moreover, methylphenidate decreases PFC glucose metabolism in normal mice, but increases it in mutants (Michaelides et al., 2010). It is unclear to what extent these metabolic changes are directly linked to the lack of D4R function in these mice, or to compensatory changes aforementioned. Interestingly, while *Drd4* homozygous mice are normal in behavioral tests of attention and impulsivity (Helms et al., 2008; Young et al., 2011), *Drd4* heterozygous mice exhibit less response inhibition in the five choice – continuous performance task (Young et al., 2011). This observation is consistent with a lack of secondary neurochemical changes in *Drd4*$^{+/-}$ mice (Thomas et al., 2007) and might therefore represent a true hypomorphic D4R phenotype that is not occluded by compensatory neural adaptations observed in the full mutants.

SYNAPTIC EFFECTS OF D4Rs

Effects on cortical microcircuits

Cellular effects of D4R signaling in neurons have mostly been studied using electrophysiological recordings in brain slices, acutely dissociated neurons or long-term neuron cultures. Importantly, many of the effects discussed below were observed using high concentrations of the D4R agonist PD168077 that may also activate adrenergic α_{1A} and α_{2C} as well as serotonergic 5-HT_{1A} receptors (Moreland et al., 2005). At the local circuit level, Onn et al. (2006) found that in PFC slices with intact synaptic connections between axon collaterals and recorded neurons, D4R inhibition causes complex evoked spike discharges in pyramidal neurons, but only with intact GABAergic transmission. The authors suggest that DA signaling bi-directionally regulates PFC pyramidal neuron excitability via D1R and D4R-dependent pathways, respectively, and that D4Rs reduce pyramidal neuron excitability through their tonic activity by low ambient levels of DA. Consistent with these results, *Drd4*$^{-/-}$ mice exhibit hyperexcitability of PFC pyramidal neurons and are more sensitive to bicuculline (Rubinstein et al., 2001). Although these findings indicate a possible role of D4Rs in promoting GABAergic transmission onto PFC pyramidal neurons, acute assessments of the effects of pharmacological D4R perturbations on GABAergic interneuron function have yielded mixed results, possibly stemming from the use of non-specific drugs (Gorelova et al., 2002; Gao, 2007). The possibility that D4R effects might vary between different cortical areas has to be considered as well, as pharmacological data from the prelimbic cortex suggest that D4Rs actually facilitate pyramidal neuron firing (Ceci et al., 1999).

Inhibition of ionotropic receptors

Various studies using neuronal preparations from the PFC, the hippocampus and the rodent globus pallidus have consistently found that pharmacological D4R activation decreases postsynaptic currents and surface expression of both excitatory and inhibitory ionotropic receptors (see **Tables 1** and **2**). GABA current amplitude and surface expression of $GABA_AR\beta_{2/3}$ clusters are decreased in PFC pyramidal neurons after treatment with relatively high concentrations (30 μM) of the D4R agonist PD168077 (Wang et al., 2002; Graziane et al., 2009). Agonist treatment also reduces $GABA_A$ IPSCs in globus pallidus GABAergic neurons in wild-type mice but not in *Drd4*$^{-/-}$ mice (Shin et al., 2003). However, D4R activation does not affect GABA current amplitude in hippocampal pyramidal neurons during ongoing kainate-induced oscillations (Andersson et al., 2012a). These data correlate with the expression of D4Rs on PFC pyramidal and globus pallidus GABAergic neurons (Mrzljak et al., 1996; Ariano et al., 1997), but not in hippocampal pyramidal neurons (Andersson et al., 2012b), and suggest that effects on GABAergic transmission are cell-autonomous.

D4R stimulation also reduces NMDA and AMPA receptor currents. NMDA receptor currents and surface expression decrease in acutely isolated PFC and hippocampal pyramidal neurons in response to agonist treatment (Kotecha et al., 2002; Wang et al., 2003; Beazely et al., 2006), although the downstream signaling pathways may differ between the PFC and hippocampus (see D4R Signaling Partnerships). Similar effects were reported in projection neurons of the lateral amygdala (Martina and Bergeron, 2008), and in hippocampal area CA1 where NMDA receptor-dependent induction of long-term potentiation (LTP) induction in *stratum oriens* was inhibited by D4R activation (Herwerth et al., 2012). Intriguingly, in PFC slices from animals treated with the NMDA receptor antagonist PCP, a psychotomimetic drug that in humans can elicit effects remarkably similar to the symptomology of SCZ (Jentsch and Roth, 1999), D4R activation no longer inhibits NMDA receptor currents, although the cellular mechanisms underlying this effect are not known (Wang et al., 2006).

In pyramidal neurons of the PFC, but not the hippocampus, acute D4R activation by low concentrations (100 nM) of PD168077 was also shown to reduce baseline AMPA receptor currents and surface expression (Kwon et al., 2008; Yuen et al., 2010; Andersson et al., 2012a; but see Rubinstein et al., 2001; Gu et al., 2006). High concentrations of PD168077 also reduce baseline AMPA receptor currents and surface expression in PFC interneurons (Graziane et al., 2009). Furthermore, recent data suggest that D4R effects on AMPA receptors may at least in part be state-dependent. In cultured hippocampal neurons expressing the D4R, agonist treatment has no effect on baseline AMPA receptor surface expression but internalizes GluA1-containing AMPA receptors following a chemical form of LTP (Kwon et al., 2008). In PFC pyramidal cells, the D4R agonist PD168077 decreases AMPA currents when slices are pretreated with bicuculline to increase overall activity by inhibiting $GABA_A$ receptors but increases AMPA currents in slices pretreated with tetrodotoxin (TTX) to reduce overall activity by inhibiting sodium channels (Yuen et al., 2010; Yuen and Yan, 2011). D4Rs also reverse early LTP at Schaeffer collateral-to-CA1 glutamatergic synapses by reversing AMPA receptor-mediated excitatory postsynaptic currents back to pre-LTP levels (Kwon et al., 2008). However, in these cases it is not known if this represents a cell-autonomous or a circuit-based effect. Taken together,

Table 1 | Postsynaptic effects of D4R activation on evoked and mini current amplitudes.

Current	Increase/decrease	Cell type recorded	Region	Reference
AMPA	↑ At low synaptic activity	Pyramidal	PFC	Yuen et al. (2010); Yuen and Yan (2011), but see Rubinstein et al. (2001); Onn et al. (2006)
AMPA	↓ At high synaptic activity	Pyramidal	PFC, hippocampus	Kwon et al. (2008); Yuen et al. (2010), Yuen and Yan (2011)
AMPA	↓	Interneuron	PFC	Yuen and Yan (2009); Yuen et al. (2010)
NMDA	↓	Pyramidal	PFC, hippocampus, lateral amygdala	Kotecha et al. (2002); Wang et al. (2003), Beazely et al. (2006); Wang et al. (2006), Martina and Bergeron (2008); Herwerth et al. (2012)
GABA	↓	Pyramidal, various others	PFC, hippocampus, septal nucleus, thalamus, globus pallidus, subthalamic nucleus, substantia nigra	Wang et al. (2002); Shin et al. (2003), Florán et al. (2004a,b), Asaumi et al. (2006), Acosta-Garcia et al. (2009), Graziane et al. (2009); Gasca-Martinez et al. (2010), Govindaiah et al. (2010); Andersson et al. (2012a)
Voltage-gated calcium channels	↓	Pyramidal, granule cells	PFC, cerebellum	Mei et al. (1995); Wang et al. (2006)
Potassium channel: inward rectifying	↑		Cultured neurons	Werner et al. (1996); Pillai et al. (1998), Lavine et al. (2002); Wedemeyer et al. (2007)
Potassium channel: outward current	↓	Fast-spiking interneurons	Hippocampus	Andersson et al. (2012a)

Table 2 | Presynaptic effects of D4R activation (frequency).

Current	Increase/decrease	Cell type recorded	Region	Reference
Glutamate	↓	Pyramidal	PFC	Rubinstein et al. (2001)
Glutamate	No change	Interneuron	PFC, hippocampus	Yuen and Yan (2009); Andersson et al. (2012a)
GABA	↓	Various	Hypothalamus, septal nucleus, thalamus	Azdad et al. (2003); Baimoukhametova et al. (2004), Asaumi et al. (2006); Gasca-Martinez et al. (2010)

with the observation that *Drd4*$^{-/-}$ mice have dramatic increases in glutamatergic signaling (Rubinstein et al., 2001), these data suggest that the D4R is involved in homeostatic processes that serve to maintain overall glutamatergic excitability within a physiological range.

Effects on ion channels

D4R activation decreases voltage-gated calcium channel currents in acute PFC slices and cultured cerebellar granule cells (Mei et al., 1995; Wang et al., 2006). Heterologously expressed D4Rs also couple to G protein inwardly rectifying potassium channels in *Xenopus* oocytes (Werner et al., 1996; Wedemeyer et al., 2007) through a Gβγ-dependent mechanism (Pillai et al., 1998). In principle, both activities are well suited to reduce neurotransmitter release from presynaptic nerve terminals. Consistent with this notion, the D4R agonist PD168077 reduces mIPSC frequency onto layer V pyramidal neurons, indicative of reduced GABA release from local interneurons (Gao, 2007). Moreover, D4Rs modulate transmitter release in various midbrain regions receiving GABAergic projections from the globus pallidus (Baimoukhametova et al., 2004; Florán et al., 2004a,b; Asaumi et al., 2006; Acosta-Garcia et al., 2009; Gasca-Martinez et al., 2010). As mentioned earlier, globus pallidus GABAergic neurons express high levels of D4R (Mrzljak et al., 1996). Ablation of globus pallidus neurons by kainate injection prevents the inhibitory effects of PD168077 in the thalamic reticular nucleus and the substantia nigra (Acosta-Garcia et al., 2009; Gasca-Martinez et al., 2010). Effects on presynaptic calcium currents may also contribute to reduced glutamate release (Rubinstein et al., 2001; Romo-Parra et al., 2005; Yuen et al., 2010).

D4R SIGNALING PARTNERSHIPS

Dopamine receptors were initially classified according to their ability to positively or negatively couple to adenylyl cyclase to promote the production of the intracellular second messenger cAMP. Additionally, D2-type receptors mediate many of their physiological functions via liberation of Gβγ subunits and subsequent modulation of effectors such ion channels and receptors either by direct binding or by activation of intracellular signaling pathways such as phospholipase C and mitogen-activated protein (MAP) kinase. For general reviews on DA signaling pathways downstream of heterodimeric G proteins the reader is referred to a number of excellent earlier reviews on the subject (Missale et al., 1998; Greengard et al., 1999; Gainetdinov et al., 2004; Beaulieu and Gainetdinov, 2011). In addition, D4Rs have been implicated in a non-canonical signaling process unique among DA receptors that involves the stimulation of inherent methyl transferase activity to modulate phospholipid methylation in response to agonist treatment (Sharma et al., 1999).

In the current review, we will focus on signaling partnerships of the D4R with other G protein-coupled receptors (GPCRs) and with receptor tyrosine kinases (RTK) in the regulation of neuronal function, as many of the described D4R effects in the central nervous system are tightly linked to the functional association with other receptor systems. These synergistic partnerships encompass both direct and functional interactions between receptors.

Partnerships with other G protein-coupled receptors

It is now widely accepted that DA receptors and other GPCRs can form homomeric and heteromeric structures, and that these multimeric aggregates can affect both ligand binding as well as signaling characteristics of their constituent subunits (Ferre et al., 2007, 2009). Recent studies have shown that the D4R heteromerizes with the both the long (D2L) and the short (D2S) forms of the D2R, alternative splice variants that differ in their third intracellular loop sequence (Borroto-Escuela et al., 2011; Gonzalez et al., 2012b). Furthermore, neurochemical evidence suggests that interactions between D4 and D2S receptors mediate DA modulation of neurotransmitter release from corticostriatal glutamatergic projections where these receptors are co-expressed (Gonzalez et al., 2012b). Intriguingly, heteromerization with D2S receptors was observed with D4R variants harboring two or four, but not seven tandem repeats, and engineered mice with the human 7R version knocked into the mouse *Drd4* locus failed to show increased glutamate release in the striatum in response to co-activation of D2Rs and D4Rs (Gonzalez et al., 2012b). These findings are especially relevant in light of the implication of the 7R allele of the human *DRD4* gene in psychiatric disorders. A second interaction was recently reported between the D4R and α_{1B} or β1 adrenergic receptors in the pineal gland where these receptors synergize to regulate circadian melatonin synthesis (Gonzalez et al., 2012a).

Partnerships with receptor tyrosine kinases

Functional interactions between GPCRs and RTKs have been known for many years (Daub et al., 1996). These interactions are typically functional, rather than physical, and mostly manifest as transactivation of a RTK by a GPCR (Ferguson, 2003). An early example of such transactivation involves the regulation of NMDA receptors by D4Rs (see Inhibition of Ionotropic Receptors). In PFC neurons, activation of D4R signaling downregulates NMDA receptor-mediated synaptic currents and surface expression via protein kinase A inhibition and activation of protein phosphatase 1, effects typically attributed to canonical inhibitory G protein signaling (Wang et al., 2003). However, in hippocampal neurons,

D4R activation downregulates NMDA receptors via transactivation of the platelet-derived growth factor receptor (PDGFR) and downstream activation of phospholipase C/inositol triphosphate (IP3)/Ca^{2+} signaling (Kotecha et al., 2002). Interestingly, downregulation of NMDA receptors in the PFC in response to PDGFR transactivation by DA is mediated by D2Rs but not D4Rs (Beazely et al., 2006).

The D4R is also tightly linked to the acute effects of the NRG-1/ErbB4 signaling pathway in the central nervous system. Neuregulins comprise a family of secreted and membrane-bound factors characterized by the presence of an epidermal growth factor-like motif and that signal through ErbB RTKs to regulate a diverse array of developmental and acute processes in the peripheral and central nervous systems (Garratt et al., 2000; Buonanno and Fischbach, 2001; Falls, 2003). Moreover, both *NRG1* and *ERBB4* have been identified as risk genes for SCZ (Mei and Xiong, 2008). NRG-1/ErbB4 signaling inhibits the induction and reverses the early expression of LTP at CA3 to CA1 glutamatergic synapses (Huang et al., 2000; Kwon et al., 2005; Shamir et al., 2012). LTP reversal by NRG-1 critically depends on the activation of D4Rs (Kwon et al., 2008). Taken together with the finding that NRG-1 infusion in the dorsal hippocampus triggers DA release, this suggests that NRG-1/ErbB4 signaling regulates hippocampal LTP via a DA/D4R pathway (Kwon et al., 2008). However, it is not known if these receptor systems interact in the same or across different cell types, or which signaling pathways link their activation to the removal of synaptic AMPA receptors that underlie LTP reversal by NRG-1 and D4R agonists. For the following reasons, a direct effect on PV interneurons is more likely than on pyramidal neurons: (1) ErbB4 receptors are undetectable in pyramidal neurons (Vullhorst et al., 2009; Neddens et al., 2011) while both ErbB4 and D4Rs are expressed in GABAergic cells including PV interneurons (Mrzljak et al., 1996; Andersson et al., 2012b). (2) Inhibition of LTP induction and reversal of LTP are absent from mice with targeted deletions of ErbB4 in PV interneurons (Chen et al., 2010; Shamir et al., 2012); (3) Both receptors also appear to synergize in the modulation of hippocampal gamma oscillations that critically depend on PV interneurons (see D4R and Gamma Oscillations for details).

D4R AND GAMMA OSCILLATIONS

Two lines of evidence suggested a possible role for the D4R in the regulation of hippocampal gamma oscillations. First, many PV interneurons contain D4R mRNA and protein (see Expression in FS Parvalbumin Interneurons). Second, earlier studies in acute rodent slices showed that NRG-1 signaling via ErbB4 potently augments kainate-induced gamma oscillations in hippocampal area CA3 (Fisahn et al., 2009). Taken together with the finding that NRG-1/ErbB4 effects on LTP reversal in CA1 critically depend on D4R signaling (see Partnerships with Receptor Tyrosine Kinases), it was plausible to hypothesize that NRG-1 effects on gamma oscillation power also depend on D4R signaling (see Buonanno, 2010). Indeed, the D4R antagonist L-745,870 largely blocks the potentiating effects of NRG-1 on gamma oscillations (Andersson et al., 2012b). Conversely, the D4R agonist PD168077 increases gamma power, albeit to a lesser extent than ErbB4 activation by NRG-1, suggesting that NRG-1/ErbB4 signaling engages multiple signaling systems that synergistically augment gamma oscillations. Neither PD168077 nor NRG-1 induce gamma oscillations in naïve slices, indicating that their modulatory effects impinge on ongoing oscillations (Fisahn et al., 2009; Andersson et al., 2012b). An investigation into the cellular effects of D4R signaling revealed that PD168077 enhances spike coherence and phase-coupling of action potentials relative to gamma cycle in FS interneurons, suggesting that D4R activation enhances gamma power by augmenting the synchronized inhibition of pyramidal neurons (Andersson et al., 2012a). Notably, pharmacological activation of other DA receptors, or direct application of DA, had no effect on gamma power. However, an enhancement of gamma power by DA was unmasked by simultaneous application of the D1-type receptor blocker SCH23390, indicating that D1-type DA receptors antagonize the effects of D4Rs on gamma oscillations (Andersson et al., 2012b).

Interestingly, D4R-mediated augmentation of gamma power is sensitive to pharmacological blockade of NMDA receptors by AP5 (D-2-amino-5-phosphonopentanoate), although AP5 has no effect on gamma power *per se* (Andersson et al., 2012b). Given that D4Rs internalize NMDA receptors (see Inhibition of Ionotropic Receptors), a simple explanation might be that D4R activation improves excitation-spiking coupling by removing NMDA receptors from excitatory synapses of FS interneurons to increase gamma power. Indeed, blockade of NMDA receptors increases gamma power *in vivo* (Pinault, 2008). Yet, if this were true, blocking NMDA receptors would occlude rather than inhibit the D4R effect on gamma power, and would increase gamma power even in the absence of D4R agonist. However, as mentioned earlier, this was not observed *in vitro* (Andersson et al., 2012a). At this time, the relationship between D4R signaling and NMDA receptor function in kainate-induced hippocampal oscillations is therefore unclear.

Studies exploring the role of D4Rs in gamma oscillations in the PFC are currently lacking. Considering the conserved expression of D4Rs and ErbB4 receptors in PV interneurons in the hippocampus and neocortex, it is reasonable to speculate that synergistic signaling downstream of both signaling pathways also modulates neocortical gamma rhythms. On the other hand, there are significant differences between the hippocampus and the PFC. For example, D4R activation reduces NMDA currents via PDGFR transactivation in the hippocampus and via canonical G_{ai} signaling the PFC (see Partnerships with Receptor Tyrosine Kinases). Furthermore, D4Rs in the PFC are expressed on both interneurons and pyramidal neurons, while in the hippocampus pyramidal neurons mostly do not express the receptor (see Expression in FS Parvalbumin Interneurons). However, indirect evidence already exists that D4Rs in the human cortex modulate gamma oscillations. Carriers of the 7R *DRD4* allele (*DRD4.7*) show enhanced evoked and induced gamma oscillations in the frontal and association cortices in response to auditory stimuli (Demiralp et al., 2007). This increase in evoked gamma oscillations does not discriminate between target and filler stimuli, suggesting that it is harder for subjects to distinguish between salient and distracting stimuli, a finding that is consistent with the association of the *DRD4.7* allele with ADHD and potentially cognitive function in other psychiatric disorders (see Pharmacological Properties and Involvement in Psychiatric Disorders). Importantly, the 7R variant

has half the potency of more common third-loop repeat variants for inhibiting cAMP (Asghari et al., 1995) and less effectively forms heterodimers with the long and short variants of the D2R (Borroto-Escuela et al., 2011; Gonzalez et al., 2012b). If the modulation of gamma rhythms in individuals carrying the *DRD4.7* polymorphism is indeed compromised, the prediction would be that normal D4R function enhances signal-to-noise ratios by suppressing gamma rhythms in response to non-salient sensory stimuli. At least in principle, the finding that D4Rs decrease AMPA currents in interneurons in the PFC (Yuen and Yan, 2009), and that decreased excitatory drive onto PV interneurons reduces gamma oscillation power (Rotaru et al., 2011), correlates well with increases in gamma in subjects carrying the *DRD4.7* allele.

OUTLOOK

The association of gamma oscillations with attention, working memory and other cognitive functions, and their perturbation in numerous psychiatric disorders, underscores the potential therapeutic value of targeting networks that generate and regulate gamma oscillations (see Andersson et al., 2012b). Based on work reviewed herein, we propose that a potential functional role for DA in modulating cognitive processes is through opposing effects on neuronal excitability, synaptic strength, and gamma oscillations mediated via D1- and D2-type DA receptors (see Kwon et al., 2008; Andersson et al., 2012b). Consequently, the regional and temporal dynamic changes of DA concentrations in the hippocampus and PFC could determine the D1-/D2-type receptor activity ratio that ultimately modulates gamma oscillation power and synchrony at rest (baseline) and evoked by salient stimuli; the relative activity of both receptor types could account for the inverted U-shaped dose–response relationship associated with DA effects. The D1-/D2-type receptor activity ratio is especially important for the regulation of GABAergic function in the PFC (Seamans et al., 2001; Goldman-Rakic et al., 2004; Trantham-Davidson et al., 2004; Enomoto et al., 2011), where excitatory/inhibitory balance is critical for the optimization of cognitive task performance and flexibility (Winterer and Weinberger, 2004). Because optimal signal-to-noise ratio of cortical microcircuits is important for cognitive tasks (Winterer and Weinberger, 2004), and alterations in gamma frequency oscillations are associated with psychiatric disorders (Herrmann and Demiralp, 2005; Uhlhaas and Singer, 2010), identifying modulators that improve signal-to-noise ratio by targeting cortical microcircuits could constitute a promising therapeutic approach.

We propose, based on the functional properties and expression of ErbB4 and D4Rs in GABAergic PV-expressing interneurons in the DLPFC (Mrzljak et al., 1996) and hippocampus (Andersson et al., 2012b), as well as their effects on kainate-induced gamma oscillations and plasticity (Kwon et al., 2008), that these receptors are well situated to modulate network activity that affects cognition (see Partnerships with Receptor Tyrosine Kinases, and D4R and Gamma Oscillations). Although D4R-specific antagonists were found to be ineffective as antipsychotics for the treatment of SCZ (see Pharmacological Properties and Involvement in Psychiatric Disorders), D4R-targeting drugs still hold potential as adjunct therapies to ameliorate the cognitive deficits seen in SCZ (see Andersson et al., 2012b). The fact that D4R activity can regulate working memory and other cognitive behaviors in rodent models (Zhang et al., 2004; Braszko, 2009; Young et al., 2011), and that their effects are state-dependent (Zhang et al., 2004), suggests that D4R modulators could be used to modulate D1-/D2-type receptor ratio and improve signal-to-noise ratio. Given that the cognitive responses to D4R-targeting drugs depend on emotional salience and baseline ability that may easily reflect basal DA concentration, it is plausible that people with different variants of the D4R may respond differently to these therapies. D4R agonists may prove more efficacious in some patients, while D4R antagonists may prove more efficacious in others, and oscillatory activity may help predict which therapy will be more effective.

While pharmacological targeting of D4Rs for regulating network activity and cognitive function appears to be a promising approach, as we have elaborated throughout this review, one must be mindful of the many complexities of D4R signaling when designing and interpreting meaningful experiments for future studies. These considerations include the likelihood that the cellular D4R expression pattern varies in different cortical regions, that drug effects on D4R function might be dose-dependent and that the receptor might engage in distinct signaling partnerships in different cells or subcellular compartments. The challenge will therefore be to find among the abundance of possibilities the ones that are pertinent to the regulation of local circuit activity and their potential to improve cognitive processes deficient in a number of neurological and psychiatric disorders. Perhaps treatments tailored to increase or reduce D4R activity in different patients holds promise for approaches that for the past decades have failed to improve working memory and other cognitive functions in these disorders (see Insel, 2010).

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REFERENCES

Acosta-Garcia, J., Hernandez-Chan, N., Paz-Bermudez, F., Sierra, A., Erlij, D., Aceves, J., et al. (2009). D4 and D1 dopamine receptors modulate [3H] GABA release in the substantia nigra pars reticulata of the rat. *Neuropharmacology* 57, 725–730. doi: 10.1016/j.neuropharm.2009.08.010

Adesnik, H., and Scanziani, M. (2010). Lateral competition for cortical space by layer-specific horizontal circuits. *Nature* 464, 1155–1160. doi: 10.1038/nature08935

Ahveninen, J., Kähkönen, S., Tiitinen, H., Pekkonen, E., Huttunen, J., Kaakkola, S., et al. (2000). Suppression of transient 40-Hz auditory response by haloperidol suggests modulation of human selective attention by dopamine D2 receptors. *Neurosci. Lett.* 292, 29–32. doi: org/10.1016/S0304-3940(00)01429-4

American Psychiatric Association. (2000). *Diagnostic and Statistical Manual of Mental Disorders: DSM-IV-TR*, 4th Edn. Washington, DC: American Psychiatric Association.

Andersson, R., Johnston, A., and Fisahn, A. (2012a). Dopamine D4 receptor activation increases hippocampal gamma oscillations by enhancing synchronization of fast-spiking interneurons. *PLoS ONE* 7:e40906. doi: 10.1371/journal.pone.0040906

Andersson, R. H., Johnston, A., Herman, P. A., Winzer-Serhan, U. H., Karavanova, I., Vullhorst, D., et al. (2012b). Neuregulin and dopamine modulation of hippocampal gamma oscillations is dependent on dopamine D4 receptors. *Proc. Natl. Acad.*

Sci. U.S.A. 109, 13118–13123. doi: 10.1073/pnas.1201011109

Arcos-Burgos, M., Castellanos, F. X., Konecki, D., Lopera, F., Pineda, D., Palacio, J. D., et al. (2004). Pedigree disequilibrium test (PDT) replicates association and linkage between DRD4 and ADHD in multigenerational and extended pedigrees from a genetic isolate. *Mol. Psychiatry* 9, 252–259. doi: 10.1038/sj.mp.4001396

Ariano, M. A., Wang, J., Noblett, K. L., Larson, E. R., and Sibley, D. R. (1997). Cellular distribution of the rat D4 dopamine receptor protein in the CNS using anti-receptor antisera. *Brain Res.* 752, 26–34. doi: 10.1016/S0006-8993(96)01422-9

Arnsten, A. F. T., Murphy, B., and Merchant, K. (2000). The selective dopamine D4 receptor antagonist, PNU-101387G, prevents stress-induced cognitive deficits in monkeys. *Neuropsychopharmacology* 23, 405–410. doi: 10.1016/S0893-133X(00)00133-0

Asaumi, Y., Hasuo, H., and Akasu, T. (2006). Dopamine presynaptically depresses fast inhibitory synaptic transmission via D4 receptor-protein kinase A pathway in the rat dorsolateral septal nucleus. *J. Neurophysiol.* 96, 591–601. doi: 10.1152/jn.00966.2005

Asghari, V., Sanyal, S., Buchwaldt, S., Paterson, A., Jovanovic, V., and Van Tol, H. H. M. (1995). Modulation of intracellular cyclic AMP levels by different human dopamine D4 receptor variants. *J. Neurochem.* 65, 1157–1165. doi: 10.1046/j.1471-4159.1995.65031157.x

Avale, M. E., Falzone, T. L., Gelman, D. M., Low, M. J., Grandy, D. K., and Rubinstein, M. (2004). The dopamine D4 receptor is essential for hyperactivity and impaired behavioral inhibition in a mouse model of attention deficit/hyperactivity disorder. *Mol. Psychiatry* 9, 718–726. doi: 10.1038/sj.mp.4001474

Azdad, K., Piet, R., Poulain, D. A., and Oliet, S. H. (2003). Dopamine D4 receptor-mediated presynaptic inhibition of GABAergic transmission in the rat supraoptic nucleus. *J. Neurophysiol.* 90, 559–565. doi: 10.1152/jn.00226.2003

Baimoukhametova, D. V., Hewitt, S. A., Sank, C. A., and Bains, J. S. (2004). Dopamine modulates use-dependent plasticity of inhibitory synapses. *J. Neurosci.* 24, 5162–5171. doi: 10.1523/JNEUROSCI.4979-03.2004

Baldessarini, R. J., and Frankenburg, F. R. (1991). Clozapine. A novel antipsychotic agent. *N. Engl. J. Med.* 324, 746–754. doi: 10.1056/NEJM199103143241107

Baldeweg, T., Spence, S., Hirsch, S. R., and Gruzelier, J. (1998). Gamma-band electroencephalographic oscillations in a patient with somatic hallucinations. *Lancet* 352, 620–621. doi: 10.1016/S0140-6736(05)79575-1

Barr, C. L., Wigg, K. G., Bloom, S., Schachar, R., Tannock, R., Roberts, W., et al. (2000). Further evidence from haplotype analysis for linkage of the dopamine D4 receptor gene and attention-deficit hyperactivity disorder. *Am. J. Med. Genet.* 96, 262–267. doi: 10.1002/1096-8628(20000612)96:3

Bartos, M., Vida, I., and Jonas, P. (2007). Synaptic mechanisms of synchronized gamma oscillations in inhibitory interneuron networks. *Nat. Rev. Neurosci.* 8, 45–56. doi: 10.1038/nrn2044

Basar-Eroglu, C., Brand, A., Hildebrandt, H., Karolina Kedzior, K., Mathes, B., and Schmiedt, C. (2007). Working memory related gamma oscillations in schizophrenia patients. *Int. J. Psychophysiol.* 64, 39–45. doi: 10.1016/j.ijpsycho.2006.07.007

Beaulieu, J. M., and Gainetdinov, R. R. (2011). The physiology, signaling, and pharmacology of dopamine receptors. *Pharmacol. Rev.* 63, 182–217. doi: 10.1124/pr.110.002642

Beazely, M. A., Tong, A., Wei, W. L., Van Tol, H., Sidhu, B., and MacDonald, J. F. (2006). D2-class dopamine receptor inhibition of NMDA currents in prefrontal cortical neurons is platelet-derived growth factor receptor-dependent. *J. Neurochem.* 98, 1657–1663. doi: 10.1111/j.1471-4159.2006.04064.x

Behrens, M. M., and Sejnowski, T. J. (2009). Does schizophrenia arise from oxidative dysregulation of parvalbumin-interneurons in the developing cortex? *Neuropharmacology* 57, 193–200. doi: 10.1016/j.neuropharm.2009.06.002

Belforte, J. E., Zsiros, V., Sklar, E. R., Jiang, Z., Yu, G., Li, Y., et al. (2010). Postnatal NMDA receptor ablation in corticolimbic interneurons confers schizophrenia-like phenotypes. *Nat. Neurosci.* 13, 76–83. doi: 10.1038/nn.2447

Benchenane, K., Peyrache, A., Khamassi, M., Tierney, P. L., Gioanni, Y., Battaglia, F. P., et al. (2010). Coherent theta oscillations and reorganization of spike timing in the hippocampal- prefrontal network upon learning. *Neuron* 66, 921–936. doi: 10.1016/j.neuron.2010.05.013

Benchenane, K., Tiesinga, P. H., and Battaglia, F. P. (2011). Oscillations in the prefrontal cortex: a gateway to memory and attention. *Curr. Opin. Neurobiol.* 21, 475–485. doi: 10.1016/j.conb.2011.01.004

Benes, F. M., and Berretta, S. (2001). GABAergic interneurons: implications for understanding schizophrenia and bipolar disorder. *Neuropsychopharmacology* 25, 1–27. doi: 10.1016/S0893-133X(01)00225-1

Beneyto, M., and Lewis, D. A. (2011). Insights into the neurodevelopmental origin of schizophrenia from postmortem studies of prefrontal cortical circuitry. *Int. J. Dev. Neurosci.* 29, 295–304. doi: 10.1016/j.ijdevneu.2010.08.003

Bernaerts, P., and Tirelli, E. (2003). Facilitatory effect of the dopamine D4 receptor agonist PD168,077 on memory consolidation of an inhibitory avoidance learned response in C57BL/6J mice. *Behav. Brain Res.* 142, 41–52. doi: 10.1016/S0166-4328(02)00371-6

Borroto-Escuela, D. O., Van Craenenbroeck, K., Romero-Fernandez, W., Guidolin, D., Woods, A. S., Rivera, A., et al. (2011). Dopamine D2 and D4 receptor heteromerization and its allosteric receptor–receptor interactions. *Biochem. Biophys. Res. Commun.* 404, 928–934. doi: 10.1016/j.bbrc.2010.12.083

Braszko, J. J. (2009). Dopamine D4 receptor antagonist L745,870 abolishes cognitive effects of intracerebroventricular angiotensin IV and des-Phe(6)-Ang IV in rats. *Eur. Neuropsychopharmacol.* 19, 85–91. doi: 10.1016/j.euroneuro.2008.08.011

Brenhouse, H. C., Sonntag, K. C., and Andersen, S. L. (2008). Transient D1 dopamine receptor expression on prefrontal cortex projection neurons: relationship to enhanced motivational salience of drug cues in adolescence. *J. Neurosci.* 28, 2375–2382. doi: 10.1523/JNEUROSCI.5064-07.2008

Bristow, L. J., Collinson, N., Cook, G. P., Curtis, N., Freedman, S. B., Kulagowski, J. J., et al. (1997). L-745,870, a subtype selective dopamine D4 receptor antagonist, does not exhibit a neuroleptic-like profile in rodent behavioral tests. *J. Pharmacol. Exp. Ther.* 283, 1256–1263.

Broderick, P. A., and Piercey, M. F. (1998). Clozapine, haloperidol, and the D4 antagonist PNU-101387G: in vivo effects on mesocortical, mesolimbic, and nigrostriatal dopamine and serotonin release. *J. Neural Transm.* 105, 749–767. doi: 10.1007/s007020050093

Browman, K. E., Curzon, P., Pan, J. B., Molesky, A. L., Komater, V. A., Decker, M. W., et al. (2005). A-412997, a selective dopamine D4 agonist, improves cognitive performance in rats. *Pharmacol. Biochem. Behav.* 82, 148–155. doi: 10.1016/j.pbb.2005.08.002

Brozoski, T. J., Brown, R. M., Rosvold, H. E., and Goldman, P. S. (1979). Cognitive deficit caused by regional depletion of dopamine in prefrontal cortex of rhesus monkey. *Science* 205, 929–932. doi: 10.1126/science.112679

Buonanno, A. (2010). The neuregulin signaling pathway and schizophrenia: from genes to synapses and neural circuits. *Brain Res. Bull.* 83, 122–131. doi: 10.1016/j.brainresbull.2010.07.012

Buonanno, A., and Fischbach, G. D. (2001). Neuregulin and ErbB receptor signaling pathways in the nervous system. *Curr. Opin. Neurobiol.* 11, 287–296. doi: 10.1016/S0959-4388(00)00210-5

Buzsaki, G., and Wang, X. J. (2012). Mechanisms of gamma oscillations. *Annu. Rev. Neurosci.* 35, 203–225. doi: 10.1146/annurev-neuro-062111-150444

Cardin, J. A., Carlen, M., Meletis, K., Knoblich, U., Zhang, F., Deisseroth, K., et al. (2009). Driving fast-spiking cells induces gamma rhythm and controls sensory responses. *Nature* 459, 663–667. doi: 10.1038/nature08002

Carlen, M., Meletis, K., Siegle, J. H., Cardin, J. A., Futai, K., Vierling-Claassen, D., et al. (2012). A critical role for NMDA receptors in parvalbumin interneurons for gamma rhythm induction and behavior. *Mol. Psychiatry* 17, 537–548. doi: 10.1038/mp.2011.31

Ceci, A., Brambilla, A., Duranti, P., Grauert, M., Grippa, N., and Borsini, F. (1999). Effect of antipsychotic drugs and selective dopaminergic antagonists on dopamine-induced facilitatory activity in prelimbic cortical pyramidal neurons. An *in vitro* study. *Neuroscience* 93, 107–115. doi: 10.1016/S0306-4522(99)00123-2

Chang, F. M., Kidd, J. R., Livak, K. J., Pakstis, A. J., and Kidd, K. K. (1996). The world-wide distribution of allele frequencies at the human dopamine D4 receptor locus. *Hum. Genet.* 98, 91–101. doi: 10.1007/s004390050166

Chattopadhyaya, B., Di Cristo, G., Wu, C. Z., Knott, G., Kuhlman, S., Fu, Y., et al. (2007). GAD67-mediated GABA synthesis and signaling regulate inhibitory synaptic innervation in the visual

cortex. *Neuron* 54, 889–903. doi: 10.1016/j.neuron.2007.05.015

Chen, Y. J., Zhang, M., Yin, D. M., Wen, L., Ting, A., Wang, P., et al. (2010). ErbB4 in parvalbumin-positive interneurons is critical for neuregulin 1 regulation of long-term potentiation. *Proc. Natl. Acad. Sci. U.S.A.* 107, 21818–21823. doi: 10.1073/pnas.1010669107

Cho, R. Y., Konecky, R. O., and Carter, C. S. (2006). Impairments in frontal cortical gamma synchrony and cognitive control in schizophrenia. *Proc. Natl. Acad. Sci. U.S.A.* 103, 19878–19883. doi: 10.1073/pnas.0609440103

Colgin, L. L. (2011). Oscillations and hippocampal-prefrontal synchrony. *Curr. Opin. Neurobiol.* 21, 467–474. doi: 10.1016/j.conb.2011.04.006

Corrigan, M. H., Gallen, C. C., Bonura, M. L., and Merchant, K. M. (2004). Effectiveness of the selective D4 antagonist sonepiprazole in schizophrenia: a placebo-controlled trial. *Biol. Psychiatry* 55, 445–451. doi: 10.1016/j.biopsych.2003.10.004

Czermak, C., Lehofer, M., Liebmann, P. M., and Traynor, J. (2006). [35S]GTPgammaS binding at the human dopamine D4 receptor variants hD4.2, hD4.4 and hD4.7 following stimulation by dopamine, epinephrine and norepinephrine. *Eur. J. Pharmacol.* 531, 20–24. doi: 10.1016/j.ejphar.2005.11.063

Daub, H., Weiss, F. U., Wallasch, C., and Ullrich, A. (1996). Role of transactivation of the EGF receptor in signalling by G-protein-coupled receptors. *Nature* 379, 557–560. doi: 10.1038/379557a0

de Almeida, J., Palacios, J. M., and Mengod, G. (2008). Distribution of 5-HT and DA receptors in primate prefrontal cortex: implications for pathophysiology and treatment. *Prog. Brain Res.* 172, 101–115. doi: 10.1016/S0079-6123(08)00905-9

del Campo, N., Chamberlain, S. R., Sahakian, B. J., and Robbins, T. W. (2011). The roles of dopamine and noradrenaline in the pathophysiology and treatment of attention-deficit/hyperactivity disorder. *Biol. Psychiatry* 69, e145–e157. doi: 10.1016/j.biopsych.2011.02.036

Demiralp, T., Herrmann, C. S., Erdal, M. E., Ergenoglu, T., Keskin, Y. H., Ergen, M., and Beydagi, H. (2007). DRD4 and DAT1 polymorphisms modulate human gamma band responses. *Cereb. Cortex* 17, 1007–1019. doi: 10.1093/cercor/bhl011

Do, K. Q., Cabungcal, J. H., Frank, A., Steullet, P., and Cuenod, M. (2009). Redox dysregulation, neurodevelopment, and schizophrenia. *Curr. Opin. Neurobiol.* 19, 220–230. doi: 10.1016/j.conb.2009.05.001

Dulawa, S. C., Grandy, D. K., Low, M. J., Paulus, M. P., and Geyer, M. A. (1999). Dopamine D4 receptor-knock-out mice exhibit reduced exploration of novel stimuli. *J. Neurosci.* 19, 9550–9556.

Dzirasa, K., Ramsey, A. J., Takahashi, D. Y., Stapleton, J., Potes, J. M., Williams, J. K., et al. (2009). Hyperdopaminergia and NMDA receptor hypofunction disrupt neural phase signaling. *J. Neurosci.* 29, 8215–8224. doi: 10.1523/JNEUROSCI.1773-09.2009

Dzirasa, K., Ribeiro, S., Costa, R., Santos, L. M., Lin, S. C., Grosmark, A., et al. (2006). Dopaminergic control of sleep–wake states. *J. Neurosci.* 26, 10577–10589. doi: 10.1523/JNEUROSCI.1767-06.2006

Ebstein, R. P., Novick, O., Umansky, R., Priel, B., Osher, Y., Blaine, D., et al. (1996). Dopamine D4 receptor (D4DR) exon III polymorphism associated with the human personality trait of novelty seeking. *Nat. Genet.* 12, 78–80. doi: 10.1038/ng0196-78

Ehrlichman, R. S., Gandal, M. J., Maxwell, C. R., Lazarewicz, M. T., Finkel, L. H., Contreras, D., et al. (2009). *N*-methyl-D-aspartic acid receptor antagonist-induced frequency oscillations in mice recreate pattern of electrophysiological deficits in schizophrenia. *Neuroscience* 158, 705–712. doi: 10.1016/j.neuroscience.2008.10.031

Enomoto, T., Tse, M. T., and Floresco, S. B. (2011). Reducing prefrontal gamma-aminobutyric acid activity induces cognitive, behavioral, and dopaminergic abnormalities that resemble schizophrenia. *Biol. Psychiatry* 69, 432–441. doi: 10.1016/j.biopsych.2010.09.038

Falls, D. L. (2003). Neuregulins: functions, forms, and signaling strategies. *Exp. Cell Res.* 284, 14–30. doi: 10.1016/S0014-4827(02)00102-7

Falzone, T. L., Gelman, D. M., Young, J. I., Grandy, D. K., Low, M. J., and Rubinstein, M. (2002). Absence of dopamine D4 receptors results in enhanced reactivity to unconditioned, but not conditioned, fear. *Eur. J. Neurosci.* 15, 158–164. doi: 10.1046/j.0953-816x.2001.01842.x

Faraone, S. V., Biederman, J., Weiffenbach, B., Keith, T., Chu, M. P., Weaver, A., et al. (1999). Dopamine D4 gene 7-repeat allele and attention deficit hyperactivity disorder. *Am. J. Psychiatry* 156, 768–770.

Ferguson, S. S. (2003). Receptor tyrosine kinase transactivation: fine-tuning synaptic transmission. *Trends Neurosci.* 26, 119–122. doi: 10.1016/S0166-2236(03)00022-5

Ferrarelli, F., Massimini, M., Peterson, M. J., Riedner, B. A., Lazar, M., Murphy, M. J., et al. (2008). Reduced evoked gamma oscillations in the frontal cortex in schizophrenia patients: a TMS/EEG study. *Am. J. Psychiatry* 165, 996–1005. doi: 10.1176/appi.ajp.2008.07111733

Ferre, S., Baler, R., Bouvier, M., Caron, M. G., Devi, L. A., Durroux, T., et al. (2009). Building a new conceptual framework for receptor heteromers. *Nat. Chem. Biol.* 5, 131–134. doi: 10.1038/nchembio0309-131

Ferre, S., Ciruela, F., Woods, A. S., Lluis, C., and Franco, R. (2007). Functional relevance of neurotransmitter receptor heteromers in the central nervous system. *Trends Neurosci.* 30, 440–446. doi: 10.1016/j.tins.2007.07.001

Fisahn, A., Neddens, J., Yan, L., and Buonanno, A. (2009). Neuregulin-1 modulates hippocampal gamma oscillations: implications for schizophrenia. *Cereb. Cortex* 19, 612–618. doi: 10.1093/cercor/bhn107

Florán, B., Florán, L., Erlij, D., and Aceves, J. (2004a). Activation of dopamine D4 receptors modulates [3H]GABA release in slices of the rat thalamic reticular nucleus. *Neuropharmacology* 46, 497–503. doi: 10.1016/j.neuropharm.2003.10.004

Florán, B., Florán, L., Erlij, D., and Aceves, J. (2004b). Dopamine D4 receptors inhibit depolarization-induced [3H]GABA release in the rat subthalamic nucleus. *Eur. J. Pharmacol.* 498, 97–102. doi: 10.1016/j.ejphar.2004.07.078

Fuchs, E. C., Zivkovic, A. R., Cunningham, M. O., Middleton, S., Lebeau, F. E., Bannerman, D. M., et al. (2007). Recruitment of parvalbumin-positive interneurons determines hippocampal function and associated behavior. *Neuron* 53, 591–604. doi: 10.1016/j.neuron.2007.01.031

Gainetdinov, R. R., Premont, R. T., Bohn, L. M., Lefkowitz, R. J., and Caron, M. G. (2004). Desensitization of G protein-coupled receptors and neuronal functions. *Annu. Rev. Neurosci.* 27, 107–144. doi: 10.1146/annurev.neuro.27.070203.144206

Gallinat, J., Winterer, G., Herrmann, C. S., and Senkowski, D. (2004). Reduced oscillatory gamma-band responses in unmedicated schizophrenic patients indicate impaired frontal network processing. *Clin. Neurophysiol.* 115, 1863–1874. doi: 10.1016/j.clinph.2004.03.013

Gan, L., Falzone, T. L., Zhang, K., Rubinstein, M., Baldessarini, R. J., and Tarazi, F. I. (2004). Enhanced expression of dopamine D(1) and glutamate NMDA receptors in dopamine D(4) receptor knockout mice. *J. Mol. Neurosci.* 22, 167–178. doi: 10.1385/JMN:22:3:167

Gandal, M. J., Edgar, J. C., Klook, K., and Siegel, S. J. (2012). Gamma synchrony: towards a translational biomarker for the treatment-resistant symptoms of schizophrenia. *Neuropharmacology* 62, 1504–1518. doi: 10.1016/j.neuropharm.2011.02.007

Gao, W. J. (2007). Acute clozapine suppresses synchronized pyramidal synaptic network activity by increasing inhibition in the ferret prefrontal cortex. *J. Neurophysiol.* 97, 1196–1208. doi: 10.1152/jn.00400.2006

Gao, W. J., Wang, Y., and Goldman-Rakic, P. S. (2003). Dopamine modulation of perisomatic and peridendritic inhibition in prefrontal cortex. *J. Neurosci.* 23, 1622–1630.

Garratt, A. N., Britsch, S., and Birchmeier, C. (2000). Neuregulin, a factor with many functions in the life of a Schwann cell. *Bioessays* 22, 987–996. doi: 10.1002/1521-1878(200011)22:11<987::AID-BIES5>3.0.CO;2-5

Gasca-Martinez, D., Hernandez, A., Sierra, A., Valdiosera, R., Anaya-Martinez, V., Floran, B., et al. (2010). Dopamine inhibits GABA transmission from the globus pallidus to the thalamic reticular nucleus via presynaptic D4 receptors. *Neuroscience* 169, 1672–1681. doi: 10.1016/j.neuroscience.2010.05.048

Gizer, I. R., Ficks, C., and Waldman, I. D. (2009). Candidate gene studies of ADHD: a meta-analytic review. *Hum. Genet.* 126, 51–90. doi: 10.1007/s00439-009-0694-x

Gloveli, T., Dugladze, T., Saha, S., Monyer, H., Heinemann, U., Traub, R. D., et al. (2005). Differential involvement of oriens/pyramidale interneurones in hippocampal network oscillations *in vitro*. *J. Physiol.* 562, 131–147. doi: 10.1113/jphysiol.2004.073007

Goldberg, E. M., Jeong, H. Y., Kruglikov, I., Tremblay, R., Lazarenko, R. M., and Rudy, B. (2011). Rapid developmental maturation of neocortical FS cell intrinsic excitability. *Cereb. Cortex* 21, 666–682. doi: 10.1093/cercor/bhq138

Goldberg, T. E., Egan, M. F., Gscheidle, T., Coppola, R., Weickert, T., Kolachana, B. S., et al. (2003). Executive subprocesses in working memory: relationship to catechol-*O*-methyltransferase Val158Met genotype and schizophrenia. *Arch.*

Gen. Psychiatry 60, 889–896. doi: 10.1001/archpsyc.60.9.889

Goldman-Rakic, P. S. (1995). Cellular basis of working memory. *Neuron* 14, 477–485. doi: 10.1016/0896-6273(95)90304-6

Goldman-Rakic, P. S., Castner, S. A., Svensson, T. H., Siever, L. J., and Williams, G. V. (2004). Targeting the dopamine D1 receptor in schizophrenia: insights for cognitive dysfunction. *Psychopharmacology* 174, 3–16. doi: 10.1007/s00213-004-1793-y

Goldstein, R. Z., and Volkow, N. D. (2011). Dysfunction of the prefrontal cortex in addiction: neuroimaging findings and clinical implications. *Nat. Rev. Neurosci.* 12, 652–669. doi: 10.1038/nrn3119

Gonzalez, S., Moreno-Delgado, D., Moreno, E., Perez-Capote, K., Franco, R., Mallol, J., et al. (2012a). Circadian-related heteromerization of adrenergic and dopamine D_4 receptors modulates melatonin synthesis and release in the pineal gland. *PLoS Biol.* 10:e1001347. doi: 10.1371/journal.pbio.1001347

Gonzalez, S., Rangel-Barajas, C., Peper, M., Lorenzo, R., Moreno, E., Ciruela, F., et al. (2012b). Dopamine D_4 receptor, but not the ADHD-associated $D_{4.7}$ variant, forms functional heteromers with the dopamine D_{2S} receptor in the brain. *Mol. Psychiatry* 17, 650–662. doi: 10.1038/mp.2011.93

Gonzalez-Burgos, G., and Lewis, D. A. (2012). NMDA receptor hypofunction, parvalbumin-positive neurons, and cortical gamma oscillations in schizophrenia. *Schizophr. Bull.* 38, 950–957. doi: 10.1093/schbul/sbs010

Gorelova, N., Seamans, J. K., and Yang, C. R. (2002). Mechanisms of dopamine activation of fast-spiking interneurons that exert inhibition in rat prefrontal cortex. *J. Neurophysiol.* 88, 3150–3166. doi: 10.1152/jn.00335.2002

Govindaiah, G., Wang, T., Gillette, M. U., Crandall, S. R., and Cox, C. L. (2010). Regulation of inhibitory synapses by presynaptic D_4 dopamine receptors in thalamus. *J. Neurophysiol.* 104, 2757–2765. doi: 10.1152/jn.00361.2010

Grace, A. A., Floresco, S. B., Goto, Y., and Lodge, D. J. (2007). Regulation of firing of dopaminergic neurons and control of goal-directed behaviors. *Trends Neurosci.* 30, 220–227. doi: 10.1016/j.tins.2007.03.003

Graziane, N. M., Yuen, E. Y., and Yan, Z. (2009). Dopamine D4 receptors regulate GABAA receptor trafficking via an actin/cofilin/myosin-dependent mechanism. *J. Biol. Chem.* 284, 8329–8336. doi: 10.1074/jbc.M807387200

Green, M. F. (1996). What are the functional consequences of neurocognitive deficits in schizophrenia? *Am. J. Psychiatry* 153, 321–330. doi: 10.1176/appi.pn.2013.6a7

Greengard, P., Allen, P. B., and Nairn, A. C. (1999). Beyond the dopamine receptor: the DARPP-32/protein phosphatase-1 cascade. *Neuron* 23, 435–447. doi: 10.1016/S0896-6273(00)80798-9

Gu, Z., Jiang, Q., Yuen, E. Y., and Yan, Z. (2006). Activation of dopamine D4 receptors induces synaptic translocation of Ca^{2+}/calmodulin-dependent protein kinase II in cultured prefrontal cortical neurons. *Mol. Pharmacol.* 69, 813–822.

Haenschel, C., Bittner, R. A., Waltz, J., Haertling, F., Wibral, M., Singer, W., et al. (2009). Cortical oscillatory activity is critical for working memory as revealed by deficits in early-onset schizophrenia. *J. Neurosci.* 29, 9481–9489. doi: 10.1523/JNEUROSCI.1428-09.2009

Haig, A. R., Gordon, E., De Pascalis, V., Meares, R. A., Bahramali, H., and Harris, A. (2000). Gamma activity in schizophrenia: evidence of impaired network binding? *Clin. Neurophysiol.* 111, 1461–1468. doi: 10.1016/S1388-2457(00)00347-3

Hall, M. H., Taylor, G., Sham, P., Schulze, K., Rijsdijk, F., Picchioni, M., et al. (2011). The early auditory gamma-band response is heritable and a putative endophenotype of schizophrenia. *Schizophr. Bull.* 37, 778–787. doi: 10.1093/schbul/sbp134

Hampson, E. C., Vaney, D. I., and Weiler, R. (1992). Dopaminergic modulation of gap junction permeability between amacrine cells in mammalian retina. *J. Neurosci.* 12, 4911–4922.

Harrison, P. J. (2004). The hippocampus in schizophrenia: a review of the neuropathological evidence and its pathophysiological implications. *Psychopharmacology* 174, 151–162. doi: 10.1007/s00213-003-1761-y

Hashimoto, T., Bazmi, H. H., Mirnics, K., Wu, Q., Sampson, A. R., and Lewis, D. A. (2008). Conserved regional patterns of GABA-related transcript expression in the neocortex of subjects with schizophrenia. *Am. J. Psychiatry* 165, 479–489. doi: 10.1176/appi.ajp.2007.07081223

Hawi, Z., McCarron, M., Kirley, A., Daly, G., Fitzgerald, M., and Gill, M. (2000). No association of the dopamine DRD4 receptor (DRD4) gene polymorphism with attention deficit hyperactivity disorder (ADHD) in the Irish population. *Am. J. Med. Genet.* 96, 268–272. doi: 10.1002/1096-8628(20000612)96

Hefft, S., and Jonas, P. (2005). Asynchronous GABA release generates long-lasting inhibition at a hippocampal interneuron-principal neuron synapse. *Nat. Neurosci.* 8, 1319–1328. doi: 10.1038/nn1542

Heise, C. E., and Mitrofanis, J. (2005). Reduction in parvalbumin expression in the zona incerta after 6OHDA lesion in rats. *J. Neurocytol.* 34, 421–434. doi: 10.1007/s11068-006-8728-y

Helms, C. M., Gubner, N. R., Wilhelm, C. J., Mitchell, S. H., and Grandy, D. K. (2008). D4 receptor deficiency in mice has limited effects on impulsivity and novelty seeking. *Pharmacol. Biochem. Behav.* 90, 387–393. doi: 10.1016/j.pbb.2008.03.013

Herrmann, C. S., and Demiralp, T. (2005). Human EEG gamma oscillations in neuropsychiatric disorders. *Clin. Neurophysiol.* 116, 2719–2733. doi: 10.1016/j.clinph.2005.07.007

Herwerth, M., Jensen, V., Novak, M., Konopka, W., Hvalby, O., and Kohr, G. (2012). D4 dopamine receptors modulate NR2B NMDA receptors and LTP in stratum oriens of hippocampal CA1. *Cereb. Cortex* 22, 1786–1798. doi: 10.1093/cercor/bhr275

Huang, Y. Z., Won, S., Ali, D. W., Wang, Q., Tanowitz, M., Du, Q. S., et al. (2000). Regulation of neuregulin signaling by PSD-95 interacting with ErbB4 at CNS synapses. *Neuron* 26, 443–455. doi: 10.1016/S0896-6273(00)81176-9

Insel, T. R. (2010). Rethinking schizophrenia. *Nature* 468, 187–193. doi: 10.1038/nature09552

Jentsch, J. D., and Roth, R. H. (1999). The neuropsychopharmacology of phencyclidine: from NMDA receptor hypofunction to the dopamine hypothesis of schizophrenia. *Neuropsychopharmacology* 20, 201–225. doi: 10.1016/S0893-133X(98)00060-8

Jones, N. C., Reddy, M., Anderson, P., Salzberg, M. R., O'Brien, T. J., and Pinault, D. (2012). Acute administration of typical and atypical antipsychotics reduces EEG gamma power, but only the preclinical compound LY379268 reduces the ketamine-induced rise in gamma power. *Int. J. Neuropsychopharmacol.* 15, 657–668. doi: 10.1017/S1461145711000848

Jonsson, E. G., Sedvall, G. C., Nothen, M. M., and Cichon, S. (2003). Dopamine D4 receptor gene (DRD4) variants and schizophrenia: meta-analyses. *Schizophr. Res.* 61, 111–119. doi: 10.1016/S0920-9964(02)00287-6

Jovanovic, V., Guan, H. C., and Van Tol, H. H. (1999). Comparative pharmacological and functional analysis of the human dopamine D4.2 *and D*4.10 receptor variants. *Pharmacogenetics* 9, 561–568. doi: 10.1097/00008571-199910000-00003

Kantrowitz, J., and Javitt, D. C. (2012). Glutamatergic transmission in schizophrenia: from basic research to clinical practice. *Curr. Opin. Psychiatry* 25, 96–102.

Kikuchi, M., Hashimoto, T., Nagasawa, T., Hirosawa, T., Minabe, Y., Yoshimura, M., et al. (2011). Frontal areas contribute to reduced global coordination of resting-state gamma activities in drug-naive patients with schizophrenia. *Schizophr. Res.* 130, 187–194. doi: 10.1016/j.schres.2011.06.003

Kissler, J., Muller, M. M., Fehr, T., Rockstroh, B., and Elbert, T. (2000). MEG gamma band activity in schizophrenia patients and healthy subjects in a mental arithmetic task and at rest. *Clin. Neurophysiol.* 111, 2079–2087. doi: 10.1016/S1388-2457(00)00425-9

Korotkova, T., Fuchs, E. C., Ponomarenko, A., von Engelhardt, J., and Monyer, H. (2010). NMDA receptor ablation on parvalbumin-positive interneurons impairs hippocampal synchrony, spatial representations, and working memory. *Neuron* 68, 557–569. doi: 10.1016/j.neuron.2010.09.017

Kotecha, S. A., Oak, J. N., Jackson, M. F., Perez, Y., Orser, B. A., Van Tol, H. H. M., et al. (2002). A D2 class dopamine receptor transactivates a receptor tyrosine kinase to inhibit NMDA receptor transmission. *Neuron* 35, 1111–1122. doi: 10.1016/S0896-6273(02)00859-0

Kramer, M. A., Roopun, A. K., Carracedo, L. M., Traub, R. D., Whittington, M. A., and Kopell, N. J. (2008). Rhythm generation through period concatenation in rat somatosensory cortex. *PLoS Comput. Biol.* 4:e1000169. doi: 10.1371/journal.pcbi.1000169

Kramer, M. S., Last, B., Getson, A., and Reines, S. A. (1997). The effects of a selective D4 dopamine receptor antagonist (L-745,870) in acutely psychotic inpatients with schizophrenia. D4 Dopamine Antagonist Group. *Arch. Gen. Psychiatry* 54, 567–572. doi: 10.1001/archpsyc.1997.01830180085011

Kröner, S., Krimer, L. S., Lewis, D. A., and Barrionuevo, G. (2007). Dopamine increases inhibition in the monkey dorsolateral prefrontal cortex through cell type-specific

modulation of interneurons. *Cereb. Cortex* 17, 1020–1032.

Kruzich, P. J., Suchland, K. L., and Grandy, D. K. (2004). Dopamine D4 receptor-deficient mice, congenic on the C57BL/6J background, are hypersensitive to amphetamine. *Synapse* 53, 131–139. doi: 10.1002/syn.20043

Kuznetsova, A., and Deth, R. (2008). A model for modulation of neuronal synchronization by D4 dopamine receptor-mediated phospholipid methylation. *J. Comput. Neurosci.* 24, 314–329. doi: 10.1007/s10827-007-0057-3

Kwon, O. B., Longart, M., Vullhorst, D., Hoffman, D. A., and Buonanno, A. (2005). Neuregulin-1 reverses long-term potentiation at CA1 hippocampal synapses. *J. Neurosci.* 25, 9378–9383. doi: 10.1523/JNEUROSCI.2100-05.2005

Kwon, O. B., Paredes, D., Gonzalez, C. M., Neddens, J., Hernandez, L., Vullhorst, D., et al. (2008). Neuregulin-1 regulates LTP at CA1 hippocampal synapses through activation of dopamine D4 receptors. *Proc. Natl. Acad. Sci. U.S.A.* 105, 15587–15592. doi: 10.1073/pnas.0805722105

LaHoste, G. J., Swanson, J. M., Wigal, S. B., Glabe, C., Wigal, T., King, N., et al. (1996). Dopamine D4 receptor gene polymorphism is associated with attention deficit hyperactivity disorder. *Mol. Psychiatry* 1, 121–124.

Lanau, F., Zenner, M. T., Civelli, O., and Hartman, D. S. (1997). Epinephrine and norepinephrine act as potent agonists at the recombinant human dopamine D4 receptor. *J. Neurochem.* 68, 804–812. doi: 10.1046/j.1471-4159.1997.68020804.x

Lauzon, N. M., Bishop, S. F., and Laviolette, S. R. (2009). Dopamine D1 versus D4 receptors differentially modulate the encoding of salient versus nonsalient emotional information in the medial prefrontal cortex. *J. Neurosci.* 29, 4836–4845. doi: 10.1523/JNEUROSCI.0178-09.2009

Lavine, N., Ethier, N., Oak, J. N., Pei, L., Liu, F., Trieu, P., et al. (2002). G protein-coupled receptors form stable complexes with inwardly rectifying potassium channels and adenylyl cyclase. *J. Biol. Chem.* 277, 46010–46019. doi: 10.1074/jbc.M205035200

Laviolette, S. R., Lipski, W. J., and Grace, A. A. (2005). A subpopulation of neurons in the medial prefrontal cortex encodes emotional learning with burst and frequency codes through a dopamine D4 receptor-dependent basolateral amygdala input. *J. Neurosci.* 25, 6066–6075. doi: 10.1523/JNEUROSCI.1168-05.2005

Lee, S. H., Wynn, J. K., Green, M. F., Kim, H., Lee, K. J., Nam, M., et al. (2006). Quantitative EEG and low resolution electromagnetic tomography (LORETA) imaging of patients with persistent auditory hallucinations. *Schizophr. Res.* 83, 111–119. doi: 10.1016/j.schres.2005.11.025

Lewis, D. A., Fish, K. N., Arion, D., and Gonzalez-Burgos, G. (2011). Perisomatic inhibition and cortical circuit dysfunction in schizophrenia. *Curr. Opin. Neurobiol.* 21, 866–872. doi: 10.1016/j.conb.2011.05.013

Li, H., Zhang, Z., Blackburn, M. R., Wang, S. W., Ribelayga, C. P., and O'Brien, J. (2013). Adenosine and dopamine receptors coregulate photoreceptor coupling via gap junction phosphorylation in mouse retina. *J. Neurosci.* 33, 3135–3150. doi: 10.1523/JNEUROSCI.2807-12.2013

Lisman, J. E., Coyle, J. T., Green, R. W., Javitt, D. C., Benes, F. M., Heckers, S., et al. (2008). Circuit-based framework for understanding neurotransmitter and risk gene interactions in schizophrenia. *Trends Neurosci.* 31, 234–242. doi: 10.1016/j.tins.2008.02.005

Ma, J., and Leung, L. S. (2000). Relation between hippocampal gamma waves and behavioral disturbances induced by phencyclidine and methamphetamine. *Behav. Brain Res.* 111, 1–11. doi: 10.1016/S0166-4328(00)00138-8

Maher, B. S., Marazita, M. L., Ferrell, R. E., and Vanyukov, M. M. (2002). Dopamine system genes and attention deficit hyperactivity disorder: a meta-analysis. *Psychiatr. Genet.* 12, 207–215. doi: 10.1097/00041444-200212000-00003

Martina, M., and Bergeron, R. (2008). D1 and D4 dopaminergic receptor interplay mediates coincident G protein-independent and dependent regulation of glutamate NMDA receptors in the lateral amygdala. *J. Neurochem.* 106, 2421–2435. doi: 10.1111/j.1471-4159.2008.05584.x

Mattay, V. S., Goldberg, T. E., Fera, F., Hariri, A. R., Tessitore, A., Egan, M. F., et al. (2003). Catechol *O*-methyltransferase val158-met genotype and individual variation in the brain response to amphetamine. *Proc. Natl. Acad. Sci. U.S.A.* 100, 6186–6191. doi: 10.1073/pnas.0931309100

Mei, L., and Xiong, W. C. (2008). Neuregulin 1 in neural development, synaptic plasticity and schizophrenia. *Nat. Rev. Neurosci.* 9, 437–452. doi: 10.1038/nrn2392

Mei, Y. A., Griffon, N., Buquet, C., Martres, M. P., Vaudry, H., Schwartz, J. C., et al. (1995). Activation of dopamine D4 receptor inhibits an L-type calcium current in cerebellar granule cells. *Neuroscience* 68, 107–116. doi: 10.1016/0306-4522(95)00116-Z

Meltzer, H. Y., and Huang, M. (2008). In vivo actions of atypical antipsychotic drug on serotonergic and dopaminergic systems. *Prog. Brain Res.* 172, 177–197. doi: 10.1016/S0079-6123(08)00909-6

Michaelides, M., Pascau, J., Gispert, J. D., Delis, F., Grandy, D. K., Wang, G. J., et al. (2010). Dopamine D4 receptors modulate brain metabolic activity in the prefrontal cortex and cerebellum at rest and in response to methylphenidate. *Eur. J. Neurosci.* 32, 668–676. doi: 10.1111/j.1460-9568.2010.07319.x

Minzenberg, M. J., Firl, A. J., Yoon, J. H., Gomes, G. C., Reinking, C., and Carter, C. S. (2010). Gamma oscillatory power is impaired during cognitive control independent of medication status in first-episode schizophrenia. *Neuropsychopharmacology* 35, 2590–2599. doi: 10.1038/npp.2010.150

Missale, C., Nash, S. R., Robinson, S. W., Jaber, M., and Caron, M. G. (1998). Dopamine receptors: from structure to function. *Physiol. Rev.* 78, 189–225.

Miura, Y., Ito, T., and Kadokawa, T. (1987). Effects of intraseptally injected dopamine and noradrenaline on hippocampal synchronized theta wave activity in rats. *Jpn. J. Pharmacol.* 44, 471–479. doi: 10.1254/jjp.44.471

Moran, L. V., and Hong, L. E. (2011). High vs low frequency neural oscillations in schizophrenia. *Schizophr. Bull.* 37, 659–663. doi: 10.1093/schbul/sbr056

Moreland, R. B., Patel, M., Hsieh, G. C., Wetter, J. M., Marsh, K., and Brioni, J. D. (2005). A-412997 is a selective dopamine D4 receptor agonist in rats. *Pharmacol. Biochem. Behav.* 82, 140–147. doi: 10.1016/j.pbb.2005.08.001

Mrzljak, L., Bergson, C., Pappy, M., Huff, R., Levenson, R., and Goldman-Rakic, P. S. (1996). Localization of dopamine D4 receptors in GABAergic neurons of the primate brain. *Nature* 381, 245–248. doi: 10.1038/381245a0

Mulert, C., Kirsch, V., Pascual-Marqui, R., McCarley, R. W., and Spencer, K. M. (2011). Long-range synchrony of gamma oscillations and auditory hallucination symptoms in schizophrenia. *Int. J. Psychophysiol.* 79, 55–63. doi: 10.1016/j.ijpsycho.2010.08.004

Nakagawa, T., Ukai, K., Ohyama, T., Gomita, Y., and Okamura, H. (2000). Effect of dopaminergic drugs on the reserpine-induced lowering of hippocampal theta wave frequency in rats. *Nihon Shinkei Seishin Yakurigaku Zasshi* 20, 71–76.

Nakazawa, K., Zsiros, V., Jiang, Z., Nakao, K., Kolata, S., Zhang, S., et al. (2012). GABAergic interneuron origin of schizophrenia pathophysiology. *Neuropharmacology* 62, 1574–1583. doi: 10.1016/j.neuropharm.2011.01.022

Naneix, F., Marchand, A. R., Di Scala, G., Pape, J. R., and Coutureau, E. (2012). Parallel maturation of goal-directed behavior and dopaminergic systems during adolescence. *J. Neurosci.* 32, 16223–16232. doi: 10.1523/JNEUROSCI.3080-12.2012

Nayak, S., and Cassaday, H. J. (2003). The novel dopamine D4 receptor agonist (PD 168,077 maleate): doses with different effects on locomotor activity are without effect in classical conditioning. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 27, 441–449. doi: 10.1016/S0278-5846(03)00031-9

Neddens, J., Fish, K. N., Tricoire, L., Vullhorst, D., Shamir, A., Chung, W., et al. (2011). Conserved interneuron-specific ErbB4 expression in frontal cortex of rodents, monkeys, and humans: implications for schizophrenia. *Biol. Psychiatry* 70, 636–645. doi: 10.1016/j.biopsych.2011.04.016

Noain, D., Avale, M. E., Wedemeyer, C., Calvo, D., Peper, M., and Rubinstein, M. (2006). Identification of brain neurons expressing the dopamine D4 receptor gene using BAC transgenic mice. *Eur. J. Neurosci.* 24, 2429–2438. doi: 10.1111/j.1460-9568.2006.05148.x

O'Donnell, P. (2010). Adolescent maturation of cortical dopamine. *Neurotox. Res.* 18, 306–312. doi: 10.1007/s12640-010-9157-3

Okaty, B. W., Miller, M. N., Sugino, K., Hempel, C. M., and Nelson, S. B. (2009). Transcriptional and electrophysiological maturation of neocortical fast-spiking GABAergic interneurons. *J. Neurosci.* 29, 7040–7052. doi: 10.1523/JNEUROSCI.0105-09.2009

Oldenhof, J., Vickery, R., Anafi, M., Oak, J., Ray, A., Schoots, O., et al. (1998). SH3 binding domains in the dopamine D4 receptor. *Biochemistry* 37, 15726–15736. doi: 10.1021/bi981634

Onn, S. P., and Grace, A. A. (1994). Dye coupling between rat striatal neurons recorded in vivo: compartmental organization and modulation by dopamine. *J. Neurophysiol.* 71, 1917–1934.

Onn, S. P., Wang, X. B., Lin, M., and Grace, A. A. (2006). Dopamine D1 and D4 receptor subtypes differentially modulate recurrent excitatory synapses in prefrontal cortical pyramidal neurons. *Neuropsychopharmacology* 31, 318–338. doi: 10.1038/sj.npp.1300829

Pillai, G., Brown, N. A., McAllister, G., Milligan, G., and Seabrook, G. R. (1998). Human D2 and D4 dopamine receptors couple through βγ G-protein subunits to inwardly rectifying K^+ channels (GIRK1) in a *Xenopus* oocyte expression system: selective antagonism by L-741,626 and L-745,870 respectively. *Neuropharmacology* 37, 983–987. doi: 10.1016/S0028-3908(98)00092-6

Pinault, D. (2008). *N*-methyl D-aspartate receptor antagonists ketamine and MK-801 induce wake-related aberrant gamma oscillations in the rat neocortex. *Biol. Psychiatry* 63, 730–735. doi: 10.1016/j.biopsych.2007.10.006

Porter, L. L., Rizzo, E., and Hornung, J. P. (1999). Dopamine affects parvalbumin expression during cortical development *in vitro*. *J. Neurosci.* 19, 8990–9003.

Reichenberg, A., Weiser, M., Rabinowitz, J., Caspi, A., Schmeidler, J., Mark, M., et al. (2002). A population-based cohort study of premorbid intellectual, language, and behavioral functioning in patients with schizophrenia, schizoaffective disorder, and nonpsychotic bipolar disorder. *Am. J. Psychiatry* 159, 2027–2035. doi: 10.1176/appi.ajp.159.12.2027

Robbins, T. W., and Arnsten, A. F. T. (2009). The neuropsychopharmacology of fronto-executive function: monoaminergic modulation. *Annu. Rev. Neurosci.* 32, 267–287. doi: 10.1146/annurev.neuro.051508.135535

Romo-Parra, H., Aceves, J., and Gutierrez, R. (2005). Tonic modulation of inhibition by dopamine D4 receptors in the rat hippocampus. *Hippocampus* 15, 254–259. doi: 10.1002/hipo.20049

Rondou, P., Haegeman, G., and Van Craenenbroeck, K. (2010). The dopamine D4 receptor: biochemical and signalling properties. *Cell. Mol. Life Sci.* 67, 1971–1986. doi: 10.1007/s00018-010-0293-y

Ross, N. R., and Porter, L. L. (2002). Effects of dopamine and estrogen upon cortical neurons that express parvalbumin in vitro. *Brain Res. Dev. Brain Res.* 137, 23–34. doi: 10.1016/S0165-3806(02)00364-4

Rotaru, D. C., Yoshino, H., Lewis, D. A., Ermentrout, G. B., and Gonzalez-Burgos, G. (2011). Glutamate receptor subtypes mediating synaptic activation of prefrontal cortex neurons: relevance for schizophrenia. *J. Neurosci.* 31, 142–156. doi: 10.1523/JNEUROSCI.1970-10.2011

Rowe, D. C., Stever, C., Giedinghagen, L. N., Gard, J. M., Cleveland, H. H., Terris, S. T., et al. (1998). Dopamine DRD4 receptor polymorphism and attention deficit hyperactivity disorder. *Mol. Psychiatry* 3, 419–426. doi: 10.1038/sj.mp.4000432

Rubinstein, M., Cepeda, C., Hurst, R. S., Flores-Hernandez, J., Ariano, M. A., Falzone, T. L., et al. (2001). Dopamine D4 receptor-deficient mice display cortical hyperexcitability. *J. Neurosci.* 21, 3756–3763.

Rubinstein, M., Phillips, T. J., Bunzow, J. R., Falzone, T. L., Dziewczapolski, G., Zhang, G., et al. (1997). Mice lacking dopamine D4 receptors are supersensitive to ethanol, cocaine, and methamphetamine. *Cell* 90, 991–1001. doi: 10.1016/S0092-8674(00)80365-7

Rutter, L., Carver, F. W., Holroyd, T., Nadar, S. R., Mitchell-Francis, J., Apud, J., et al. (2009). Magnetoencephalographic gamma power reduction in patients with schizophrenia during resting condition. *Hum. Brain Mapp.* 30, 3254–3264. doi: 10.1002/hbm.20746

Salvador, R., Anguera, M., Gomar, J. J., Bullmore, E. T., and Pomarol-Clotet, E. (2010). Conditional mutual information maps as descriptors of net connectivity levels in the brain. *Front. Neuroinform.* 4:115. doi: 10.3389/fninf.2010.00115

Sawaguchi, T., and Goldman-Rakic, P. S. (1994). The role of D1-dopamine receptor in working memory: local injections of dopamine antagonists into the prefrontal cortex of rhesus monkeys performing an oculomotor delayed-response task. *J. Neurophysiol.* 71, 515–528.

Schreiber, S., Fellous, J. M., Tiesinga, P., and Sejnowski, T. J. (2004). Influence of ionic conductances on spike timing reliability of cortical neurons for suprathreshold rhythmic inputs. *J. Neurophysiol.* 91, 194–205. doi: 10.1152/jn.00556.2003

Schulz, S. B., Heidmann, K. E., Mike, A., Klaft, Z. J., Heinemann, U., and Gerevich, Z. (2012). First and second generation antipsychotics influence hippocampal gamma oscillations by interactions with 5-HT3 and D3 receptors. *Br. J. Pharmacol.* 167, 1480–1491. doi: 10.1111/j.1476-5381.2012.02107.x

Seamans, J. K., Gorelova, N., Durstewitz, D., and Yang, C. R. (2001). Bidirectional dopamine modulation of GABAergic inhibition in prefrontal cortical pyramidal neurons. *J. Neurosci.* 21, 3628–3638.

Seeman, P., Guan, H. C., and Van Tol, H. H. (1993). Dopamine D4 receptors elevated in schizophrenia. *Nature* 365, 441–445. doi: 10.1038/365441a0

Shah, A. A., Sjovold, T., and Treit, D. (2004). Selective antagonism of medial prefrontal cortex D4 receptors decreases fear-related behaviour in rats. *Eur. J. Neurosci.* 19, 3393–3397. doi: 10.1111/j.0953-816X.2004.03447.x

Shamir, A., Kwon, O. B., Karavanova, I., Vullhorst, D., Leiva-Salcedo, E., Janssen, M. J., et al. (2012). The importance of the NRG-1/ErbB4 pathway for synaptic plasticity and behaviors associated with psychiatric disorders. *J. Neurosci.* 32, 2988–2997. doi: 10.1523/JNEUROSCI.1899-11.2012

Sharma, A., Kramer, M. L., Wick, P. F., Liu, D., Chari, S., Shim, S., et al. (1999). D4 dopamine receptor-mediated phospholipid methylation and its implications for mental illnesses such as schizophrenia. *Mol. Psychiatry* 4, 235–246. doi: 10.1038/sj.mp.4000522

Shin, R. M., Masuda, M., Miura, M., Sano, H., Shirasawa, T., Song, W. J., et al. (2003). Dopamine D4 receptor-induced postsynaptic inhibition of GABAergic currents in mouse globus pallidus neurons. *J. Neurosci.* 23, 11662–11672.

Simpson, E. H., Kellendonk, C., and Kandel, E. (2010). A possible role for the striatum in the pathogenesis of the cognitive symptoms of schizophrenia. *Neuron* 65, 585–596. doi: 10.1016/j.neuron.2010.02.014

Smalley, S. L., Bailey, J. N., Palmer, C. G., Cantwell, D. P., McGough, J. J., Del'Homme, M. A., et al. (1998). Evidence that the dopamine D4 receptor is a susceptibility gene in attention deficit hyperactivity disorder. *Mol. Psychiatry* 3, 427–430. doi: 10.1038/sj.mp.4000457

Sohal, V. S., Zhang, F., Yizhar, O., and Deisseroth, K. (2009). Parvalbumin neurons and gamma rhythms enhance cortical circuit performance. *Nature* 459, 698–702. doi: 10.1038/nature07991

Spencer, K. M., Niznikiewicz, M. A., Nestor, P. G., Shenton, M. E., and McCarley, R. W. (2009). Left auditory cortex gamma synchronization and auditory hallucination symptoms in schizophrenia. *BMC Neurosci.* 10:85. doi: 10.1186/1471-2202-10-85

Sullivan, E. M., and O'Donnell, P. (2012). Inhibitory interneurons, oxidative stress, and schizophrenia. *Schizophr. Bull.* 38, 373–376. doi: 10.1093/schbul/sbs052

Swanson, J. M., Sunohara, G. A., Kennedy, J. L., Regino, R., Fineberg, E., Wigal, T., et al. (1998). Association of the dopamine receptor D4 (DRD4) gene with a refined phenotype of attention deficit hyperactivity disorder (ADHD): a family-based approach. *Mol. Psychiatry* 3, 38–41. doi: 10.1038/sj.mp.4000354

Symond, M. P., Harris, A. W., Gordon, E., and Williams, L. M. (2005). Gamma synchrony in first-episode schizophrenia: a disorder of temporal connectivity? *Am. J. Psychiatry* 162, 459–465. doi: 10.1176/appi.ajp.162.3.459

Tarazi, F. I., and Baldessarini, R. J. (2000). Comparative postnatal development of dopamine D1, D2 and D4 receptors in rat forebrain. *Int. J. Dev. Neurosci.* 18, 29–37. doi: 10.1016/S0736-5748(99)00108-2

Tarazi, F. I., Zhang, K., and Baldessarini, R. J. (2004). Dopamine D4 receptors: beyond schizophrenia. *J. Recept. Signal Transduct. Res.* 24, 131–147. doi: 10.1081/RRS-200032076

Thomas, T. C., Grandy, D. K., Gerhardt, G. A., and Glaser, P. E. (2009). Decreased dopamine D4 receptor expression increases extracellular glutamate and alters its regulation in mouse striatum. *Neuropsychopharmacology* 34, 436–445. doi: 10.1038/npp.2008.74

Thomas, T. C., Kruzich, P. J., Joyce, B. M., Gash, C. R., Suchland, K., Surgener, S. P., et al. (2007). Dopamine D4 receptor knockout mice exhibit neurochemical changes consistent with decreased dopamine release. *J. Neurosci. Methods* 166, 306–314. doi: 10.1016/j.jneumeth.2007.03.009

Tierney, P. L., Thierry, A. M., Glowinski, J., Deniau, J. M., and Gioanni, Y. (2008). Dopamine modulates temporal dynamics of feedforward inhibition in rat prefrontal cortex in vivo. *Cereb. Cortex* 18, 2251–2262. doi: 10.1093/cercor/bhm252

Tiesinga, P., and Sejnowski, T. J. (2009). Cortical enlightenment: are attentional gamma oscillations driven by ING or PING? *Neuron* 63, 727–732. doi: 10.1016/j.neuron.2009.09.009

Tost, H., Ende, G., Ruf, M., Henn, F. A., and Meyer-Lindenberg, A. (2005). Functional imaging research in schizophrenia. *Int. Rev. Neurobiol.*

67, 95–118. doi: 10.1016/S0074-7742(05)67004-3

Towers, S. K., and Hestrin, S. (2008). D1-like dopamine receptor activation modulates GABAergic inhibition but not electrical coupling between neocortical fast-spiking interneurons. *J. Neurosci.* 28, 2633–2641. doi: 10.1523/JNEUROSCI.5079-07.2008

Trantham-Davidson, H., Kröner, S., and Seamans, J. K. (2008). Dopamine modulation of prefrontal cortex interneurons occurs independently of DARPP-32. *Cereb. Cortex* 18, 951–958.

Trantham-Davidson, H., Neely, L. C., Lavin, A., and Seamans, J. K. (2004). Mechanisms underlying differential D1 versus D2 dopamine receptor regulation of inhibition in prefrontal cortex. *J. Neurosci.* 24, 10652–10659. doi: 10.1523/JNEUROSCI.3179-04.2004

Traub, R. D., Whittington, M. A., Colling, S. B., Buzsáki, G., and Jefferys, J. G. (1996). Analysis of gamma rhythms in the rat hippocampus in vitro and in vivo. *J. Physiol.* 493, 471–484.

Tseng, K. Y., Mallet, N., Toreson, K. L., Le Moine, C., Gonon, F., and O'Donnell, P. (2006). Excitatory response of prefrontal cortical fast-spiking interneurons to ventral tegmental area stimulation in vivo. *Synapse* 59, 412–417. doi: 10.1002/syn.20255

Tseng, K. Y., and O'Donnell, P. (2005). Post-pubertal emergence of prefrontal cortical up states induced by D1–NMDA co-activation. *Cereb. Cortex* 15, 49–57. doi: 10.1093/cercor/bhh107

Tseng, K. Y., and O'Donnell, P. (2007). Dopamine modulation of prefrontal cortical interneurons changes during adolescence. *Cereb. Cortex* 17, 1235–1240. doi: 10.1093/cercor/bhl034

Tye, S. J., Covey, D. P., and Griessenauer, C. J. (2009). A balancing act: D4 receptor activation and the neurobiological basis of emotional learning. *J. Neurosci.* 29, 10785–10787. doi: 10.1523/JNEUROSCI.2822-09.2009

Uhlhaas, P. J., Roux, F., Singer, W., Haenschel, C., Sireteanu, R., and Rodriguez, E. (2009). The development of neural synchrony reflects late maturation and restructuring of functional networks in humans. *Proc. Natl. Acad. Sci. U.S.A.* 106, 9866–9871. doi: 10.1073/pnas.0900390106

Uhlhaas, P. J., and Singer, W. (2010). Abnormal neural oscillations and synchrony in schizophrenia. *Nat. Rev. Neurosci.* 11, 100–113. doi: 10.1038/nrn2774

Uhlhaas, P. J., and Singer, W. (2012). Neuronal dynamics and neuropsychiatric disorders: toward a translational paradigm for dysfunctional large-scale networks. *Neuron* 75, 963–980. doi: 10.1016/j.neuron.2012.09.004

Van Tol, H. H., Bunzow, J. R., Guan, H. C., Sunahara, R. K., Seeman, P., Niznik, H. B., et al. (1991). Cloning of the gene for a human dopamine D4 receptor with high affinity for the antipsychotic clozapine. *Nature* 350, 610–614. doi: 10.1038/350610a0

Van Tol, H. H., Wu, C. M., Guan, H. C., Ohara, K., Bunzow, J. R., Civelli, O., et al. (1992). Multiple dopamine D4 receptor variants in the human population. *Nature* 358, 149–152. doi: 10.1038/358149a0

Vijayraghavan, S., Wang, M., Birnbaum, S. G., Williams, G. V., and Arnsten, A. F. T. (2007). Inverted-U dopamine D1 receptor actions on prefrontal neurons engaged in working memory. *Nat. Neurosci.* 10, 376–384. doi: 10.1038/nn1846

von Stein, A., and Sarnthein, J. (2000). Different frequencies for different scales of cortical integration: from local gamma to long range alpha/theta synchronization. *Int. J. Psychophysiol.* 38, 301–313. doi: 10.1016/S0167-8760(00)00172-0

Vreugdenhil, M., Jefferys, J. G., Celio, M. R., and Schwaller, B. (2003). Parvalbumin-deficiency facilitates repetitive IPSCs and gamma oscillations in the hippocampus. *J. Neurophysiol.* 89, 1414–1422. doi: 10.1152/jn.00576.2002

Vullhorst, D., Neddens, J., Karavanova, I., Tricoire, L., Petralia, R. S., McBain, C. J., et al. (2009). Selective expression of ErbB4 in interneurons, but not pyramidal cells, of the rodent hippocampus. *J. Neurosci.* 29, 12255–12264. doi: 10.1523/JNEUROSCI.2454-09.2009

Vysokanov, A., Flores-Hernandez, J., and Surmeier, D. J. (1998). mRNAs for clozapine-sensitive receptors co-localize in rat prefrontal cortex neurons. *Neurosci. Lett.* 258, 179–182. doi: 10.1016/S0304-3940(98)00882-9

Wang, H. X., and Gao, W. J. (2009). Cell type-specific development of NMDA receptors in the interneurons of rat prefrontal cortex. *Neuropsychopharmacology* 34, 2028–2040. doi: 10.1038/npp.2009.20

Wang, H. X., and Gao, W. J. (2010). Development of calcium-permeable AMPA receptors and their correlation with NMDA receptors in fast-spiking interneurons of rat prefrontal cortex. *J. Physiol.* 588, 2823–2838. doi: 10.1113/jphysiol.2010.187591

Wang, X., Gu, Z., Zhong, P., Chen, G., Feng, J., and Yan, Z. (2006). Aberrant regulation of NMDA receptors by dopamine D4 signaling in rats after phencyclidine exposure. *Mol. Cell. Neurosci.* 31, 15–25. doi: 10.1016/j.mcn.2005.08.018

Wang, X., Zhong, P., Gu, Z., and Yan, Z. (2003). Regulation of NMDA receptors by dopamine D4 signaling in prefrontal cortex. *J. Neurosci.* 23, 9852–9861.

Wang, X., Zhong, P., and Yan, Z. (2002). Dopamine D4 receptors modulate GABAergic signaling in pyramidal neurons of prefrontal cortex. *J. Neurosci.* 22, 9185–9193.

Wang, X. H., Levitt, P., Grayson, D. R., and Murphy, E. H. (1995). Intrauterine cocaine exposure of rabbits: persistent elevation of GABA-immunoreactive neurons in anterior cingulate cortex but not visual cortex. *Brain Res.* 689, 32–46. doi: 10.1016/0006-8993(95)00528-X

Wedemeyer, C., Goutman, J. D., Avale, M. E., Franchini, L. F., Rubinstein, M., and Calvo, D. J. (2007). Functional activation by central monoamines of human dopamine D(4) receptor polymorphic variants coupled to GIRK channels in *Xenopus* oocytes. *Eur. J. Pharmacol.* 562, 165–173. doi: 10.1016/j.ejphar.2007.01.055

Weiss, T., Veh, R. W., and Heinemann, U. (2003). Dopamine depresses cholinergic oscillatory network activity in rat hippocampus. *Eur. J. Neurosci.* 18, 2573–2580. doi: 10.1046/j.1460-9568.2003.02970.x

Werner, P., Hussy, N., Buell, G., Jones, K. A., and North, R. A. (1996). D2, D3, and D4 dopamine receptors couple to G protein-regulated potassium channels in *Xenopus* oocytes. *Mol. Pharmacol.* 49, 656–661.

Whittington, M. A., Roopun, A. K., Traub, R. D., and Davies, C. H. (2011). Circuits and brain rhythms in schizophrenia: a wealth of convergent targets. *Curr. Opin. Pharmacol.* 11, 508–514. doi: 10.1016/j.coph.2011.04.010

Williams, G. V., and Goldman-Rakic, P. S. (1995). Modulation of memory fields by dopamine Dl receptors in prefrontal cortex. *Nature* 376, 572–575. doi: 10.1038/376572a0

Winterer, G., and Weinberger, D. R. (2004). Genes, dopamine and cortical signal-to-noise ratio in schizophrenia. *Trends Neurosci.* 27, 683–690. doi: 10.1016/j.tins.2004.08.002

Winterer, G., Ziller, M., Dorn, H., Frick, K., Mulert, C., Wuebben, Y., Herrmann, W. M., and Coppola, R. (2000). Schizophrenia: reduced signal-to-noise ratio and impaired phase-locking during information processing. *Clin. Neurophysiol.* 111, 837–849. doi: 10.1016/S1388-2457(99)00322-3

Wójtowicz, A. M., van den Boom, L., Chakrabarty, A., Maggio, N., Haq, R. U., Behrens, C. J., and Heinemann, U. (2009). Monoamines block kainate- and carbachol-induced gamma-oscillations but augment stimulus-induced gamma-oscillations in rat hippocampus in vitro. *Hippocampus* 19, 273–288.

Womelsdorf, T., and Fries, P. (2007). The role of neuronal synchronization in selective attention. *Curr. Opin. Neurobiol.* 17, 154–160. doi: 10.1016/j.conb.2007.02.002

Woolley, M. L., Waters, K. A., Reavill, C., Bull, S., Lacroix, L. P., Martyn, A. J., et al. (2008). Selective dopamine D4 receptor agonist (A-412997) improves cognitive performance and stimulates motor activity without influencing reward-related behaviour in rat. *Behav. Pharmacol.* 19, 765–776. doi: 10.1097/FBP.0b013e32831c3b06

Wu, J., and Hablitz, J. J. (2005). Cooperative activation of D1 and D2 dopamine receptors enhances a hyperpolarization-activated inward current in layer I interneurons. *J. Neurosci.* 25, 6322–6328. doi: 10.1523/JNEUROSCI.1405-05.2005

Wulff, P., Ponomarenko, A. A., Bartos, M., Korotkova, T. M., Fuchs, E. C., Bähner, F., et al. (2009). Hippocampal theta rhythm and its coupling with gamma oscillations require fast inhibition onto parvalbumin-positive interneurons. *Proc. Natl. Acad. Sci. U.S.A.* 106, 3561–3566.

Xu, T., Stephane, M., and Parhi, K. K. (2013). Multidimensional analysis of the abnormal neural oscillations associated with lexical processing in schizophrenia. *Clin. EEG Neurosci.* 44, 135–143. doi: 10.1177/1550059412465078

Yeragani, V. K., Cashmere, D., Miewald, J., Tancer, M., and Keshavan, M. S. (2006). Decreased coherence in higher frequency ranges (beta and gamma) between central and frontal EEG in patients with schizophrenia: a preliminary report. *Psychiatry Res.* 141, 53–60. doi: 10.1016/j.psychres.2005.07.016

Yizhar, O., Fenno, L. E., Prigge, M., Schneider, F., Davidson, T. J., O'Shea, D. J., et al. (2011). Neocortical excitation/inhibition balance in information processing and social

dysfunction. *Nature* 477, 171–178. doi: 10.1038/nature10360

Young, J. W., Powell, S. B., Scott, C. N., Zhou, X., and Geyer, M. A. (2011). The effect of reduced dopamine D4 receptor expression in the 5-choice continuous performance task: separating response inhibition from premature responding. *Behav. Brain Res.* 222, 183–192. doi: 10.1016/j.bbr.2011.03.054

Yuen, E. Y., and Yan, Z. (2009). Dopamine D4 receptors regulate AMPA receptor trafficking and glutamatergic transmission in GABAergic interneurons of prefrontal cortex. *J. Neurosci.* 29, 550–562. doi: 10.1523/JNEUROSCI.5050-08.2009

Yuen, E. Y., and Yan, Z. (2011). Cellular mechanisms for dopamine D4 receptor-induced homeostatic regulation of alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors. *J. Biol. Chem.* 286, 24957–24965. doi: 10.1074/jbc.M111.221416

Yuen, E. Y., Zhong, P., and Yan, Z. (2010). Homeostatic regulation of glutamatergic transmission by dopamine D4 receptors. *Proc. Natl. Acad. Sci. U.S.A.* 107, 22308–22313. doi: 10.1073/pnas.10100 25108

Zhang, K., Grady, C. J., Tsapakis, E. M., Andersen, S. L., Tarazi, F. I., and Baldessarini, R. J. (2004). Regulation of working memory by dopamine D4 receptor in rats. *Neuropsychopharmacology* 29, 1648–1655. doi: 10.1038/sj.npp.1300491

Zhang, K., Tarazi, F. I., and Baldessarini, R. J. (2001). Role of dopamine D(4) receptors in motor hyperactivity induced by neonatal 6-hydroxydopamine lesions in rats. *Neuropsychopharmacology* 25, 624–632. doi: 10.1016/S0893-133X(01)00262-7

Zhou, F. M., and Hablitz, J. J. (1999). Dopamine modulation of membrane and synaptic properties of interneurons in rat cerebral cortex. *J. Neurophysiol.* 81, 967–976.

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Associations between purine metabolites and monoamine neurotransmitters in first-episode psychosis

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Schizophrenia (SZ) is a biochemically complex disorder characterized by widespread defects in multiple metabolic pathways whose dynamic interactions, until recently, have been difficult to examine. Rather, evidence for these alterations has been collected piecemeal, limiting the potential to inform our understanding of the interactions amongst relevant biochemical pathways. We herein review perturbations in purine and neurotransmitter metabolism observed in early SZ using a metabolomic approach. Purine catabolism is an underappreciated, but important component of the homeostatic response of mitochondria to oxidant stress. We have observed a homeostatic imbalance of purine catabolism in first-episode neuroleptic-naïve patients with SZ (FENNS). Precursor and product relationships within purine pathways are tightly correlated. Although some of these correlations persist across disease or medication status, others appear to be lost among FENNS suggesting that steady formation of the antioxidant uric acid (UA) via purine catabolism is altered early in the course of illness. As is the case for within-pathway correlations, there are also significant cross-pathway correlations between respective purine and tryptophan (TRP) pathway metabolites. By contrast, purine metabolites show significant cross-pathway correlation only with tyrosine, and not with its metabolites. Furthermore, several purine metabolites (UA, guanosine, or xanthine) are each significantly correlated with 5-hydroxyindoleacetic acid (5-HIAA) in healthy controls, but not in FENNS at baseline or 4-week after antipsychotic treatment. Taken together, the above findings suggest that purine catabolism strongly associates with the TRP pathways leading to serotonin (5-hydroxytryptamine, 5-HT) and kynurenine metabolites. The lack of a significant correlation between purine metabolites and 5-HIAA, suggests alterations in key 5-HT pathways that may both be modified by and contribute to oxidative stress via purine catabolism in FENNS.

Keywords: schizophrenia, first-episode psychosis, neuroleptic-naïve, oxidative stress, purine catabolism, monoamine neurotransmitters

INTRODUCTION

Schizophrenia (SZ) is a common and highly disabling mental disorder without a clearly identified pathophysiology. A number of putative mechanisms have been proposed to explain the etiopathogenesis and illness presentation of SZ including abnormal neuronal development, impaired neurotransmission, viral infections *in utero*, autoimmune dysfunction, and many others. Extensive, albeit fragmentary, findings from neurochemical and neuroendocrine studies of SZ (Javitt and Laruelle, 2006) have not provided conclusive evidence for any specific etiologic theory of SZ, perhaps due to etiopathogenetic heterogeneity (Tandon et al., 2009). However, there exists a point of convergence for many of these theoretical models, one that occurs at the level of the neuronal membrane, which is the site of neurotransmitter receptors, ion channels, signal transduction, and drug effects. Membrane deficits, specifically free radical-mediated, can significantly alter a broad range of membrane functions. There is abundant evidence that alterations in key neurotransmitters can both be modified by and contribute to oxidative stress and membrane dysfunction (**Figure 1**), suggesting a link among oxidative stress, membrane dysfunction, and multi-neurotransmitter pathologies in SZ (Yao and Keshavan, 2011).

METABOLOMIC INVESTIGATION

Schizophrenia is a heterogeneous disease with various abnormal metabolites involving multiple biochemical pathways. Therefore, to identify candidate pathological process(es) that account for the constellation of clinical and biological features in SZ, it is necessary to simultaneously evaluate multiple metabolites in a network of interacting biochemical pathways. The development of

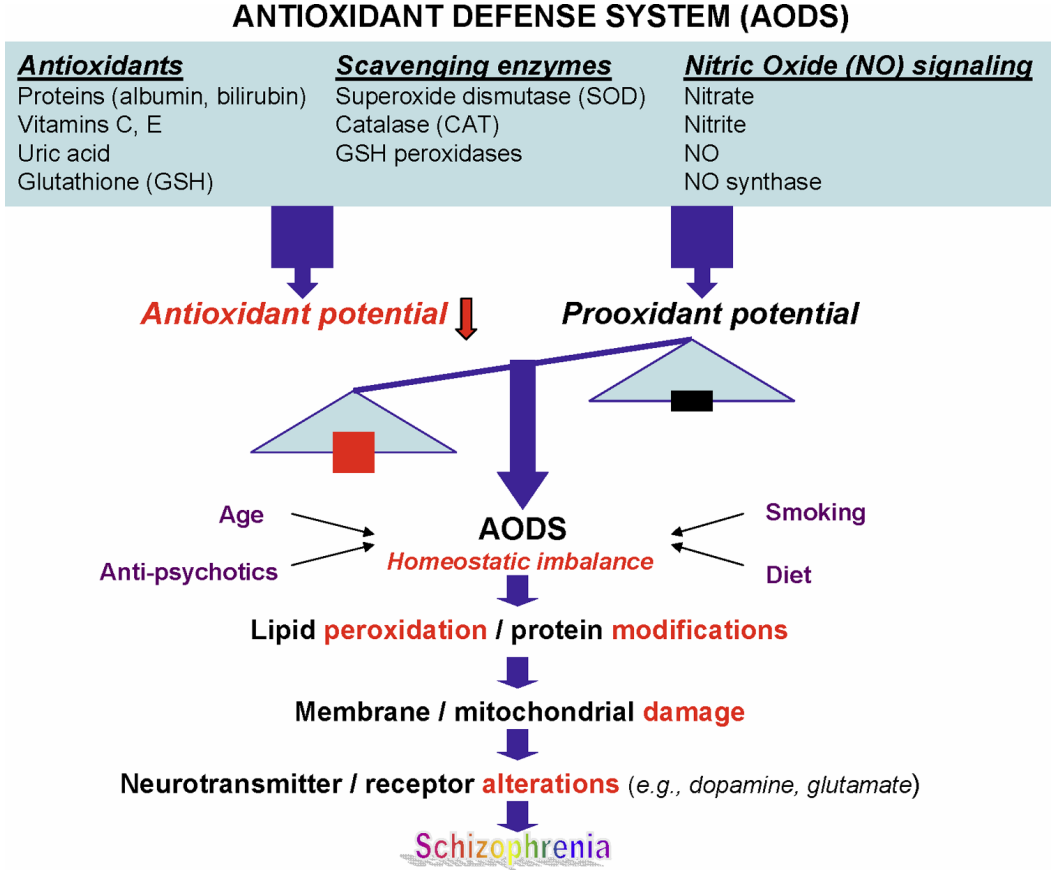

FIGURE 1 | A schematic diagram linking antioxidant defense system, membrane phospholipids, and neurotransmission to pathophysiology in schizophrenia. Reprinted by permission from Yao and Keshavan (2011).

high-resolution multidimensional separation techniques such as high-pressure liquid chromatography coupled with a 16-channel coulometric multi-electrode array system (HPLC–CMEAS), can lead to revolutionary changes in our understanding at the molecular level (Matson et al., 1984; Kristal et al., 1998; Yao and Cheng, 2004; Rozen et al., 2005; Kaddurah-Daouk et al., 2008). The resolving power of these methods is superior to one-dimensional approaches, enabling the comprehensive metabolic analyses particularly in the targeted biochemical pathways. The HPLC–CMEAS allows quantitative assays of hundreds to thousands of low molecular-weight metabolites, in turn permitting identification of biomarkers and metabolic maps associated with disease processes. The data collected from HPLC–CMEAS system reflect fingerprinting of the disorder or state/trait-related markers, which will greatly improve the predictive diagnostics for phenotypes that directly involve in the oxidative stress. More significantly, these comprehensive analyses that generate metabolic profiles represent not only biomarkers for disease but also metabolic maps that can be used to identify specific genes responsible for disease. Such metabolic maps provide a different perspective to biomedical research in further understanding the effects of therapeutic, nutritional, toxicological, and environmental interventions.

ANTIOXIDANT DEFENSE SYSTEM

GLUTATHIONE REDOX COUPLING AND NITRIC OXIDE SIGNALING

Free radicals are unstable atoms or molecules with odd (unpaired) electron(s) that can start a toxic chain reaction on important cellular components such as DNA, or the cell membrane. Biological systems have evolved complex protective strategies against free radical toxicity. Under physiological conditions the potential for free radical-mediated damage is kept in check by the antioxidant defense system (AODS), comprising a series of enzymatic and non-enzymatic components. These enzymes act cooperatively at different sites in the free radical pathways. A dynamic state is kept in check during the redox coupling under normal conditions (Yao et al., 2006). By contrast, lack of such correlations in brains of patients with SZ point to a disturbance of redox coupling mechanisms in the AODS, possibly resulting from a decreased level of glutathione (GSH) as well as age-related decreases of oxidized GSH and GSH reductase activities. Taken together, our previous data showing altered membrane dynamics and AODS enzyme activities, and findings from other investigators (Ranjekar et al., 2003; Othmen et al., 2008; Virit et al., 2009; Matsuzawa and Hashimoto, 2011) are consistent with the notion of free radical-mediated neurotoxicity in SZ (Yao et al., 2001).

There are multiple pathways to the production of excess free radical generation and subsequent oxidative stress. One such pathway is the formation of peroxynitrite by a reaction of nitric oxide (NO) and superoxide radical. In human brain, NO is metabolized primarily in the form of nitrate. A significantly increased level of NO was found in brains with SZ than those of normal and non-schizophrenic psychiatric controls (Yao et al., 2004a). Because the reaction of NO with free thiols competes with the same substrate (e.g., GSH), the excessive NO formation may further lead to significant depletion of GSH in SZ.

PURINE CATABOLISM

In addition to GSH redox coupling mechanism and NO signaling, purine catabolism (**Figure 2**) may be a previously unappreciated component of the homeostatic response of mitochondria to oxidant stress and may play a critical role in slowing progressive mitochondrial dysfunction in certain disease states (Kristal et al., 1999). Mitochondria process most of the cellular oxygen to provide energy that drives almost all metabolic processes, and also are the site of significant free radical production. About 3% of all oxygen consumed is converted to superoxide, and subsequently to hydrogen peroxide (Floyd, 1996). Thus there is an enormous and continuous free-radical burden. Antioxidant systems keep this in check. When the equilibrium between pro-oxidant and antioxidant systems are disturbed in favor of the former, mitochondrial damage can occur. Mitochondrial membranes, similar to neuronal membranes, are vulnerable to lipid peroxidation. Any impairment in mitochondrial oxidative phosphorylation can lead to a broad range of cellular disturbances, including altered neurotransmission, increased DNA damage (Bogdanov et al., 2000; Schulz et al., 2000) and decreased DNA repair, and finally cell death. Cytochrome *c* oxidase is a key enzyme in the mitochondrial electron transport chain. Decreased activity of this enzyme has been reported in the frontal cortex and caudate nucleus of schizophrenic patients. Several lines of evidence suggest decreased oxidative metabolism in some brain areas in SZ (Yao et al., 2004a; Yao et al., 2006), and may be explained in part by mitochondrial dysfunction.

An early study by Kristal et al. (1999) indicated that purine catabolism may contribute to mitochondrial antioxidant defense by producing uric acid (UA). Failure to maintain elevated xanthine (Xan) and UA occurred contemporaneously with progressive mitochondrial dysfunction. Thus, purine catabolism appears to be a homeostatic response of mitochondria to oxidant stress and may protect against progressive mitochondrial dysfunction in certain disease states (Kristal et al., 1999).

During the *de novo* synthesis of purine nucleotides, many reactions require a great deal of energy utilizing the hydrolysis of adenosine triphosphate (ATP). To provide "energy saving" for the cell, the purine bases can be reutilized via "salvage pathways" (Cory, 1982) by converting adenine, guanine (G), or hypoxanthine (Hx) to adenosine monophosphate (AMP), guanosine monophosphate (GMP), or inosine monophosphate (IMP), respectively (shown dotted arrow in **Figure 2**). The unsalvaged Hx is then converted to Xan, which is further converted to UA by Xan oxidase. In man, UA is the final product of purine catabolism (Linden and Rosin, 2006), which has been implicated as a risk factor and cause of numerous pathological conditions (see below).

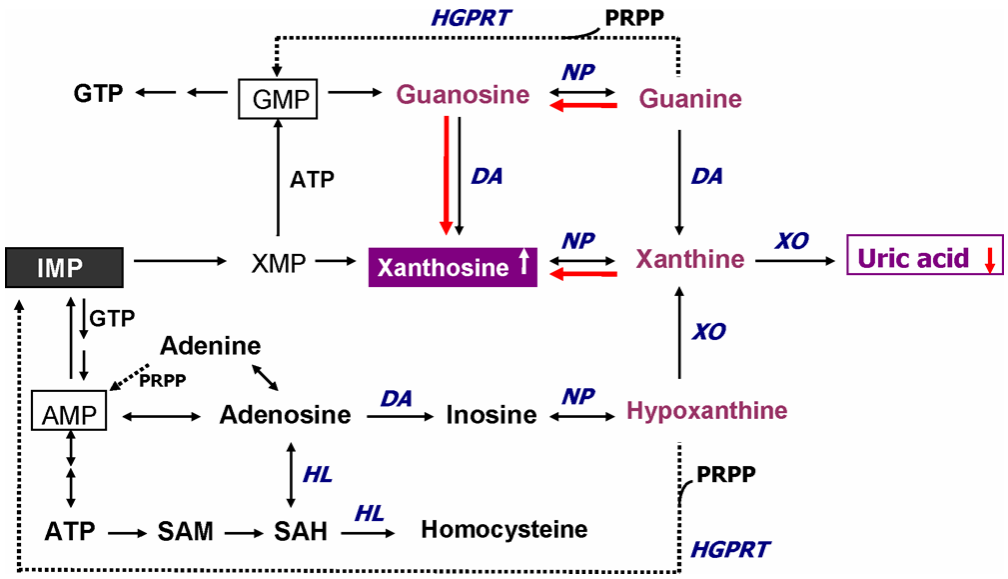

FIGURE 2 | Altered purine catabolism in first-episode neuroleptic-naïve patients with schizophrenia. Red arrows indicate shifts toward an increase of xanthosine and a decrease of uric acid productions in FENNS patients at baseline. Reactions shown with dotted lines represent the "salvage pathways," which purine bases can be reutilized resulting in considerably energy saving for the cell. ADP, adenosine diphosphate; AMP, adenosine monophosphate; ATP, adenosine triphosphate; DA, deaminase; GMP, guanosine monophosphate; GTP, guanosine triphosphate; HGPRT, hypoxanthine-guanine phosphoribosyltransferase; HL, hydrolase; IMP, inosine monophosphate; NP, nucleoside phosphorylase; PRPP, 5-phosphoribosyl pyrophosphate; SAH, *S*-adenosylhomocysteine; SAM, S-adenosylmethionine; XMP, xanthosine monophosphate; XO, xanthine oxidase. Reprinted by permission from Yao et al. (2010b).

DUAL ROLES OF URIC ACID IN AODS

Contrary to the traditional understanding as a metabolically inert and waste compound without any physiological significance, UA is a natural antioxidant contributing to approximately 60% of the free radical scavenging activity in human blood (Ames et al., 1981). Past studies have demonstrated that UA and inosine (precursor of UA) may be beneficial in the treatment of oxidative stress-related neurodegenerative diseases (Hooper et al., 2000; Spitsin et al., 2001; Scott et al., 2002; Liu et al., 2006; Du et al., 2007).

Uric acid is a selective antioxidant (**Figure 3**) that removes superoxide by preventing the degradation of superoxide dismutase and subsequently inhibits its reaction with NO to form peroxynitrite (van der Veen et al., 1997). Moreover, UA can neutralize peroxynitrite (Keller et al., 1998) and hydroxyl radicals (Davies et al., 1986) to inhibit protein nitration (Pacher et al., 2007) and lipid peroxidation (Muraoka and Miura, 2003), respectively. Recent investigations further indicated that UA may operate as a protective factor mediated through astroglia for dopaminergic neurons from glutamate toxicity (de Lau et al., 2005; Du et al., 2007). Moreover, UA prevents the propagation of oxidative stress from the extracellular to the intracellular milieu by preserving the integrity of the plasma membrane at the lipid–aqueous interface boundary (Guerreiro et al., 2009). High K^+-induced depolarization amplifies neuroprotection provided by UA through a mechanism involving Ca^2 elevation and extracellular signal-regulated kinases½ ($ERK_{1/2}$) activation (**Figure 3**). Thus, decreased plasma UA levels may reflect decreased ability of the body to prevent superoxide and peroxynitrite from acting on cellular components and damaging the cell (Kutzing and Firestein, 2007). Previously, we have demonstrated significant decreases of plasma UA levels in either first-episode neuroleptic-naïve patients with SZ (FENNS) patients (Reddy et al., 2003) or clinically stable patients with chronic SZ (Yao et al., 1998). Similarly, low levels of UA have been linked to a variety of neurodegenerative diseases including Alzheimer's disease, multiple sclerosis, optic neuritis, and Parkinson's disease (Church and Ward, 1994; Toncev et al., 2002; Knapp et al., 2004; de Lau et al., 2005; Kim et al., 2006; Bogdanov et al., 2008; Ascherio et al., 2009).

On the other hand, at increased levels, UA is considered as a marker of oxidative stress (Becker, 1993; Strazzullo and Puig, 2007) due to accumulation of reactive oxygen species (Hayden and Tyagi, 2004). Abnormally high levels of UA have been related to cardiovascular disease, gout, hypertension, and renal disease (Jossa et al., 1994; Freedman et al., 1995; Kang et al., 2002; Choi et al., 2005; Bos et al., 2006). Although some studies have indicated that UA may play a role in the development or progression of such diseases (Saito et al., 1978; Jossa et al., 1994; Waring et al., 2000; Kang et al., 2002; Bos et al., 2006), it remains unclear whether an increased UA contributes to the cause or simply a consequence of these pathologic conditions (Kutzing and Firestein, 2007).

In addition, an altered purine catabolism has also been demonstrated in subjects with cocaine addition (Patkar et al., 2009) or with opioid dependence (Mannelli et al., 2009), although plasma UA levels remained unchanged. It is not clear whether such changes in purine metabolites without affecting plasma UA levels would eventually lead to oxidative damage in substance abusers. Nevertheless, taken together, UA may serve as either antioxidant or pro-oxidant in the AODS as illustrated in **Figure 3**.

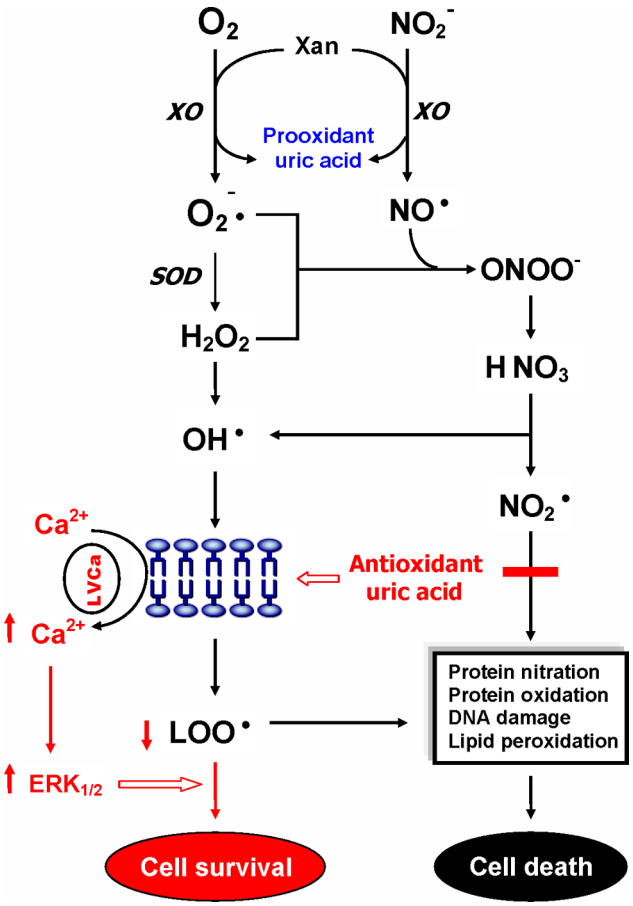

FIGURE 3 | Dual role of uric acid in the antioxidant defense system. Uric acid can neutralize peroxynitrite and hydroxyl radicals to inhibit protein nitration and lipid peroxidation, respectively. At increased levels, however, uric acid may be considered as a marker of oxidative stress due to accumulation of reactive oxygen species. CAT, catalase; GSH, glutathione; $ERK_{1/2}$, extracellular signal-regulated kinases½, LOO•, lipid peroxyl radical; LVCa, L-type voltage-gated calcium channel; NO, nitric oxide; $NO_2^•$, nitrogen dioxide radical; NO_2^-, nitrite; O_2^-, superoxide anion; $ONOO^-$, peroxynitrite; OH•, hydroxyl radical; SOD, superoxide dismutase; Xan, xanthine; XO, xanthine oxidase. Reprinted by permission from Yao et al. (2010b).

HOMEOSTATIC IMBALANCE OF PURINE CATABOLISM

Using a targeted electrochemistry based metabolomics (HPLC–CMEAS) platform, we compared metabolic signatures consisting of six plasma purine metabolites simultaneously between FENNS ($n = 25$) and healthy controls (HC, $n = 30$). We also compared these metabolites between FENNS at baseline (FENNS-BL) and 4 weeks (FENNS-4w) after antipsychotic treatment (Yao et al., 2010b). Significantly higher levels of xanthosine (Xant) and lower levels of G were seen in both patient groups compared to HC subjects. Moreover, the ratios of G/guanosine (Gr), UA/Gr, and UA/Xant were significantly lower, whereas the ratio of Xant/G was significantly higher in FENNS-BL patients than

in HC subjects (**Table 1**). Such changes remained in these same patients after 4 weeks of treatment (FENNS-4w) with the exception that the ratio of UA/Gr was completely normalized. During purine catabolism, both conversions from Gr to G and from Xant to Xan are reversible. Decreased ratios of product to precursor suggested a shift favorable to the Xant production resulting in decreased UA levels in the FENNS (**Figure 2**). More importantly, such an imbalance in purine catabolism is observed independent of treatment since patients were neuroleptic-naïve at entry into the study.

In addition, within the purines' pathway, all three groups had significant correlations between G and UA, and Xan and Hx. By contrast, correlations of UA with each of Xan and Hx, and correlation of Xan with Gr were all quite significant for the HC group but not for the FENNS group before or after treatment. Thus, there are tightly correlated precursor and product relationships within purine pathways; although some of these correlations persist across disease or medication status, others appear to be lost among FENNS patients. Taken together, the potential for steady formation of antioxidant UA from purine catabolism is altered early in the course of illness (Yao et al., 2010b).

CROSS-PATHWAY CORRELATIONS BETWEEN PURINE METABOLITES AND MONOAMINE NEUROTRANSMITTERS

The purinergic neurotransmission hypothesis was originally proposed in 1972 (Burnstock, 1972). Although ATP is widely recognized as an intracellular energy source for carrying out many biochemical reactions, it is also considered as a co-transmitter with glutamate, noradrenaline, acetylcholine, dopamine, and gamma-aminobutyric acid (GABA) in both central and peripheral nervous systems (Burnstock, 2007, 2009). Following the stimulation (e.g., electrical excitation) of brain, the adenine nucleosides that are stored in vesicles in nerve varicosities are released (Pull and McIlwain, 1972; Sulakhe and Phillis, 1975) by exocytosis to act on postjunctional receptors for ATP on smooth muscle. ATP is broken down by ATPases and 5′-nucleotidase to adenosine, which is taken up by varicosities to be resynthesized and reincorporated into vesicles. Adenosine is further broken down extracellularly by adenosine deaminase to inosine and Hx (**Figure 2**) and then removed by circulation (Burnstock, 1972).

In the study of normal behavior, purinergic signaling has been linked to learning and memory, sleep and arousal, locomotor activity and exploration, feeding behavior, and mood and motivation (Burnstock et al., 2011). On the other hand, a disordered purinergic signaling has been implicated in a variety of neurodegenerative diseases (Alzheimer's, Parkinson's, and Huntington's disease, multiple sclerosis, and amyotrophic lateral sclerosis) as well as neuropsychiatric diseases (SZ and mood disorders). Previously, a conspicuous relationship was observed between purine and monoamine metabolite concentrations in cerebrospinal fluid (CSF) during depressive illness suggesting the presence of a parallel purinergic and monoaminergic activation in the brain (Niklasson et al., 1983).

To test whether plasma purine and monoamine metabolite concentrations were correlated in SZ, we studied previously published measurements (Yao et al., 2010a,b) of six purine metabolites (Hx, Xan, Xant, G, Gr, UA) for which concomitant measurements of 14 monoamine metabolites, tryptophan (TRP), serotonin (5-hydroxytryptamine, 5-HT), 5-hydroxyindoleacetic acid (5-HIAA), tryptamine (TRPA), melatonin (MEL), kynurenine (KYN), 3-hydroxykynurenine (3-OHKY), tryptophol (TPOL), tyrosine (TYR), L-3,4-dihydroxyphenylalanine (L-DOPA), Normetanephrine (NMET), homovanillic acid (HVA), 3-methoxy-4-hydroxyphenylglycol (MHPG), and vanillylmandelic acid (VMA), were also available from HC ($n = 30$) and FENNS-BL ($n = 25$) and FENNS-4w ($n = 25$). Using Q–Q plots and a univariate correlation test (Johnson and Wichern, 1998), we found these data not to be approximately normal nor consistently transformable to approximate normality for all three datasets (HC, BL, 4w). Kendall's tau values and the p-values for rejection of H_0: tau = 0, were thus computed for all pairs consisting of one purine metabolite and one monoamine metabolite within each of the three datasets. Correction of alpha for multiple tests (252) was done by the Bonferroni procedure.

The Kendall's tau analysis found positive correlations that were significantly different from 0 in the HC group, for cross-pathway

Table 1 | Comparisons of ratios of product to precursor in purine pathway.

Ratios	HC	FENNS-BL	FENNS-4w	*p*		
				HC vs BL*	HC vs 4w*	BL vs 4w†
G/Gr	0.89 ± 0.61§	0.37 ± 0.30	0.48 ± 0.72	0.0004¶	0.0009	0.8949
Xan/G	46.33 ± 85.46	81.92 ± 98.86	66.68 ± 50.91	0.0211	0.0015	0.7112
UA/Gr	7371 ± 4325	4152 ± 2193	7047 ± 5556	0.0015	0.4967	0.0025
UA/G	11998 ± 11525	16529 ± 14751	23771 ± 14948	0.0614	<0.0001	0.0236
UA/Xant	5073 ± 4845	1298 ± 972	2184 ± 4310	0.0021	0.0067	0.5782
Xant/G	10.48 ± 15.58	42.02 ± 75.08	31.35 ± 27.93	0.0009	0.0001	0.2752

**Wilcoxon rank sum test.*
† Wilcoxon signed rank sum test.
§ Data obtained from Yao et al. (2010b).
¶ Significance with $p < 0.0033$ after the Bonferroni correction.
G, guanine; Gr, guanosine; Xan, xanthine; UA, uric acid; Xant, xanthosine.

purine and monoamine metabolite pairs (**Table 2**) as follows: (1) for UA with TRP, 5-HIAA, MEL, KYN, and TYR; (2) for G with TRP, TYR, and possibly (trend) with MEL and KYN; (3) for GR with TYR; and (4) for Xan with TYR, and possibly (trend) with 5-HIAA. Many of these same correlations were also significant or possibly significant for the BL and 4w groups, with the following notable exceptions. The correlations between each of UA and Xan with 5-HIAA were much weaker and far from significance for BL and 4w patients, suggesting possible group differences among HC, BL, and 4w. Formal testing for equality of correlations among these groups, the next step, will require larger group numbers than are available with the present dataset.

To summarize, in HC, the purine and TRP pathways show extensive cross-correlations (all positive) among their respective member metabolites, whereas the TYR pathway shows significant cross-correlation with purines *only via tyrosine*. These relationships are generally seen for the BL and 4w groups as well. It may be that there are general dietary (precursor amino acids and purines are both high in many foods), hydration, hepatic, or other influences that affect purines and indoleamines and TYR similarly. However, the correlation of 5-HIAA with UA and Xan appear to be much weaker in the BL and 4w groups. We have already observed that BL patients have weaker correlations within the TRP pathway, e.g., 5-HIAA with TRP (tau = 0.09 BL, tau = 0.69 HC), which may occur when dietary associations are overcome by other rate-limiting pathway controls based on physiological needs for serotonin neurotransmitter (Yao et al., 2010a). The 4w group appears to have very little association between 5-HIAA and Xan, perhaps due to treatment with atypical neuroleptic drugs, which block serotonin 5-HT_2, as well as dopamine D_2, receptors, bringing more variables to influence the 5-HIAA metabolic product of 5-HT. The positive correlations in human CSF of Xan and several monoamines including 5-HIAA have been noted earlier (Niklasson et al., 1983) and between UA and 5-HIAA (Degrell and Nagy,

Table 2 | Across pathway correlations between 6 purine and 14 monoamine metabolites by the Kendall's tau method.

Metabolites		Kendall's tau rank correlations					
		HC ($n = 30$)		FENNS-BL ($n = 25$)		FENNS-4w ($n = 25$)	
I	II	tau	*p*	tau	*p*	tau	*p*
Significant correlations among all three groups							
UA	TRP	0.6598	<0.0001	0.7122	<0.0001	0.6400	<0.0001
UA	MEL	0.5034	0.0001	0.5800	<0.0001	0.5400	0.00017
UA	KYN	0.6184	<0.0001	0.6800	<0.0001	0.6333	<0.0001
UA	TYR	0.7287	<0.0001	0.7200	<0.0001	0.6467	<0.0001
G	TRP	0.5034	0.0001	0.5843	<0.0001	0.5667	<0.0001
G	TYR	0.5816	<0.0001	0.5333	0.0002	0.5733	<0.0001
G	MEL	0.4667	0.0003	0.5267	0.0002	0.5600	<0.0001
Significant correlations present only in HC and FENNS-BL but not FENNS-4w							
Gr	TYR	0.5681	<0.0001	0.6118	<0.0001	0.3022	0.0516
G	KYN	0.4805	0.0002	0.5200	0.0003	0.4933	0.0006
Significant correlations present only in HC but not FENNS groups							
UA	5-HIAA	0.5310	<0.0001	0.1733	0.2336	0.3667	0.0109
Xan	5-HIAA	0.4759	0.0002	0.1733	0.2336	0.0133	0.9441
Xan	TYR	0.5264	<0.0001	0.4000	0.0054	0.4133	0.0041
Significant correlations present only in FENNS-BL but not in HC and FENNS-4w							
UA	TRPA	0.3563	0.0060	0.6333	<0.0001	0.4267	0.0030
Gr	TRP	0.4613	0.0006	0.6440	<0.0001	0.3255	0.0359
Gr	MEL	0.4127	0.0021	0.6118	<0.0001	0.2480	0.1112
Gr	KYN	0.4127	0.0021	0.6256	<0.0001	0.3952	0.0107
Gr	3-OHKY	0.3885	0.0038	0.6403	<0.0001	0.3649	0.0187
Significant correlations present only in FENNS groups but not in HC							
UA	3-OHKY	0.3977	0.0022	0.7114	<0.0001	0.5710	<0.0001

Data were obtained from Yao et al. (2010a,b).
Significance with $p < 0.000197$ after the Bonferroni correction.
HC, healthy control subjects; FENNS, first-episode neuroleptic-naïve patients with schizophrenia; BL, baseline; 4w, 4-week after antipsychotic treatment; UA, uric acid; G, guanine; Gr, guanosine; Xan, xanthine; TRP, tryptophan; MEL, melatonin; KYN, kynurenine; TYR, tyrosine; 5-HIAA, 5-hydroxyindoleacetic acid; TRPA, tryptamine; 3-OHKY, 3-hydroxykynurenine.

1990). It is notable that correlations are maintained between UA or G and metabolites in other branches of the TRP pathway (MEL, KYN) which are not involved in serotonin neurotransmission, for HC and patient groups.

PURINERGIC SIGNALING, CLINICAL IMPROVEMENT, AND NEUROLOGICAL DEFICITS

Associations between purine metabolites and clinical and neurological symptoms were examined before and after 4w antipsychotic treatment (Yao et al., 2012). A lower initial proportion of product (UA) to precursor (guanine) measured at baseline was associated with *greater* improvement in clinical functioning 1 month later (**Figure 4**). Improvement in clinical functioning was associated with initial levels of UA and G in the FENNS patients. The initial severity of clinical dysfunction may thus be important to this relationship. As a group, the average level of clinical functioning reflected impairment at both time points, with mean values (<40) falling within the range typically observed for former inpatients likely to be readmitted to hospital (Endicott et al., 1976). Descriptively, degree of clinical improvement achieved by the patient group in the above study (Yao et al., 2012) represented an increase from "*Unable to function in almost all areas. . .*" at baseline to "*Major impairment in several areas. . .*" 1 month later (Global Assessment Scale or GAS anchor points). It may be appropriate, therefore, to qualify interpretation of findings based on this degree of severity.

Neurological abnormalities are a core feature of SZ even at the time of their first episode of psychosis without antipsychotic drug treatment (Rubin et al., 1994; Gupta et al., 1995; Keshavan et al., 2003; Mohr et al., 2003; Sanders et al., 2004). Moreover, neurological signs are correlated with clinical symptoms in unmedicated patients (Sanders et al., 2000). Significant heritability, or familial influence, has also been reported for several aspects of neurologic-related responding (Sanders et al., 2006), which suggest that neurological deficits may represent a biological marker of SZ risk. Recently, we have shown that purine metabolites were also linked to neurological and cognitive symptoms in the FENNS patients (Yao et al., 2012). Firstly, motor neurological signs (Buchanan and Heinrichs, 1989) recorded at baseline were associated with initial or baseline level of the ratio of Xant to Gr, indicating the higher a patient's initial or baseline ratio of Xan to Gr, the greater his or her motor neurological signs was before initiating treatment with antipsychotic medications. Secondly, sensory-integrative neurological signs were predicted by baseline level of UA, which suggests that lower levels of UA were associated with *greater* impairment in sensory processing tasks. The above findings thus suggest an association between optimal levels of purine byproducts and dynamics in clinical symptoms and adjustment, as well as in the integrity of sensory and motor processing.

PURINERGIC SIGNALING AND PLATELET ACTIVATION

Purinergic signaling is an important link among platelet activation, vascular thrombosis, and inflammation (Eltzschig et al., 2012). Mammalian cells contain high levels of ATP. Under pathologic conditions such as inflammation, there is an increased release of ATP. Extracellular adenosine is formed predominately from a series of enzymatic conversion from ATP, adenosine diphosphate (ADP), and AMP to adenosine (**Figure 2**). Adenosine signaling is terminated by uptaking adenosine from extracellular space to intracellular space and is then rapidly metabolized to inosine through adenosine deaminase (Eltzschig et al., 2006) or converted back to AMP through adenosine kinase (Morote-Garcia et al., 2008). Inhibition of adenosine kinase by cyclosporine resulting in increased levels of extracellular adenosine may contribute, at least in part, to the anti-inflammatory effects of cyclosporine (Spychala and Mitchell, 2002).

In human platelets, serotonin (5-HT) amplifies the aggregation induced by ADP (McBride et al., 1989; de Clerck, 1990), which is mediated by the 5-HT_2 receptor complex. Thus, the magnitude

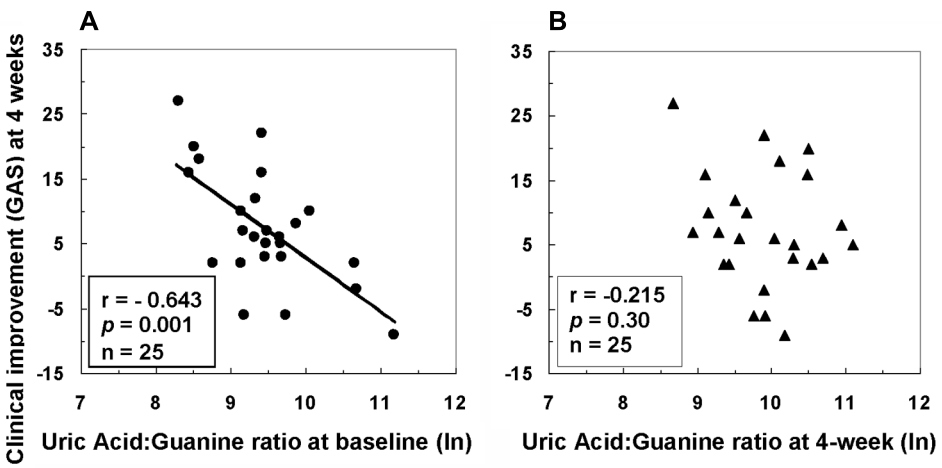

FIGURE 4 | Associations between clinical improvement at 4 weeks and ratio of uric acid to guanine in first-episode neuroleptic-naïve patients with schizophrenia at baseline (A) or at 4-week (B) after antipsychotic treatment. GAS, Global Assessment Scale; ln, natural logarithm. Reprinted by permission from Yao et al. (2012).

of serotonin amplification of ADP-induced platelet aggregation and dense granule secretion (DGS) may provide us with an index to evaluate the platelet serotonin responsivity. In both normal control subjects and clinically stable patients with chronic SZ (with antipsychotic treatment), our laboratory demonstrated a robust increase of platelet aggregation in response to synergistic effects of ADP and 5-HT (Yao et al., 1996). Moreover, increases in 5-HT amplification were inversely correlated with the psychosis severity. The magnitude of 5-HT amplification, however, was not significantly different in those same patients after haloperidol withdrawal. Recently, we have further shown that FENNS patients have significantly lower 5-HT amplification than the normal control subjects (Reddy et al., 2007). The blunted platelet serotonergic responsivity may thus be associated with SZ *per se*, independent of drug effects. The magnitude of 5-HT amplification on ADP-induced platelet aggregation, however, can be augmented in SZ patients after eicosapentaenoic acid (EPA) supplementation (Yao et al., 2004b).

DO PERIPHERAL INDICES OF METABOLIC DEFICITS ALSO REFLECT SIMILAR CHANGES IN THE BRAIN?

Whether peripheral indices of abnormal metabolites reflect similar changes in the brain and/or are related to presumed brain events are frequently raised by the reviewers in the grant applications and manuscript submissions. This issue has been vigorously debated because of examples in the literature, where peripheral measures either failed to adequately reflect central pathophysiology or did not serve as reliable biological markers. Therefore, in principle, the majority of research investigators believe that peripheral findings do not reflect the similar changes in the brain. However, in an editorial in Molecular Psychiatry, Wong and Licinio (2005) have eloquently stated that this belief has pervaded the field and has undermined our ability to confidently use the powerful tools of contemporary biology in order to dissect the biology of psychiatric disorders through investigation of peripheral markers, particularly those measured in peripheral blood.

Substantial evidence has been accumulated that reveals metabolic defects in both the peripheral and central tissue of patients with SZ (see reviews by Skosnik and Yao, 2003; Yao and van Kammen, 2004; Mahadik and Yao, 2006; Yao and Keshavan, 2011). Moreover, direct correlations between the peripheral (red blood cell, RBC) and central (31-phosphorus magnetic resonance spectroscopy, ^{31}P MRS) phospholipids and polyunsaturated fatty acids (PUFAs) were shown in SZ patients (Richardson et al., 2001; Yao et al., 2002). Additionally, platelets and fibroblasts have been used as models for nerve cells in a variety of neuropsychiatric diseases (Farmer, 1980; Mahadik and Mukherjee, 1996). These findings support the notion that metabolic defects are present in both neural and extra-neural tissues, but the functional consequences may differ. For example, changes in peripheral metabolites may play a role in clinical presentation and outcome during the early course of SZ (Condray et al., 2011; Yao et al., 2012).

Moreover, there are several paradigmatic conditions such as Down syndrome, phenylketonuria, and various lipidoses (Scriver et al., 1989) where the metabolic abnormalities are expressed in both neural and peripheral tissues, but the functional consequences are most profound in the central nervous system (CNS). A recent review by Andrews and Neises (2012) also suggest that research examining the mechanism of how traumatic events are linked to peripheral blood mononuclear cell functions and biomarkers may offer improved diagnoses and treatments for post-traumatic stress disorder patients. This paradigm may also apply to SZ. A recent study by comparison of peripheral and central SZ biomarker profiles, Harris et al. (2012) have concluded that the systemic nature of SZ provides added validity of investigating blood-based biomarkers in SZ. If peripheral indices parallel central metabolic defects, and perhaps also neuromorphometric and/or neuroimaging findings, then there exists the possibility that alterations in peripheral indices on longitudinal follow-up (repeated measures) can usefully reflect central membrane function over the course of illness.

CONCLUSION AND PERSPECTIVES

During the purine catabolism, there are three major purine bases and their corresponding ribonucleosides, which consist of adenine/adenosine, G/Gr, and Hx/inosine (**Figure 2**). As mentioned above, we have observed that a homeostatic imbalance of purine catabolism is present in FENNS. There are tightly correlated precursor and product relationships *within* purine pathways. Although some of these correlations persist across disease or medication status, others appear to be lost among FENNS (Yao et al., 2010b). Similar findings of lacking a control mechanism used by HC subjects were also demonstrated in the TRP pathway from these same FENNS patients (Yao et al., 2010a). When taken together, these observations suggest that a steady formation of the important antioxidant UA via purine catabolism is altered early in the course of illness.

Moreover, we have applied Kendall's tau to assess correlations between purine metabolites and monoamine neurotransmitters with the Bonferroni corrections. Correlations between TYR, TRP, and some purines may originate in the diet or other common organism-wide influences, but some of these appear to be lost as these compounds undergo further transformations along their respective pathways. For both HC and patients, purine metabolites normally show significant cross-pathway correlation only with TYR, not with its metabolites, where correlations may be lost due to internal influences over neurotransmitter production. Furthermore, several purine metabolites (UA, Gr, or Xan) are each significantly correlated with TRP in all subjects. But purine correlations with 5-HIAA seem to be present only in HC subjects, not in FENNS at baseline or 4 weeks after antipsychotic treatment. Again, the loss of correlations in the pathway metabolite may be lost in patients due to illness-related, and also perhaps treatment influences, on 5-HIAA, since the TRP–5-HIAA correlation is appears weakened in patients (Yao et al., 2010a).

In conclusion, SZ is a heterogeneous disease with various abnormal metabolites involving multiple biochemical pathways. There is abundant evidence that alterations in key neurotransmitters can both be modified by and contribute to oxidative stress and membrane dysfunction (**Figure 1**), suggesting a link between these pathophysiological processes in SZ. GSH redox coupling, NO signaling, and purine catabolism are the key pathways involving the AODS. We have previously demonstrated a homeostatic

imbalance of purine catabolism (Yao et al., 2010b) and blunted platelet serotonergic responsivity (Yao et al., 1996; Yao et al., 2004b; Reddy et al., 2007) in FENNS. In this "Hypothesis and Theory" paper, we propose that the altered purine metabolites have significantly impacts on not only within the purine catabolism but also across the TRP pathways involving the serotonin and KYN metabolism.

Firstly, several purine metabolites (UA, Gr, or Xan) are each significantly correlated with TRP in all subjects. However, purine correlations with 5-HIAA seem to be present only in HC subjects, not in FENNS at baseline or 4 weeks after antipsychotic treatment (**Table 2**). Conversion of serotonin to *N*-acetylserotonin by serotonin *N*-acetyltransferase may be upregulated in the same set of FENNS patients, possibly related to the observed alteration in TRP–5-HIAA correlation (Yao et al., 2010a). Lacking significant correlations between purine metabolites (UA and Xan) and 5-HIAA suggest that alterations in key serotonin pathways may both be modified by and contribute to oxidative stress via purine catabolism in FENNS.

Secondly, we have shown that a neurotoxic product of TRP metabolism, 3-OHKY, predicts severity of clinical symptoms during the early phase of illness and before exposure to antipsychotic drugs (Condray et al., 2011). Baseline level of 3-OHKY may also predict the degree of clinical improvement following brief treatment with antipsychotics. In the present paper, we have further demonstrated that levels of 3-OHKY were significantly correlated with levels of either Gr or UA in this same sample set. Considering the unique functional roles of UA (the end product of purine catabolism) as both antioxidant and pro-oxidant, the homeostatic balance of UA appears to play a vital role of regulatory functions in not only the AODS but also the KYN pathway. The KYN pathway that produces neurotoxic and neuroinhibitory compounds is regulated by the dopamine metabolites, VMA and HVA, which has been implicated in the pathogenic mechanisms underlying SZ.

Lastly, optimum levels of purine metabolites have been associated with the dynamics of clinical symptoms and therapeutic improvements, which may lead to discovery of novel targets for drug development. Interestingly, allopurinol, which is a structural isomer of Hx and is an inhibitor of the Xan oxidase, can improve SZ symptoms either when given alone or as add-on medication to haloperidol (Lara et al., 2001). Xan oxidase is responsible for the successive oxidation of Hx and Xan (http://en.wikipedia.org/wiki/Xanthine), leading to the formation of UA (Pacher et al., 2006). Therefore, allopurinol may regulate levels of not only UA but also the extracellular adenosine (via feedback inhibition). Future investigations are required to establish therapeutic target for purinergic drugs in treatment of SZ patients.

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REFERENCES

Ames, B. N., Cathcart, R., Schwiers, E., and Hochstein, P. (1981). Uric acid provides an antioxidant defense in humans against oxidant- and radical-caused aging and cancer: a hypothesis. *Proc. Natl. Acad. Sci. U.S.A.* 78, 6858–6862. doi: 10.1073/pnas.78.11.6858

Andrews, J. A., and Neises, K. D. (2012). Cells, biomarkers, and post-traumatic stress disorder: evidence for peripheral involvement in a central disease. *J. Neurochem.* 120, 26–36. doi: 10.1111/j.1471-4159.2011.07545.x

Ascherio, A., LeWitt, P. A., Xu, K., Eberly, S., Watts, A., Matson, W. R., et al. (2009). Urate as a predictor of the rate of clinical decline in Parkinson disease. *Arch. Neurol.* 66, 1460–1468. doi: 10.1001/archneurol.2009.247

Becker, B. F. (1993). Towards the physiological function of uric acid. *J. Free Radic. Biol. Med.* 14, 615–631. doi: 10.1016/0891-5849(93)90143-I

Bogdanov, M., Brown, R. H., Matson, W., Smart, R., Hayden, D., O'Donnell, H., et al. (2000). Increased oxidative damage to DNA in ALS patients. *Free Radic. Biol. Med.* 29, 652–658. doi: 10.1016/S0891-5849(00)00349-X

Bogdanov, M., Matson, W. R., Wang, L., Matson, T., Saunders-Pullman, R., Bressman, S. S., et al. (2008). Metabolomic profiling to develop blood biomarkers for Parkinson's disease. *Brain* 131, 389–396. doi: 10.1093/brain/awm304

Bos, M. J., Koudstaal, P. J., Hofman, A., Witteman, J. C., and Breteler, M. M. (2006). Uric acid is a risk factor for myocardial infarction and stroke: the Rotterdam study. *Stroke* 37, 1503–1507. doi: 10.1161/01.STR.0000221716.55088.d4

Buchanan, R. W., and Heinrichs, D. W. (1989). The Neurological Evaluation Scale (NES): A structured instrument for the assessment of neurological signs in schizophrenia. *Psychiatry Res.* 27, 335–350. doi: 10.1016/0165-1781(89)90148-0

Burnstock, G. (1972). Purinergic nerves. *Pharmacol. Rev.* 24, 509–581.

Burnstock, G. (2007). Physiology and pathophysiology of purinergic neurotransmission. *Physiol. Rev.* 87, 659–797. doi: 10.1152/physrev.00043.2006

Burnstock, G. (2009). Purinergic cotransmission. *Exp. Physiol.* 94, 20–24. doi: 10.1113/expphysiol.2008.043620

Burnstock, G., Krugel, U., Abbracchio, M. P., and Illes, P. (2011). Purinergic signalling: from normal behaviour to pathological brain function. *Prog. Neurobiol.* 95, 229–274. doi: 10.1016/j.pneurobio.2011.08.006

Choi, H. K., Mount, D. B., and Reginato, A. M. (2005). Pathogenesis of gout. *Ann. Intern. Med.* 143, 499–516. doi: 10.7326/0003-4819-143-7-200510040-00009

Church, W. H., and Ward, V. L. (1994). Uric acid is reduced in the substantia nigra in Parkinson's disease: effect on dopamine oxidation. *Brain Res. Bull.* 33, 419–425. doi: 10.1016/0361-9230(94)90285-2

Condray, R., Dougherty, G. G., Keshavan, M. S., Reddy, R. D., Haas, G. L., Montrose, D. M., et al. (2011). 3-Hydroxykynurenine and clinical symptoms in first-episode neuroleptic-naïve patients with schizophrenia. *Int. J. Neuropsychopharmacol.* 14, 756–767. doi: 10.1017/S1461145710001689

Cory, J. G. (1982). "Purine and pyrimidine nucleotide metabolism," in *Text Book of Biochemistry with Clinical Correlations*, ed. T. M. Devlin (New York: John Wiley & Sons), 489–528.

Davies, K. J., Sevanian, A., Muakkassah-Kelly, S. F., and Hochstein, P. (1986). Uric acid–iron ion complexes. A

new aspect of the antioxidant functions of uric acid. *Biochem. J.* 235, 747–754.

de Clerck, F. (1990). The role of serotonin in thrombogenesis. *Clin. Physiol. Biochem.* 8(Suppl. 3), 40–49.

Degrell, I., and Nagy, E. (1990). Concentration gradients for HVA, 5-HIAA, ascorbic acid, and uric acid in cerebrospinal fluid. *Biol. Psychiatry* 27, 891–896. doi: 10.1016/0006-3223(90)90470-M

de Lau, L. M., Koudstaal, P. J., Hofman, A., and Breteler, M. M. (2005). Serum uric acid levels and the risk of Parkinson disease. *Ann. Neurol.* 58, 797–800. doi: 10.1002/ana.20663

Du, Y., Chen, C. P., Tseng, C. Y., Eisenberg, Y., and Firestein, B. L. (2007). Astroglia-mediated effects of uric acid to protect spinal cord neurons from glutamate toxicity. *Glia* 55, 463–472. doi: 10.1002/glia.20472

Eltzschig, H. K., Faigle, M., Knapp, S., Karhausen, J., Ibla, J., Rosenberger, P., et al. (2006). Endothelial catabolism of extracellular adenosine during hypoxia: the role of surface adenosine deaminase and CD26. *Blood* 108, 1602–1610. doi: 10.1182/blood-2006-02-001016

Eltzschig, H. K., Sitkovsky, M. V., and Robinson, S. C. (2012). Purinergic signaling during inflammation. *New Engl. J. Med.* 367, 2322–2333. doi: 10.1056/NEJMra1205750

Endicott, J., Spitzer, R. L., Fleiss, J. L., and Cohen, J. (1976). The Global Assessment Scale: a procedure for measuring overall severity of psychiatric disturbance. *Arch. Gen. Psychiatry* 33, 766–771. doi: 10.1001/archpsyc.1976.01770060086012

Farmer, P. M. (1980). Platelet enzyme abnormalities in neuropsychiatric disease. *Ann. Clin. Lab. Sci.* 10, 263–268.

Floyd, R. A. (1996). "Mitochondrial damage in neurodegenerative disease," in *Free Radicals in Brain Physiology and Disorders*, eds L. Packer, M. Hiramatsu, and T. Yoshikawa (San Diego, CA: Academic Press), 313–329.

Freedman, D. S., Williamson, D. F., Gunter, E. W., and Byers, T. (1995). Relation of serum uric acid to mortality and ischemic heart disease. The NHANES I Epidemiologic Follow-up Study. *Am. J. Epidemiol.* 141, 637–644.

Guerreiro, S., Ponceau, A., Toulorge, D., Martin, E., Alvarez-Fischer, D., Hirsch, E. C., et al. (2009). Protection of midbrain dopaminergic neurons by the end-product of purine metabolism uric acid: potentiation by low-level depolarization. *J. Neurochem.* 109, 1118–1128. doi: 10.1111/j.1471-4159.2009.06040.x

Gupta, S., Andreasen, N. C., Arndt, S., Flaum, M., Schultz, S. K., Hubbard, W. C., et al. (1995). Neurological soft signs in neuroleptic-naïve and neuroleptic-treated schizophrenic patients and in normal comparison subjects. *Am. J. Psychiatry* 152, 191–196. doi: 10.1176/appi.pn.2013.5b12

Harris, L. W., Pietsch, S., Cheng, T. M. K., Schwarz, E., Guest, P. C., and Bahn, S. (2012). Comparison of peripheral and central schizophrenia biomarker profiles. *PLoS ONE* 7:e46368. doi: 10.1371/journal.pone.0046368.

Hayden, M. R., and Tyagi, S. C. (2004). Uric acid: a new look at an old risk marker for cardiovascular disease, metabolic syndrome, and type 2 diabetes mellitus: the urate redox shuttle. *Nutr. Metab. (Lond.)* 1, 10. doi: 10.1186/1743-7075-1-10

Hooper, D. C., Scott, G. S., Zborek, A., Mikheeva, T., Kean, R. B., Koprowski, H., et al. (2000). Uric acid, a peroxynitrite scavenger, inhibits CNS inflammation, blood–CNS barrier permeability changes, and tissue damage in a mouse model of multiple sclerosis. *FASEB J.* 14,691–698.

Javitt, D. C., and Laruelle, M. (2006). "Neurochemical theories," in *Text Book of Schizophrenia*, eds J. A. Lieberman, T. S. Stroup, and D. O. Perkins (Washington, DC: The American Psychiatric Publishing), 85–116.

Johnson, R. A., and Wichern, D. W. (1998). *Applied Multivariate Statistical Analysis*, 4th Edn. Upper Saddle River, NJ: Prentice Hall, 193, 222–223.

Jossa, F., Farinaro, E., Panico, S., Krogh, V., Celentano, E., Galasso, R., et al. (1994). Serum uric acid and hypertension: the Olivetti Heart Study. *J. Hum. Hypertens.* 8, 677–681.

Kaddurah-Daouk, R., Kristal, B. S., and Weinshilboum, R. M. (2008). Metabolomics: a global biochemical approach to drug response and disease. *Annu. Rev. Pharmacol. Toxicol.* 48, 653–683. doi: 10.1146/annurev.pharmtox.48.113006.094715

Kang, D. H., Nakagawa, T., Feng, L., Watanabe, S., Han, L., Mazzali, M., et al. (2002). A role for uric acid in the progression of renal disease. *J. Am. Soc. Nephrol.* 13, 2888–2897. doi: 10.1097/01.ASN.0000034910.58454.FD

Keller, J. N., Kindy, M. S., Holtsberg, F. W., St Clair, D. K., Yen, H. C., Germeyer, A., et al. (1998). Mitochondrial manganese superoxide dismutase prevents neural apoptosis and reduces ischemic brain injury: suppression of peroxynitrite production, lipid peroxidation, and mitochondrial dysfunction. *J. Neurosci.* 18, 687–697.

Keshavan, M. S., Sanders, R. D., Sweeney, J. A., Diwadkar, V. A., Goldstein, G., Pettegrew, J. W., et al. (2003). Diagnostic specificity and neuroanatomical validity of neurological abnormalities in first-episode psychoses. *Am. J. Psychiatry* 160, 1298–1304. doi: 10.1176/appi.ajp.160.7.1298

Kim, T. S., Pae, C. U., Yoon, S. J., Jang, W. Y., Lee, N. J., Kim, J. J., et al. (2006). Decreased plasma antioxidants in patients with Alzheimer's disease. *Int. J. Geriatr. Psychiatry* 21, 344–348. doi: 10.1002/gps.1469

Knapp, C. M., Constantinescu, C. S., Tan, J. H., McLean, R., Cherryman, G. R., and Gottlob, I. (2004). Serum uric acid levels in optic neuritis. *Mult. Scler.* 10, 278–280. doi: 10.1191/1352458504ms1042oa

Kristal, B. S., Vigneau-Callahan, K. E., and Matson, W. R. (1998). Simultaneous analysis of the majority of low-molecular-weight, redox-active compounds from mitochondria. *Anal. Biochem.* 263, 18–25. doi: 10.1006/abio.1998.2831

Kristal, B. S., Vigneau-Callahan, K. E., Moskowitz, A. J., and Matson, W. R. (1999). Purine catabolism: links to mitochondrial respiration and antioxidant defenses? *Arch. Biochem. Biophys.* 370, 22–33. doi: 10.1006/abbi.1999.1387

Kutzing, M. K., and Firestein, B. L. (2007). Altered uric acid levels and disease states. *J. Pharmacol. Exp. Ther.* 324, 1–7. doi: 10.1124/jpet.107.129031

Lara, D. R., Brunstein, M. G., Ghisolfi, E. S., Lobato, M. I., Belmonte-de-Abreu, P., and Souza, D. O. (2001). Allopurinol augmentation for poorly responsive schizophrenia. *Int. Clin. Psychopharmacol.* 16, 235–237. doi: 10.1097/00004850-200107000-00008

Linden, J., and Rosin, D. L. (2006). "Purinergic system," in *Basic Neurochemistry, Molecular, Cellular and Medical Aspects*, 7th Edn., eds G. J. Siegel, R. W. Albers, S. T. Brady, D. L. Price (Boston: Elsevier), 303–316.

Liu, F., You, S. W., Yao, L. P., Liu, H. L., Jiao, X. Y., Shi, M., et al. (2006). Secondary degeneration reduced by inosine after spinal cord injury in rats. *Spinal Cord* 44, 421–426. doi: 10.1038/sj.sc.3101878

Mahadik, S. P., and Mukherjee, S. (1996). Cultured skin fibroblasts as a cell model for investigating schizophrenia. *J. Psychiatry Res.* 30, 421–439. doi: 10.1016/S0022-3956(96)00025-8

Mahadik, S. P., and Yao, J. K. (2006). "Phospholipids in schizophrenia," in *Textbook of Schizophrenia*, eds J. A. Lieberman, T. S. Stroup, D. O. Perkins (Washington, DC: The American Psychiatric Publishing, Inc.), 117–135.

Mannelli, P., Patkar, A., Rozen, S., Matson, W., Krishnan, R., and Kaddurah-Daouk, R. (2009). Opioid use affects antioxidant activity and purine metabolism. *Hum. Psychopharmacol.* 24, 666–675. doi: 10.1002/hup.1068

Matson, W. R., Langlais, P., Volicer, L., Gamache, P. H., Bird, E., and Mark, K. A. (1984). n-Electrode three-dimensional liquid chromatography with electrochemical detection for determination of neurotransmitters. *Clin. Chem.* 30, 1477–1488.

Matsuzawa, D., and Hashimoto, K. (2011). Magnetic resonance spectroscopy study of the antioxidant defense system in schizophrenia. *Antioxid. Redox Signal.* 15, 2057–2065. doi: 10.1089/ars.2010.3453

McBride, P. A., Anderson, G. M., Hertzig, M., Sweeney, J. A., Kream, J., Cohen, D. J., et al. (1989). Serotonergic responsivity in male young adults with autistic disorder. *Arch. Gen. Psychiatry* 46, 213–221. doi: 10.1001/archpsyc.1989.01810030019003

Mohr, F., Hubmann, W., Albus, M., Franz, U., Hecht, S., Scherer, J., et al. (2003). Neurological soft signs and neuropsychological performance in patients with first episode schizophrenia. *Psychiatry Res.* 121, 21–30. doi: 10.1016/S0165-1781(03)00203-8

Morote-Garcia, J. C., Rosenberger, P., Kuhlicke, J., and Eltzchig, H. K. (2008). HIF-1-dependent repression of adenosine kinase attenuates hypoxia-induced vascular leak. *Blood* 111, 5571–5580. doi: 10.1182/blood-2007-11-126763

Muraoka, S., and Miura, T. (2003). Inhibition by uric acid of free radicals that damage biological molecules. *Pharmacol. Toxicol.* 93, 284–289. doi: 10.1111/j.1600-0773.2003.pto930606.x

Niklasson, F., Agren, H., and Hallgren, R. (1983). Purine and monoamine metabolites in cerebrospinal fluid:

parallel Purinergic and monoaminergic activation in depressive illness? *J. Neurol. Neurosurg. Psychiatry* 46, 255–260. doi: 10.1136/jnnp.46.3.255

Othmen, L. B., Mechri, A., Fendri, C., Bost, M., Chazot, G., Gaha, L., et al. (2008). Altered antioxidant defense system in clinically stable patients with schizophrenia and their unaffected siblings. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 32, 155–159. doi: 10.1016/j.pnpbp.2007.08.003

Pacher, P., Beckman, J. S., and Liaudet, L. (2007). Nitric oxide and peroxynitrite in health and disease. *Physiol. Rev.* 87, 315–424. doi: 10.1152/physrev.00029.2006

Pacher, P., Nivorozhkin, A., and Szabó, C. (2006). Therapeutic effects of xanthine oxidase inhibitors: renaissance half a century after the discovery of allopurinol. *Pharmacol. Rev.* 58, 87–114. doi: 10.1124/pr.58.1.6

Patkar, A. A., Rozen, S., Mannelli, P., Matson, W., Pae, C.-U., Krishnan, R., et al. (2009). Alterations in tryptophan and purine metabolism in cocaine addiction: a metabolomic study. *Psychopharmacology (Berl.)* 206, 479–489. doi: 10.1007/s00213-009-1625-1

Pull, I., and McIlwain, H. (1972). Metabolism of (14C)adenine and derivatives by cerebral tissues, superfused and electrically stimulated. *Biochem. J.* 126, 965–973.

Ranjekar, P. K., Hinge, A., Hegde, M. V., Ghate, M., Kale, A., Sitasawad, S., et al. (2003). Decreased antioxidant enzymes and membrane essential polyunsaturated fatty acids in schizophrenic and bipolar mood disorder patients. *Psychiatry Res.* 121, 109–122. doi: 10.1016/S0165-1781(03)00220-8

Reddy, R. D., Keshavan, M. S., and Yao, J. K. (2003). Reduced plasma antioxidants in first-episode patients with schizophrenia. *Schizophr. Res.* 62, 205–212. doi: 10.1016/S0920-9964(02)00407-3

Reddy, R. D., Keshavan, M. S., and Yao, J. K. (2007). Blunted serotonergic responsivity in neuroleptic-naïve patients at first-episode of schizophrenia. *Schizophr. Res.* 90, 81–85. doi: 10.1016/j.schres.2006.11.013

Richardson, A. J., Allen, S. J., Hajnal, J. V., Cox, I. J., Easton, T., and Puri, B. K. (2001). Associations between central and peripheral measures of phospholipid breakdown revealed by cerebral 31-phosphorus magnetic resonance spectroscopy and fatty acid composition of erythrocyte membranes. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 25, 1513–1521. doi: 10.1016/S0278-5846(01)00211-1

Rozen, S., Cudkowicz, M. E., Bogdanov, M., Matson, W. R., Kristal, B. S., Beecher, C., et al. (2005). Metabolomic analysis and signatures in motor neuron disease. *Metabolomics* 1, 101–108. doi: 10.1007/s11306-005-4810-1

Rubin, P., Vorstrup, S., Hemmingsen, R., Andersen, H. S., Bendsen, B. B., Stromso, N., et al. (1994). Neurological abnormalities in patients with schizophrenia or schizophreniform disorder at first admission to hospital: correlations with computerized tomography and regional cerebral blood flow findings. *Acta Psychiatr. Scand.* 90, 385–390. doi: 10.1111/j.1600-0447.1994.tb01611.x

Saito, I., Saruta, T., Kondo, K., Nakamura, R., Oguro, T., Yamagami, K., et al. (1978). Serum uric acid and the renin–angiotensin system in hypertension. *J. Am. Geriatr. Soc.* 26, 241–247.

Sanders, R. D., Joo, Y. H., Almasy, L., Wood, J., Keshavan, M. S., Pogue-Geile, M. F., et al. (2006). Are neurologic examination abnormalities heritable? A preliminary study. *Schizophr. Res.* 86, 172–180. doi: 10.1016/j.schres.2006.06.012

Sanders, R. D., Keshavan, M. S., Forman, S. D., Pieri, J. N., McLaughlin, N., Allen, D. N., et al. (2000). Factor structure of neurological examination abnormalities in unmedicated schizophrenia. *Psychiatry Res.* 95, 237–243. doi: 10.1016/S0165-1781(00)00176-1

Sanders, R. D., Schuepbach, D., Goldstein, G., Haas, G. L., Sweeney, J. A., and Keshavan, M. S. (2004). Relationships between cognitive and neurological performance in neuroleptic-naïve psychosis. *J. Neuropsychiatry Clin. Neurosci.* 16, 480–487. doi: 10.1176/appi.neuropsych.16.4.480

Schulz, J. B., Dehmer, T., Schols, L., Mende, H., Hardt, C., Vorgerd, M., et al. (2000). Oxidative stress in patients with Friedreich ataxia. *Neurology* 55, 1719–1721. doi: 10.1212/WNL.55.11.1719

Scott, G. S., Spitsin, S. V., Kean, R. B., Mikheeva, T., Koprowski, H., and Hooper, D. C. (2002). Therapeutic intervention in experimental allergic encephalomyelitis by administration of uric acid precursors. *Proc. Natl. Acad. Sci. U.S.A.* 99, 16303–16308. doi: 10.1073/pnas.212645999

Scriver, C. R., Beaudet, A. L., Sly, W. S., and Valle, D. (1989). *The Metabolic Bases of Inherited Disease*, 6th Edn. New York: McGraw-Hill.

Skosnik, P. D., and Yao, J. K. (2003). From phospholipid and fatty acid defects to altered neurotransmission: Is arachidonic acid a nexus in the pathophysiology of schizophrenia? *Prostaglandins Leukot. Essent. Fatty Acids* 69, 367–384.

Spitsin, S., Hooper, D. C., Leist, T., Streletz, L. J., Mikheeva, T., and Koprowskil, H. (2001). Inactivation of peroxynitrite in multiple sclerosis patients after oral administration of inosine may suggest possible approaches to therapy of the disease. *Mult. Scler.* 7, 313–319. doi: 10.1177/135245850100700507

Spychala, J., and Mitchell, B. S. (2002). Cyclosporin A and FK506 decrease adenosine kinase activity and adenosine uptake in T-lymphocytes. *J. Lab. Clin. Med.* 140, 84–91. doi: 10.1016/S0022-2143(02)00022-7

Strazzullo, P., and Puig, J. G. (2007). Uric acid and oxidative stress: relative impact on cardiovascular risk? *Nutr. Metab. Cardiovasc. Dis.* 17, 409–414. doi: 10.1016/j.numecd.2007.02.011

Sulakhe, P. V., and Phillis, J. W. (1975). The release of 3H-adenosine and its derivatives from cat sensorimotor cortex. *Life Sci.* 17, 551–555. doi: 10.1016/0024-3205(75)90089-2

Tandon, R., Nasrallah, H. A., and Keshavan, M. S. (2009). Schizophrenia, "just the facts" 4. Clinical features and conceptualization. *Schizophr. Res.* 110, 1–23. doi: 10.1016/j.schres.2009.03.005

Toncev, G., Milicic, B., Toncev, S., and Samardzic, G. (2002). Serum uric acid levels in multiple sclerosis patients correlate with activity of disease and blood–brain barrier dysfunction. *Eur. J. Neurol.* 9, 221–226. doi: 10.1046/j.1468-1331.2002.00384.x

van der Veen, R. C., Hinton, D. R., Incardonna, F., and Hofman, F. M. (1997). Extensive peroxynitrite activity during progressive stages of central nervous system inflammation. *J. Neuroimmunol.* 77, 1–7. doi: 10.1016/S0165-5728(97)00013-1

Virit, O., Altindag, A., Yumru, M., Dalkilic, A., Savas, H. A., Selek, S., et al. (2009). A defect in the antioxidant defense system in schizophrenia. *Neuropsychobiology* 60, 87–93. doi: 10.1159/000239684

Waring, W. S., Webb, D. J., and Maxwell, S. R. (2000). Uric acid as a risk factor for cardiovascular disease. *Q. J. Med.* 93, 707–713. doi: 10.1093/qjmed/93.11.707

Wong, M.-L., and Licinio, J. (2005). Peripheral is central to the question. *Mol. Psychiatry* 10, 421–422. doi: 10.1038/sj.mp.4001674

Yao, J. K., and Cheng, P. (2004). Determination of multiple redox-active compounds by high-performance liquid chromatography with coulometric multi-electrode array system. *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.* 810, 93–100. doi: 10.1016/j.jchromb.2004.07.021

Yao, J. K., Condray, R., Dougherty, G. G., Keshavan, M. S., Montrose, D. M., Matson, W. R., et al. (2012). Associations between purine metabolites and clinical symptoms in schizophrenia. *PLoS ONE* 7:e42165. doi: 10.1371/journal.pone.0042165.

Yao, J. K., Dougherty, G. G., Reddy, R. D., Keshavan, M. S., Montrose, D. M., Matson, W. R., et al. (2010a). Altered interactions of tryptophan metabolites in first-episode neuroleptic-naïve patients with schizophrenia. *Mol. Psychiatry* 15, 938–953. doi: 10.1038/mp.2009.33

Yao, J. K., Dougherty, G. G., Reddy, R. D., Keshavan, M. S., Montrose, D. M., Matson, W. R., et al. (2010b). Homeostatic imbalance of purine catabolism in first-episode neuroleptic-naïve patients with schizophrenia. *PLoS ONE* 5:e9508. doi: 10.1371/journal.pone.0009508

Yao, J. K., and Keshavan, M. S. (2011). Antioxidants, redox signaling, and pathophysiology in schizophrenia: an integrative view. *Antioxid. Redox Signal.* 15, 2011–2035. doi: 10.1089/ars.2010.3603

Yao, J. K., Leonard, S., and Reddy, R. D. (2004a). Increased nitric oxide radicals in postmortem brains from schizophrenic patients. *Schizophr. Bull.* 30, 923–934. doi: 10.1093/oxfordjournals.schbul.a007142

Yao, J. K., Magan, S., Sonel, A. F., Gurklis, J. A., Sanders, R., and Reddy, R. D. (2004b). Effects of omega-3 fatty acid on platelet serotonin responsivity in patients with schizophrenia. *Prostaglandins Leukot. Essent. Fatty Acids* 71, 171–176. doi: 10.1016/j.plefa.2004.03.011

Yao, J. K., Leonard, S., and Reddy, R. D. (2006). Altered glutathione redox state in schizophrenia. *Dis. Markers* 22, 83–93.

Yao, J. K., Reddy, R., and van Kammen, D. P. (1998). Reduced level of plasma antioxidant uric acid in schizophrenia. *Psychiatry Res.* 80, 29–39. doi: 10.1016/S0165-1781(98)00051-1

Yao, J. K., Reddy, R. D., and van Kammen, D. P. (2001). Oxidative damage and schizophrenia: an overview of the

evidence and its therapeutic implications. *CNS Drugs* 15, 287–310. doi: 10.2165/00023210-200115040-00004

Yao, J. K., Stanley, J. A., Reddy, R. D., Keshavan, M. S., and Pettegrew, J. W. (2002). Correlations between peripheral polyunsaturated fatty acid content and in vivo membrane phospholipid metabolites. *Biol. Psychiatry* 52, 823–830. doi: 10.1016/S0006-3223(02)01397-5

Yao, J. K., and van Kammen, D. P. (2004). Membrane phospholipids and cytokine interaction in schizophrenia. *Int. Rev. Neurobiol.* 59, 297–326. doi: 10.1016/S0074-7742(04)59012-8

Yao, J. K., van Kammen, D. P., Moss, H. B., and Sokulski, D. E. (1996). A decreased serotonergic responsivity in platelets of drug-free schizophrenic patients. *Psychiatry Res.* 63, 123–132. doi: 10.1016/0165-1781(96)02862-4

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Biomarker investigations related to pathophysiological pathways in schizophrenia and psychosis

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Post-mortem brain investigations of schizophrenia have generated swathes of data in the last few decades implicating candidate genes and protein. However, the relation of these findings to peripheral biomarker indicators and symptomatology remain to be elucidated. While biomarkers for disease do not have to be involved with underlying pathophysiology and may be largely indicative of diagnosis or prognosis, the ideal may be a biomarker that is involved in underlying disease processes and which is therefore more likely to change with progression of the illness as well as potentially being more responsive to treatment. One of the main difficulties in conducting biomarker investigations for major psychiatric disorders is the relative inconsistency in clinical diagnoses between disorders such as bipolar and schizophrenia. This has led some researchers to investigate biomarkers associated with core symptoms of these disorders, such as psychosis. The aim of this review is to evaluate the contribution of post-mortem brain investigations to elucidating the pathophysiology pathways involved in schizophrenia and psychosis, with an emphasis on major neurotransmitter systems that have been implicated. This data will then be compared to functional neuroimaging findings as well as findings from blood based gene expression investigations in schizophrenia in order to highlight the relative overlap in pathological processes between these different modalities used to elucidate pathogenesis of schizophrenia. In addition we will cover some recent and exciting findings demonstrating microRNA (miRNA) dysregulation in both the blood and the brain in patients with schizophrenia. These changes are pertinent to the topic due to their known role in post-transcriptional modification of gene expression with the potential to contribute or underlie gene expression changes observed in schizophrenia. Finally, we will discuss how post-mortem studies may aid future biomarker investigations.

Keywords: schizophrenia, psychosis, gene expression regulation, biomarkers, postmortem brain

INTRODUCTION

The official national institute of health (NIH) definition of a biomarker is, "a characteristic that is objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention" (Nih, 2001). Investigations of blood-based biomarkers for schizophrenia have great potential in discovering diagnostic or prognostic indicators of clinical utility and may provide clues to the underlying pathophysiology as the blood is a dynamic, sentinel tissue that is known to reflect immune and pathological status (Gladkevich et al., 2004). Data from post-mortem investigations of gene and protein expression in schizophrenia can act two-fold in order to aid blood-based biomarker discovery. They may act to drive blood-based biomarker investigations by the genes, proteins, or structures that have been previously implicated, or conversely, as a means of extending findings seen in blood-based biomarker investigations. While biomarkers for disease do not need to have pathophysiological roles and can be utilized to detect presence or absence of disease, the ideal scenario maybe the identification of biomarkers that are involved in etiological pathways and are therefore likely to change according to disease status as well as potentially being responsive to treatments. Over the last two decades numerous post-mortem neuropathological investigations have implicated a number of different proteins in the pathogenesis of major psychiatric disorders, including schizophrenia, bipolar disorder, and major depressive disorder. Although some of these findings have been corroborated independently, many of them remain controversial and conflict with follow-up investigations. Similarly, gene association studies have been plagued with inconsistencies, with regards to genes implicated in individual populations falling out in meta-analyses or large independent replication efforts failing to validate initial findings. The advent of microarray technology, coupled with the mapping of the human genome, has greatly aided the ability

to investigate larger numbers of genes simultaneously. While a number of these investigations have led to the generation of false-positive and -negative findings in terms of disease related gene expression changes some consistent trends are beginning to emerge.

In post-mortem gene expression investigations, alterations in the expression of transcripts encoding for oligodendrocyte functioning and myelination have been observed in the PFC in schizophrenia (Hakak et al., 2001; Tkachev et al., 2003, 2007; Aston et al., 2004, 2005; Kerns et al., 2010) and also in bipolar and major depressive disorder (Tkachev et al., 2003; Aston et al., 2005; Sun et al., 2006; Kim and Webster, 2011). These include genes encoding for major myelin components such as myelin basic protein (MBP) as well as myelin oligodendrocyte basic protein (MOBP). Such genes have been postulated to be involved in higher cognitive functions (Nave, 2010), functions which are compromised in patients with schizophrenia. While the functions of genes such as MOBP are not fully understood they are likely to have a significant role in the compaction of myelin sheaths, thereby aiding brain connectivity and signaling. Post-mortem schizophrenia investigations have also revealed decreased myelin thickness (Flynn et al., 2003) and damaged myelin sheaths (Uranova et al., 2011). In addition, recent diffusor tensor imaging (DTI) studies have demonstrated improvement in myelin integrity upon exposure to anti-psychotics in treatment responsive patients (Garver et al., 2008; Bartzokis et al., 2012), suggesting an alternative mechanism of action that may provide new targets for future development (Walterfang et al., 2011; Ren et al., 2013).

Genes related to metabolism and mitochondrial pathways are another ontological group that have been found by a number of studies to be reduced in expression in the PFC in schizophrenia (Pongrac et al., 2002; Tkachev et al., 2003; Prabakaran et al., 2004; Iwamoto et al., 2005; Konradi, 2005) and by some in bipolar disorder (Iwamoto et al., 2005). While the association of these genes with the disorders themselves may be evidenced, their mechanism of action in causing symptoms associated with the disorders remains to be elucidated. However, some studies have demonstrated that changes in gene expression are also a function of duration of illness which could be related to changing symptom profile that occurs with progression of illness (Narayan et al., 2008; Tang et al., 2009, 2012). Teasing out this etiological component is confounded by major psychiatric disorders having overlapping symptomatology, and in the case of schizophrenia and bipolar disorder sometimes leading to differential diagnosis. This problem, has prompted some researchers to look at key characteristic such as psychosis that provide a more tangible target to assess similarities and differences in etiology between disorders. This review will begin with an overview of the main post-mortem findings contributing to our understanding of the etiology and pathophysiology of schizophrenia and psychosis. Following this, current literature relating to blood-based gene expression studies in patients with schizophrenia and how they relate to post-mortem data will be discussed as well as the future direction of post-mortem investigations to validate blood-based biomarker findings. In relation to regulation of gene expression we will also discuss recent interesting findings of microRNA (miRNA) dysregulation in schizophrenia.

POST-MORTEM DATA ELUCIDATING PATHOPHYSIOLOGY PATHWAYS IN SCHIZOPHRENIA

DOPAMINERGIC SYSTEM

Numerous post-mortem investigations in schizophrenia have implicated several neurotransmitter systems in having a pathophysiological role. The first of these centered on the dopaminergic system, primarily driven by the fact that neuroleptic medications had a high affinity for D2 receptors which correlated with their therapeutic effect at alleviating positive symptoms (Meltzer and Stahl, 1976). While this is supported by the observation that drugs such as amphetamine, cocaine and methamphetamine (METH) all increase dopaminergic transmission and are capable of inducing psychosis (Janowsky and Risch, 1979; Chen et al., 2003), other drugs such lysergic acid diethylamide (LSD), ketamine and phencyclidine (PCP) that also have the ability to produce psychosis do so without pronounced effects on the dopaminergic system. While early studies reported increased D2 receptor density in patients with schizophrenia vs. controls, these investigations did not control for the patients receiving neuroleptic medication and subsequent studies controlling for this confound (Reynolds et al., 1981; MacKay et al., 1982; Kornhuber et al., 1989) or having appropriately designed methodology (Dean et al., 1997) failed to detect differences. Post-mortem investigations have also found evidence for dysregulation in the gene for catechol-o-methyl transferase (*COMT*), encoding for the enzyme that degrades dopamine. These investigations have found that *COMT* gene expression is inversely correlated with gene expression of regulator of g-protein signalling-4 (*RGS4*) (Lipska et al., 2006), with substitutions at Val158Met in *COMT* significantly reducing the activity of COMT (Chen et al., 2004). These investigations are in keeping with a substantial gene association literature implicating polymorphism in COMT to schizophrenia susceptibility (Shifman et al., 2002; Tunbridge et al., 2006). However, as for many genes in the schizophrenia literature meta-analytic review of gene association investigations implicating *COMT* have shown that the findings of association do not hold up when comparing across studies (Glatt et al., 2003).

CHOLINERGIC SYSTEM

The cholinergic muscarinic receptors (CHRM) have been implicated in the pathophysiology of schizophrenia as well as mood disorders, where psychosis can present as part of the symptomatology (Crook et al., 2000; Gibbons et al., 2009). In schizophrenia, post-mortem studies of the cortex point to the involvement of CHRM1, with the recent identification of a sub-type of schizophrenia with widespread loss of cortical and sub-cortical CHRM1 protein expression (Scarr et al., 2009; Gibbons et al., 2013). These post-mortem studies have been supported by a neuroimaging study demonstrating a reduction in muscarinic receptor availability in unmedicated patients with schizophrenia (Raedler et al., 2003) Whereas, post-mortem, neuroimaging, and genetic association studies implicate CHRM2 in the pathophysiology of mood disorders (Wang et al., 2004; Gibbons et al., 2009). Interestingly, elucidating the role of the cholinergic system in the etiology of psychosis has been aided by studies into Alzheimer's disease where the CHRM1/CHRM4 partial agonist Xanomeline can reduce psychotic symptoms (Bymaster et al., 1997; Shekhar

et al., 2008). The cholinergic system has also been implicated in acute stress with inhibitors of the acetylcholine-hydrolyzing enzyme acetylcholinesterase (AChE) producing symptoms of depression and cognitive decline in mice via potentiation of acetylcholine signaling (Kaufer et al., 1998). In addition, a recent study in mice found that anxiety and depression behaviors elicited through administration of physostigmine, an inhibitor of AChE could be reversed with administration of nicotinic and muscarinic receptor antagonists (Mineur et al., 2013). These studies point to the potential of drugs that target the cholinergic system in alleviating some of the negative symptoms associated with schizophrenia, something that is poorly addressed by current antipsychotic medications.

Murine knockout models have shown that the antipsychotic effects of Xanomeline are predominantly mediated via CHRM4, which in turn modulates dopaminergic activity (Woolley et al., 2009). Post-mortem studies have also reported reduced binding of the radioligand [^{3}H]4-DAMP, under CHRM1/CHRM3/CHRM4/CHRM5 selective assay conditions, in the orbitofrontal cortex from subjects with Alzheimer's disease who had significant psychotic symptoms (Tsang et al., 2008). Conversely, elevated levels of the cortical autoreceptor CHRM2 (Zhang et al., 2002) are associated with behavioral disturbances in both Alzheimer's disease and Lewy body dementia (Lai et al., 2001; Teaktong et al., 2005) pointing to a hypocholinergic state associated with psychosis. In schizophrenia, subjects with deficits in CHRM1 protein expression also show a reduction in cortical and subcortical binding of [^{3}H]AF-DX 384, a CHRM2/CHRM4 selective radioligand (Gibbons et al., 2013). The reduction in hippocampal CHRM4 mRNA levels in schizophrenia suggests this loss of binding reflects a loss of CHRM4 protein expression (Scarr et al., 2007). Within the cortex, [^{3}H]AF-DX 384 binding is reported to be unchanged in the ACC in schizophrenia. However, there is contrasting data relating to the role of CHRM4 in schizophrenia (Crook et al., 1999; Zavitsanou et al., 2005; Gibbons et al., 2013). Thus, cholinergic involvement in psychosis may be specific to a sub-type within schizophrenia. Importantly, a SPECT study has reported reduced binding densities of the broadly selective CHRM radioligand [^{123}I]IQNB in patients with schizophrenia (Raedler et al., 2003) raising the possibility of clinical identification of individuals within this subtype using neuroimaging.

GLUTAMATERGIC SYSTEM

A role for glutamate, the primary excitatory neurotransmitter in the brain, has long been evidenced from observations that drugs such as ketamine and phencyclidine (PCP) that primarily block iontropic n-methyl-d-aspartate receptors (NMDARs) and are capable of causing negative and positive symptoms that resemble those seen in psychotic disorders such as schizophrenia (Luby et al., 1962; Vollenweider and Geyer, 2001; Pomarol-Clotet et al., 2006). This observation led to the NMDA hypofunction hypothesis of schizophrenia (Olney and Farber, 1995) with the involvement of the glutamatergic system in psychosis stemming from neuroimaging, genetic, and post-mortem investigations. In support of a glutamatergic dysfunction in schizophrenia, kainate receptor expression is decreased in the dlPFC in schizophrenia (Scarr et al., 2005), while hippocampal expression of the NR1 and NR2B subunits of the NMDA receptor are increased and decreased, respectively (Gao et al., 2000). Other studies have shown that NMDARs are likely involved with schizophrenia including early studies demonstrating that glutamate carboxypeptidase II (GCPII) that catabolizes N-Acetylaspartylglutamic acid (NAAG) to NAA and glutamate was reduced in the frontal cortex, hippocampus and temporal lobe in patients with schizophrenia vs. controls (Tsai et al., 1995) as well as more recent evidence demonstrating that GAD67 neurons co-expressing NR2A are reduced in the prefrontal cortex in patients with schizophrenia (Woo et al., 2004, 2008). Increased mRNA and protein levels of the glutamate transporters EAAT1, EAAT3, and EAAT4 have also been reported in schizophrenia (Rao et al., 2012). Furthermore, many of the genes that have consistently been associated with schizophrenia, such as *NRG1*, *RGS4*, *DTNBP1*, and *DAAO* are involved in regulation of glutamatergic neurotransmission, with the antipsychotic clozapine being shown to increase levels of *NRG1* as well as vesicle-associated membrane protein-1 *(VAMP1)* in human primary cultures exposed at steady state plasma levels (Chana et al., 2009), indicating that *NRG1* may be a possible therapeutic target. Indeed NRG1 signaling through ErbB4 receptors have the capacity to determine NMDA efficacy, with NMDA receptor activity playing a key role in the development and stability of synaptic spines (Bennett, 2009), with stress responses and activation of the glucocorticoid receptor system also causing synaptic regression (Bennett, 2008).

With regards to modulation of NMDA receptors and the NMDA hypofunctioning hypothesis of schizophrenia, DAAO is of particular interest due to its metabolism of d-serine, a known allosteric modulator and necessary co-agonist for NMDA receptors (Oliet and Mothet, 2009). DAAO levels have been found to be elevated in both the brain and cerebrospinal fluid (CSF) of patients with schizophrenia (Bendikov et al., 2007; Madeira et al., 2008; Habl et al., 2009). This in turn results in a reduction in the breakdown of d-serine and could facilitate NMDA hypofunctioning. In relation to psychosis, *DAOA* has recently been linked to progression to a first episode of psychosis in prodromal subjects (Mossner et al., 2010). Another two important proteins that support such a hypothesis are d-serine racemase, a predominantly astrocytic enzyme that converts l-serine to d-, and d-amino-acid oxidase activator (DAOA) that as it name suggests may be responsible for the activation of DAAO and hence subsequent breakdown of d-serine. However, while one would expect that serine racemase would be reduced in a scenario of NMDA hypofunctioning i.e., reduced d-serine production and therefore reduced NMDA activation, increased serine racemase has been reported within the frontal cortex in a post-mortem study of schizophrenic subjects (Verrall et al., 2007) as well as decreased (Bendikov et al., 2007). In addition, an earlier study reported no change in the cortex but an increase in expression within the hippocampus (Steffek et al., 2006). Therefore, as a correlate for NMDA hypofunctioning further characterization is required in independent brain cohorts before a firm conclusion can be reached. The evidence for DAOAs involvement in psychosis has been growing with several genetic studies finding an association between polymorphisms in the

G72 gene which encodes DAOA, and the development of psychosis (Addington et al., 2004; Craddock et al., 2005; Mossner et al., 2010) as well as severity of positive symptoms (Chiesa et al., 2011). In addition, a recent investigation demonstrated that polymorphisms within the *G72* gene were associated with a vulnerability to methamphetamine psychosis (Kotaka et al., 2009). While the data from the study may seem damning to aspirations of DAOA as a susceptibility gene for psychosis, the authors for this study did not differentiate between brain regions other than the amygdala and the caudate nucleus. Therefore, it was unclear whether key brain regions such as the PFC were included and in what proportions. Furthermore, there is also a need for separation of cortical and subcortical matter due to potential differences in expression that may exist between them.

GABAergic SYSTEM

Gamma-amino-butyric-acid (GABA) has been implicated for a number of years in schizophrenia following a number of post-mortem investigations demonstrating its dysregulation within the brain. The most consistent of these changes and indeed one of the most consistent post-mortem neuropathological changes observed for schizophrenia were reductions in GABAergic markers such as glutamic acid decarboxylase-67 (GAD-67), the synthesizing enzyme for GABA, within the PFC (Akbarian et al., 1995; Guidotti et al., 2000; Volk et al., 2000; Veldic et al., 2005; Akbarian and Huang, 2006) as well as the temporal cortex (Impagnatiello et al., 1998; Heckers et al., 2002; Akbarian and Huang, 2006) and hippocampus (Thompson et al., 2011). In relation to these studies, and as a potential link for GABA's involvement in psychosis, Veldic et al. (2005) demonstrated a decreased number of neurons expressing mRNA for GAD-67 within the PFC of brains from schizophrenia and bipolar subjects with a history of psychosis with a concomitant increase in cortical DNA-methyltransferase I (DNMT1), an enzyme preferentially expressed by interneurons, causing a possible down-regulation of promoter functioning within these cells (Veldic et al., 2005). Reductions in GABAergic functioning have also been evidenced by reduced expression of a number of the subunits making up the GABAA receptor by (Charych et al., 2009). Furthermore, while GAD-65 and GAD-67 mRNA expression has been shown to be either reduced or unchanged within the layers of the hippocampus in subjects with schizophrenia (Benes et al., 2007), increases in GAD-65/67 immunoreactive neuronal density has been reported in the subiculum and parahippocampal gyrus from chronically medicated cases (Schreiber et al., 2011); suggesting that increasing GABA synthesis may be a function of antipsychotics.

SEROTONINERGIC SYSTEM

While blockade of D2 receptors would seem to be a requirement for both typical and atypical antipsychotic medications in alleviating positive symptoms, atypicals such as clozapine are also known to have a high affinity for serotonergic receptors, in particular $5HT_{2A}$ receptors (2ARs) (Meltzer, 1992). This observation has led some researchers to elucidate a possible role for serotonin in both the positive and negative aspects of schizophrenia. While many post-mortem studies have demonstrated decreases in 2ARs (Burnet et al., 1996; Dean et al., 1996; Kouzmenko et al., 1997) a recent study by Gonzalez-Maeso et al. (2008) demonstrated an upregulation of 2ARs, and a concomitant decrease in mGLUR2 within the PFC of untreated patients who had schizophrenia. (Gonzalez-Maeso et al., 2008). One important aspect of this study that distinguishes it from previous post-mortem investigations of 2AR was that the brains of their subjects who had schizophrenia were anti-psychotic naïve and therefore changes in expression could not be explained medication effects. Indeed in order to better understand the discrepancy between their findings and others the authors investigated 2AR expression changes associated with treating mice with the atypical antipsychotic clozapine and typical antipsychotic haloperidol. They found that clozapine downregulated 2AR expression in the somatosensory cortex while this effect was not seen for haloperidol. While the potential cross-talk between mGLUR2 and 2AR may have been demonstrated within this investigation and is of interest, a major oversight by the authors was in not commenting on the impact of a high number of suicide victims in their schizophrenia cohort, with 87.5% of all cases dying by this mode, compared to a rate of 4.9% estimated in a recent re-examination of rates within the schizophrenia population (Palmer et al., 2005). This is of importance given that 2ARs have been demonstrated to be elevated within the frontal cortex of victims of suicide (Stanley and Mann, 1983; Arranz et al., 1994; Turecki et al., 1999), which in turn could potentially explain the elevated 2ARs seen in their study. However, the proposed role for 2AR in psychotic illness is concordant with findings in Parkinson's disease where binding the 2AR radioligand [^{3}H] ketanserin is increased in cases with a history of visual hallucinations (Huot et al., 2010). While evidence has been mounting for the independent contributions of each of the aforementioned neurotransmitter systems to the development of psychosis the coordination or dysregulation between neurotransmitters is a more logical likely causation of psychosis. This interactive approach when viewing neurochemical abnormalities in schizophrenia and psychosis has come about in part due to our understanding of individual neurotransmitter systems and how they intermingle to regulate the action of one another. It has also come about following a realization that psychosis-related disorders are too complex for their pathophysiology to be underpinned by a single neurotransmitter.

FUNCTIONAL NEURO-IMAGING DATA ELUCIDATING PATHOPHYSIOLOGY PATHWAYS IN SCHIZOPHRENIA

Over the last decade the power of functional neuroimaging studies to characterize pathophysiological pathways in schizophrenia has greatly increased, especially in the advancement of studies using positron emission tomography (PET) and single-photon emission computed tomography (SPECT) that are techniques allowing the visualization in real-time of changes in receptor densities related to neurotransmitter and signaling systems. Importantly, this has allowed the validation of post-mortem findings as well as expansion of implicated pathways for schizophrenia.

DOPAMINERGIC SYSTEM

Probably the most well-investigated neurotransmitter system using these imaging modalities has been the dopaminergic

system. As a main molecular target for antipsychotic medications, dopamine, and D2 receptor binding has been studied by a number of investigations utilizing nuclear magnetic spectroscopy (NMS) as well as PET and SPECT, with the caudate nucleus being the brain area most heavily investigated due to it's high D2 receptor density. While some investigations utilizing NMS have reported increased binding in drug naïve and late onset schizophrenia patients (Tune et al., 1993, 1996; Pearlson et al., 1995) others have failed to detect such changes (Hietala et al., 1994; Nordstrom et al., 1995). This lack of congruence amongst studies may represent differing methodologies and pharmacology of radioligands used (Dean, 2012), but may also be indicative of a similar lack of consistency for D2 receptor density and occupancy in post-mortem brain tissue. Potential confounders of these investigations are age and duration of illness, with post-mortem studies showing that D2 occupancy decreases with age (Rinne et al., 1993; Dean et al., 1997) and more recent evidence demonstrating that gene expression of D2 changes according to increasing duration of illness (Dean et al., 2007). Similar controversy has surrounded investigations in schizophrenia that assessed levels of dopamine-D1 like receptors, with decreases reported in a number of different brain regions (Okubo et al., 1997; Kosaka et al., 2010) in contrast shown to be increased by others (Abi-Dargham et al., 2012). In addition, post-mortem studies also reflect variability in findings related to D1 receptor levels (Seeman et al., 1987; Knable et al., 1994; Dean, 2012). A major confounding variable of these studies is likely related to the variation in medication histories between individuals. As for dopamine receptors, PET and SPECT studies assessing levels of dopamine transporter (DAT) have also produced inconsistency in findings, with some investigations reporting decreased DAT levels (Laakso et al., 2001; Mateos et al., 2007) but with the majority of investigations reporting no differences (Kuepper et al., 2012). Therefore, the change in DAT levels, if present in schizophrenia, are likely to be of a small magnitude. While dopamine receptor and transporter studies have failed to show consistent changes in patients with schizophrenia, investigations aimed at assessing dopamine synthesis and release have provided evidence for neurotransmitter changes in the striatum. Using [18F]flurodopamine a number of studies have demonstrated increased uptake of this radioligand in the striatum in patients with schizophrenia (Hietala et al., 1995, 1999; Laruelle et al., 1996). These findings are also consistent with amphetamine-induced dopamine release being significantly increased in the striatum of patients with schizophrenia using the ligand [123]iodobenzamide (Laruelle et al., 1996) and while it could be argued that these changes may be related to medication effects, increased dopamine release has also been observed in drug-naïve and drug free patients (Abi-Dargham et al., 1998, 2009). These findings warrant further investigation in additional cohorts.

CHOLINERGIC SYSTEM

While post-mortem investigations of the cholinergic system have revealed evidence for decreases in M1 receptors in schizophrenia (Scarr et al., 2009; Gibbons et al., 2013), functional neuroimaging investigations of the cholinergic system have been less numerous. Nevertheless, a study using the ligand [^{123}]iodoquinclidinyl benzilate ([123I]IQNB) reported widespread reductions in muscarinic receptors in the brains of drug free schizophrenia patients(Raedler et al., 2003). Given that reductions in muscarinic receptors have also been observed in bipolar disorder and major depressive disorder (Gibbons et al., 2009) the investigation of the muscarinic system as a common system affected in major psychiatric disorders may be warranted.

GLUTAMATERGIC SYSTEM

With ketamine and PCP capable of causing psychosis through NMDA receptor blockade the limited number of functional imaging studies for glutamate in schizophrenia have focused on characterizing potential changes in NMDA signaling. These studies have shown that there is no change in the thalamus and striatum using the ligand [^{123}] CNS 1261 (Bressan et al., 2003), with the same group showing that there was a decrease in NMDA receptors in the left hippocampus (Pilowsky et al., 2006). With growing evidence that the glutamatergic system plays an important role in the etiology of schizophrenia, including the role of metabotropic glutamate receptors and their cross-talk with NMDA receptors, further functional neuroimaging investigations generating ligands for other glutamatergic targets is a likely outcome within the next decade.

GABAergic SYSTEM

While GABAergic dysfunction in schizophrenia has been elucidated by a number of post-mortem investigations demonstrating alterations in interneuronal populations in the frontal and cingulate cortices, imaging studies have been limited with regards to assessing functional changes in GABA transmission. Investigations that have looked at $GABA_A$ receptors have found that there are no changes in levels of this receptor in the brain in patients with schizophrenia (Busatto et al., 1997; Abi-Dargham et al., 1999; Verhoeff et al., 1999). In addition, a PET investigation that investigated levels of the central benzodiazepine receptor (cBZr) located on the $GABA_A$ receptor in patients with anxiety and controls found no differences (Abadie et al., 1999).

SEROTONERGIC SYSTEM

The focus of functional neuroimaging studies in relations to the serotonergic system has been on the 5HT2AR receptors, due to clozapine, as the first of the second generation antipsychotic medications demonstrating a high affinity at 2ARs (Meltzer, 1992). Interestingly, as for the post-mortem studies, decreased 2ARs have also been observed by PET studies (Ngan et al., 2000; Rasmussen et al., 2010), with the latter of these studies being carried out in drug-naïve subjects. Decreased 2AR density has additionally also been seen in individuals at risk of developing mental illness (Hurlemann et al., 2008). Contrary to these investigations, however, a study conducted in first-episode neuroleptic naïve subjects found a 40% increase in 2ARs (Erritzoe et al., 2008). This controversy in functional neuroimaging studies mirrors that for post-mortem brain tissue and also reflects findings above for other neurotransmitter systems. With an increase in superior ligands for neurotransmitter receptors in the coming years, a consensus on *in vivo* receptor changes and how they relate to post-mortem findings will be important in order for

us to better understand longitudinal changes in these receptor populations according to disease.

BLOOD BASED GENE EXPRESSION INVESTIGATIONS IN SCHIZOPHRENIA

For psychotic and brain disorders the CSF represents the most obvious choice as a tissue source for detecting biomarkers in living individuals. However, as obtaining CSF samples is a substantially more invasive procedure than simply drawing blood, a rapidly growing number of investigations have focused on the use of blood to search for biological indicators of psychosis and schizophrenia. Early blood based biomarker investigations found evidence for alterations in neurotransmitter expression within blood platelets from patients with schizophrenia. These studies included a positive correlation between dopamine uptake from platelets from patients with schizophrenia and delusional state (Tateno et al., 2013) as well as increases in platelet 5HT-2 receptors that is increased further following treatment with fluphenazine and trifluoperazine (Amato et al., 2011). A more recent study has demonstrated that loxapine treatment downregulates both D2-like and 5HT-2A in platelets from patients with schizophrenia (Nord and Farde, 2011), with the latter finding being contradictory to that by Pandey et al. Further evidence indicating that platelets of patients with schizophrenia may provide a suitable tissue for a source of biomarker discovery has come from another recent study demonstrating that platelets derived from patients with schizophrenia demonstrated hypoglutamatergic release of Ca^{2+} (Miyake et al., 2011). Further characterization work is needed in order to uncover what other neurotransmitter receptors these cells express.

A number of recent blood-based expression studies have utilized lymphoblastoid cells cultured from patients with schizophrenia and bipolar disorder. The earliest one of these studies was that by Kakiuchi et al. (2003) in which a reduction in expression of X-box binding protein 1 (*XBP1*) in a pair of twins discordant for bipolar disorder vs. a control twin pair was reported. XBP1 is a pivotal gene in the endoplasmic reticulum (ER) stress response and therefore is of interest given elevation of stress response molecules such as cortisol in patients with bipolar disorder. In addition, they found that a polymorphism substitution at position 116 (C—G) in the promoter region of *XBP1* lymphoblasts derived from Japanese patients with bipolar disorder conferred a reduced ER stress response that was rescued by treatment with the mood stabilizer valproate (Kakiuchi et al., 2003). More recently, they extended their findings by demonstrating that lithium treatment also was more effective in patients with the 116C allele as opposed to patients homozygous for 116G (Kakiuchi et al., 2006). While this finding directly relates the codon substitution to a known mood-stabilizing agent, validation of this interesting finding in a separate larger independent patient cohort is warranted. In the largest microarray study of lymphoblastoid cells so far by Vawter et al. (2004) it was found that the expression of neuropeptide Y (*NPY*) and the gene that encodes for Guanine nucleotide-binding protein G(o) subunit alpha (*GNA01)* were reduced in schizophrenia and there was an increase in the mitochondrial-related gene malate dehydrogenase 1, NAD (*MDH1*) (Vawter et al., 2004). Changes in NPY have been observed by some post-mortem investigations in schizophrenia and bipolar disorder and are therefore of interest (Frederiksen et al., 1991; Iritani et al., 2000; Kuromitsu et al., 2001). It should be noted that even for this study sample numbers were relatively small with only 5 patients with schizophrenia and 9 controls. Moreover, studies that used lymphoblastoid cell lines *utilized* Epstein Barr virus (EBV) to transform PBMCs, an effect which in itself could lead to changes in gene expression.

Peripheral blood microarray findings have implicated sensory and motor neuron derived factor (*SMDF*), a splice variant of *NRG1*, in patients with schizophrenia (Petryshen et al., 2005). In addition, decreased gene expression of *NRG1* and dystrobrevin binding protein 1 (*DTNBP1*) were shown to be lower in immortalized lymphocytes from patients with schizophrenia vs. controls before and after treatment with the antipsychotic olanzapine (Chagnon et al., 2008). However, a follow-up study failed to report changes in *NRG1*, *DTNBP1*, or *GNAO1* in patients with schizophrenia vs. controls (Yamamori et al., 2011). Reduced expression of *NRG1* has also been observed in PBMCs from first-onset schizophrenia patients compared to controls, which normalized following treatment with antipsychotic medications (Zhang et al., 2008). Interestingly, another microarray gene expression investigation using whole blood from patients with schizophrenia and assessing gene expression changes related to positive symptomatology, found that *NRG1* was increased relative to higher delusional states (Kurian et al., 2011). This contradictory finding may be a consequence of a lack of control group for the latter investigation or may be related to the separation of symptomatology that was carried out. This finding for *NRG1* reflects the genetic association studies that have found a susceptibility for schizophrenia as well as recent evidence for reduced *NRG1* levels in the PFC of brains of patients with schizophrenia and unipolar depression (Bertram et al., 2007). While some studies have failed to demonstrate association of *NRG1* to schizophrenia, (Thiselton et al., 2004; Duan et al., 2005; Rosa et al., 2007) recent meta-analyses have supported a relationship (Li et al., 2006; Munafo et al., 2006). Impaired *NRG1* migration of B-lymphocytes isolated from patients with schizophrenia vs. controls has also been demonstrated that was associated with polymorphisms in *NRG1* and the gene for catechol-o-methyl-transferase [*COMT* (Sei et al., 2007)], an enzyme required for degradation of neurotransmitters including dopamine and that has also been heavily implicated in schizophrenia via association studies (Williams et al., 2007). However, it is important to mention that findings for *COMT* have been relatively inconsistent and a recent meta-analysis failed to find an association with *COMT* and susceptibility to schizophrenia (Okochi et al., 2009). Of relation to biomarkers of dopaminergic signaling, another investigation also demonstrated that the number of leukocytes expressing dopamine and cyclic adenosine 3″:5″-monophosphate regulated phosphoprotein of relative molecular mass 32,000 (DARPP-32) was reduced within PBMC sub-populations in schizophrenia and bipolar disorder (Williams et al., 2007; Torres et al., 2009).

In a comparative blood and brain gene expression study we demonstrated that the gene for selenium binding protein-1 (*SELENBP1*) was upregulated in both compartments in

schizophrenia and was validated in the blood using qRT-PCR and immunohistochemistry (Glatt et al., 2005). In a follow-up qRT-PCR study looking at a separate brain cohort, our group also observed increased *SELENBP1* expression in the dlPFC of patients with schizophrenia (Kanazawa et al., 2008). Interestingly, the expression of *SELENBP1* was significantly correlated with psychosis in a combined sample of schizophrenia and bipolar disorder patients regardless of their diagnosis. In contrast to these findings, a study of 30 patients first hospitalized for a psychotic episode with symptoms consistent with schizophrenia ($n = 15$), schizophreniform disorder ($n = 13$) or schizoaffective disorder ($n = 2$) investigated levels of *SELENBP1* in PBCs after commencement of treatment. Using qRT-PCR, *SELENBP1* was compared between patients with a psychotic disorder and controls; there was no significant difference between the groups (Yao et al., 2008). Nevertheless, a recent study investigated the expression of *SELENBP1* in BA46 from 13 subjects with major depressive disorder (MDD), 11 subjects with MDD and psychotic features and 12 controls (Teyssier et al., 2011). There was no significant difference in the expression of *SELENBP1* between subjects with psychotic features and controls, or subjects with a depressive disorder and controls (Teyssier et al., 2011). The authors argued that results demonstrate that *SELENBP1* expression is not a marker of psychosis; however, the sample size was small and may not have sufficient power to detect a difference in the expression of *SELENBP1*. *SELENBP1* is localized to chromosome 1q21, which has previously been identified as a susceptibility locus for schizophrenia (Brzustowicz et al., 2000), which indicates that the upregulation of *SELENBP1* in psychotic disorders may be involved in the susceptibility to psychosis. While increased expression of *SELENBP1* in the dlPFC in schizophrenia may be present, the functional relevance of this upregulation remains to be elucidated. One potential mechanism may be related to its potential role as a neurogenic factor evidenced by two investigations demonstrating its ability to promote neurite outgrowth; in the rat cerebral cortex (Zhao et al., 2000) as well as co-localization with actin in growing tips of human neuroblastoma SH-SY5Y cells (Miyaguchi, 2004). Given that reductions in synaptic and dendritic arbors have been observed in the brains of patients with schizophrenia, elevated *SELENBP1* may play a compensatory role in restoring neuronal connectivity and functioning. Further work to better define this tentative mechanism is being carried out. SELENBP1 has also been implicated in ubiquitination from a study demonstrating the binding of SELENBP1 to von Hippel- Lindau protein (pVHL)- interacting deubiquitinating enzyme 1 (VDU1) that was abolished with the incubation of β-mercaptoethanol, which dissociates selenium (Jeong et al., 2009), suggesting a selenium dependent interaction of SELENBP1 and VDU1.

Another recent blood based finding has been a reported increase in lipid biosynthesis in schizophrenia patients treated with olanzapine vs. untreated patients (Vik-Mo et al., 2008). In particular the authors found an increase in gene expression of fatty acid synthase (FASN) and stearoyl-CoA desaturase (SCD) potentially implicating these genes in adverse side effects such as weight gain. Calcineurin (CaN) has been shown to be down regulated within the hippocampus of subjects with schizophrenia (Eastwood et al., 2005) and has also been found to be correlated with psychopathology as measured by the brief psychiatric rating scale (BPRS) (Murata et al., 2008). Another blood-based biomarker investigation with a linkage to the brain was carried out by Hattori et al., who demonstrated that in platelets from patients with schizophrenia gene expression of Fyn, a member of the Src-kinase family which targets NMDA receptors for phosphorylation was increased (Hattori et al., 2009). In a microarray study looking at gene expression changes in leukocytes of both patients with schizophrenia and bipolar disorder vs. controls we identified dysregulation of the ubiquitin proteasome system (UPS) using pathway analysis (Bousman et al., 2010a). Follow-up examination of individual genes within the UPS showed ubiquitin conjugating enzyme 2 k (UBE2K) and seven in absentia homolog-2 (SIAH2) had positive correlations with positive symptom severity (Bousman et al., 2010b). These findings support previous schizophrenia post-mortem studies that have reported dsyregulated expression of several ubiquitin-related genes (Middleton et al., 2002; Altar et al., 2005). A role for the involvement of GSK3β signaling in schizophrenia has come from a number of studies including the demonstration that decreased AKT1 expression was present in the brain and lymphocytes of patients with schizophrenia (Emamian et al., 2004) that was recently replicated for lymphocytes at the gene expression level (van Beveren et al., 2012). Given GSK3β's role in neural development, neurogenesis, neuronal differentiation as well as synaptic plasticity and axonal growth (Castano et al., 2010; Hur and Zhou, 2010) and that Akt1 phoshporylates GSK3β to inactivate these findings within the blood may reflect mechanisms taking place in the brain. These roles are of relevance to schizophrenia as reductions in synaptic and dendritic markers are amongst the most consistent post-mortem brain observations for the disorder (Jarskog et al., 2007). Lastly, there is growing evidence that there may be immune dysregulation in patients with schizophrenia and recent gene expression investigations within the blood have demonstrated changes in immune pathways in patients with schizophrenia (Gardiner et al., 2013; Xu et al., 2012). **Table 1** below summarizes gene expression of biomarkers for schizophrenia that overlap in the blood and brain.

While blood based gene expression investigation offer great potential in identifying biomarkers for schizophrenia and psychosis, which could then ultimately be used as diagnostic and prognostic indicators the discovery of such markers may take some time to achieve. For blood-based gene expression studies there are several parameters that can substantially affect gene expression including diet, genetics, time to last meal, time of day, any medications used, and frequency of exercise (Radich et al., 2004). As well as these factors, technology such as microarrays while having the utility of assessing global gene expression changes, present problems in terms of consistency across studies and laboratorys. Indeed, a number of studies to date, looking at cross platform comparisons have unfortunately seen a very low level of correlation in gene expression (Tan et al., 2003; Hollingshead et al., 2005), even with mRNA samples derived from homogenous tissue sources such as cell lines. This has led to the conclusion that gene expression data cannot be combined reliably between platforms. Knowledge of this result has often

Table 1 | Gene expression biomarkers for schizophrenia in blood and brain.

		Tissue		Sample size			Gene expression	
Gene	**Platform**	**Category**	**Type**	**N**	**SZ**	**Controls**	**Relative to controls**	**References**
CHRNA7	qRT-PCR	Blood	PBMC	55	34	21	↓ TNS & SZ	Perl et al., 2003
	qRT-PCR	Blood	PBMC	60	44	16	↓ SZ	Perl et al., 2006
	qRT-PCR	Brain	DLPFC	91	30	61	× SZ	
	qRT-PCR	Brain	HIP	91	30	61	× SZ	Mathew et al., 2007
DARPP-32	qRT-PCR	Blood	PBMC	15	8	7	↓ SZ	Torres et al., 2009
	qRT-PCR	Brain	DLPFC (BA46)	70	35	35	↓ SZ-S	Feldcamp et al., 2008
	qRT-PCR	Brain	PFC	67	33	34	↑ SZ	Zhan et al., 2011
	qRT-PCR	Brain	DLPFC	502	176	326	↑ SZ	Kunii et al., 2013
DISC1	qRT-PCR	Blood	PBMC	22	10	11	↑ TNS & SZ	Kumarasinghe et al., 2013
	qRT-PCR	Brain	HIP	91	30	61	↑ SZ	Nakata et al., 2009
DRD2	Custom	Blood	PBL	23	10	13	↑ TNS	Zvara et al., 2005
	qRT-PCR	Blood	PBMC	56	30	26	× SZ	Yao et al., 2008
	Affy U133 plus 2.0	Blood	PBMC	21	9	12	↑ SZ	Glatt et al., 2011
	qRT-PCR	Blood	PBMC	22	10	11	× TNS & SZ	Kumarasinghe et al., 2013
	qRT-PCR	Brain	PFC	67	33	34	× SZ	Zhan et al., 2011
DTNBP1	Agilent 18K	Blood	LCL	24	12	12	↓ SZ	Chagnon et al., 2008
	qRT-PCR	Blood	LCL	90	45	45	× SZ	Yamamori et al., 2011
	QISH	Brain	DLPFC	29	14	15	↓ SZ	Weickert et al., 2004
ERRB3	qRT-PCR	Blood	LCL	59	25	34	↓ SZ	Law et al., 2012
	Affy HuGeneFL	Brain	DLPFC	24	12	12	↓ SZ	Hakak et al., 2001
	Affy HgU95A	Brain	MTG (BA21)	26	12	14	↓ SZ	Aston et al., 2004
	Affy U133A&B	Brain	BA24/32, HIP	26	113	13	↓ SZ	Katsel et al., 2005
	qRT-PCR	Brain	DLPFC	103	31	72	↓ SZ	Law et al., 2012
ERRB4	qRT-PCR	Blood	LCL	59	25	34	↑ SZ	
	qRT-PCR	Brain	DLPFC	103	31	72	× SZ	Law et al., 2012
MAL	Affy HuGeneFL	Brain	DLPFC (BA46)	24	12	12	↓ SZ	Hakak et al., 2001
	qRT-PCR	Blood	PBMC	22	10	11	↓ TNS; × SZ	Kumarasinghe et al., 2013
MBP	qRT-PCR	Blood	PBMC	84	39	45	× TNS & SZ	Gutierrez-Fernandez et al., 2010
	Affy U133 plus 2.0	Blood	PBMC	21	9	12	↓ SZ	Glatt et al., 2011
	qRT-PCR	Blood	PBMC	22	10	11	↑ TNS; × SZ	Kumarasinghe et al., 2013
	qRT-PCR	Brain	PVC (BA17)	30	15	15	↓ SZ	Matthews et al., 2012
MDH1	Custom	Blood	LCL	14	5	9	↑ SZ	Vawter et al., 2004
	Affy HuGeneFL	Brain	DLPFC (BA46)	24	12	12	↑ SZ	Hakak et al., 2001
	UniGEM V2	Brain	PFC (BA9)	20	10	10	↓ SZ	Middleton et al., 2002
	qRT-PCR	Brain	DLPFC	43	22	21	↓ SZ	Vawter et al., 2004
NPY1R	Custom	Blood	LCL	14	5	9	↑ SZ	Vawter et al., 2004
	qRT-PCR	Blood	LCL	90	45	45	× SZ	Yamamori et al., 2011
	Affy HuGeneFL	Brain	PFC (BA10)	30	15	15	↓ SZ	Kuromitsu et al., 2001
	qRT-PCR	Brain	DLPFC	164	81	82	↓ SZ	Choi et al., 2008
NRG1	Affy U133 plus 2.0	Blood	PBL	38	33	5	↑ SZ	Petryshen et al., 2005
	Affy U133 plus 2.0	Blood	PBL	78	31	47	↑ FEP	Zhang et al., 2008
	Agilent 18K	Blood	LCL	24	12	12	↓ SZ	Chagnon et al., 2008
	qRT-PCR	Blood	LCL	90	45	45	× SZ	Yamamori et al., 2011
	qRT-PCR	Blood	PBL	163	80	83	↓ TNS; × SZ	Zhang et al., 2008

(Continued)

Table 1 | Continued

Gene	Platform	Tissue		Sample size			Gene expression	References
		Category	Type	N	SZ	Controls	Relative to controls	
	qRT-PCR	Brain	DLPFC	39	20	19	↑ SZ[a]	Hashimoto et al., 2004
	qRT-PCR	Brain	HIP	91	38	53	↑ SZ[a]	Law et al., 2006
	qRT-PCR	Brain	PFC (BA10)	19	11	8	↓ SZ[a]	
	qRT-PCR	Brain	PFC (BA10)	19	11	8	↑ SZ[b]	
	qRT-PCR	Brain	PFC (BA9)	19	11	8	× SZ	
	qRT-PCR	Brain	HIP	19	11	8	× SZ	Parlapani et al., 2010
	qRT-PCR	Brain	PFC (BA9)	12	6	6	↑ SZ	
	qRT-PCR	Brain	HIP	11	6	5	× SZ	Marballi et al., 2012
PICK1	Affy U133 plus 2.0	Blood	PBMC	21	9	12	↑ SZ	Glatt et al., 2011
	qRT-PCR	Brain	DLPFC	70	35	35	↑ SZ	Sarras et al., 2010
PIK3CD	qRT-PCR	Blood	LCL	59	25	34	↑ SZ	
	qRT-PCR	Brain	DLPFC	103	31	72	× SZ	Law et al., 2012
PIK3R3	qRT-PCR	Blood	LCL	59	25	34	↑ SZ	
	qRT-PCR	Brain	DLPFC	103	31	72	↑ SZ	Law et al., 2012
SELENBP1	qRT-PCR	Blood	PBMC	58	34	24	↑ SZ	Glatt et al., 2005
	qRT-PCR	Blood	PBMC	56	30	26	× SZ	Yao et al., 2008
	Affy U133A	Brain	DLPFC	46	19	27	↑ SZ	Yao et al., 2008
	qRT-PCR	Brain	DLPFC	68	34	34	↑ SZ	Kanazawa et al., 2008

BA, Brodmann area; DLPFC, dorsolateral prefrontal cortex; FEP, first episode psychosis; HIP, hippocampus; LCL, lymphblastoid cell line; MTG, middle temporal gyrus; PAC, parietal cortex; PBL, peripheral blood lymphocyte; PBMC, peripheral blood mononuclear cell; PFC, prefrontal cortex; PVC, primary visual cortex; QISH, quantitative in situ hybridization; SZ, schizophrenia; SZ-S, schizophrenia suicide; TNS, treatment niave schizophrenia.

[a] *NRG1 Type I only.*

[b] *NRG1 Type II only.*

led researchers to choose platforms on the basis of what past investigations have utilized. While the logic behind this choice is concrete, it does to a certain extent preclude an informed decision based on merits of individual platforms. In addition, many gene expression findings that are discovered through microarray investigations are often not validated by independent and more sensitive techniques such quantitative real-time PCR (qRT-PCR). Nevertheless, with the advent of more sensitive and comprehensive ways to analyse gene expression through next generation sequencing, the potential of blood based biomarker investigations may be realized sooner than expected.

MicroRNAs AND DYSREGULATION IN SCHIZOPHRENIA

Current evidence suggests that psychiatric illnesses are not the result of a dysfunctional single monoamine system but the disruption of an entire cellular network, schizophrenia being a prime example of this (Hunsberger et al., 2009). The wide-ranging effects of miRNA, with their ability to target hundreds of genes (Hunsberger et al., 2009) make it a molecule of great interest in schizophrenia research. miRNA are single stranded, non-coding RNA molecules typically 21-23nt in length (Dinan, 2010). miRNA are able to regulate gene expression by attaching to mRNA strands based on complementarity of sequence. The degree to which there is complementarity will influence whether the mRNA strand targeted is silenced or degraded. Evaluating miRNAs as biomarkers for schizophrenia is a logical next step as they have been shown to be key components of gene regulation in the development of the central nervous system (CNS) (Smrt et al., 2010; Meza-Sosa et al., 2012). Greater than 50% of identified miRNAs are expressed either solely or principally in the brain (Dinan, 2010). Thus, miRNAs could represent a link between the numerous genes implicated in schizophrenia as they are able to target hundreds of genes making it capable of explaining the varied aspects of the disease process (Hunsberger et al., 2009). The dysregulation of miRNA biogenesis leading to abnormal levels of miRNA in those with schizophrenia or schizoaffective disorders is an area providing very promising results. A key protein implicated in this dysregulation is Dgcr8, which is located on chromosome 22, (Shiohama et al., 2003) within a section (22q11.2) known to be deleted in some patients with schizophrenia (Bassett and Chow, 2008). Santarelli et al. (2011) was able to corroborate the up-regulation of Dgcr8 while also demonstrating the higher abundance of Dicer, another molecule involved in miRNA biogenesis in schizophrenia patients as compared to normal controls through utilization of qRT-PCR (Santarelli et al., 2011).

POSTMORTEM miRNA DYSREGULATION IN SCHIZOPHRENIA

Work by (Beveridge et al., 2010) investigating miRNA expression profiles in both the superior temporal gyrus (STG) and dorsolateral dlPFC illustrated an important global increase in

miRNA expression in schizophrenia compared to healthy controls. A total of 59 miRNA were demonstrated to be up-regulated with the miR-105 family of miRNA as well as miR-107 most noticeably expressed at higher levels. This miRNA increase is despite normal pri-miRNA transcripts and gene expression, the difference lies in the increased pre-miRNA concentration. Key genes in the biogenesis of miRNA are also up-regulated leading to the described results. Dgcr8 up-regulation can be found in the STG while up-regulation of Dgcr8 as well as Drosha and Dicer1 were demonstrated in the dlPFC (Xu et al., 2008). It has also been demonstrated that miR-195, which is known to suppress colorectal carcinoma tumor development in the periphery (Liu et al., 2010), as well as miR-30a-5p down-regulates the expression of brain derived neurotrophic factor (BDNF) which consequently cascades into a decreased concentration of GABAergic genes, principally NPY and somatostatin (SST), in the dlPFC of schizophrenic patients as compared to unaffected controls (Mellios et al., 2009; Mellios and Sur, 2012). Investigation revealed that miR-346, a miRNA that targets genes associated with schizophrenia, is encoded within the glutamate receptor ionotropic delta 1 (GRID1) gene (Zhu et al., 2009) which itself was shown previously to be a factor in schizophrenia susceptibility (Treutlein et al., 2009). An investigation of Scandinavian populations (Danish, Swedish, and Norwegian) looking at 18 single nucleotide polymorphisms (SNPs) revealed two SNPs (rs17578796 and rs1700) related to miRNAs miR-206 and miR-198, respectively. These miRNAs showed a significant correlation to schizophrenia as compared to the control group in Danish and Norwegian populations. Of the genes targeted by both these miRNAs, eight of the 15 are known to be transcription factors or interaction partners in the network associated with schizophrenia (Hansen et al., 2007). A further 16 miRNAs that have been found to be differentially expressed in the PFC between schizophrenia and normal controls were identified by Perkins et al. (2007), 15 were seen to be expressed at lower levels in those affected and one was up-regulated. Of the 16 miRNAs, 12 were further investigated using qRT-PCR with seven of the original miRNAs being shown to express at significantly lower concentrations. An additional seven miRNAs have been identified as being dysregulated in the PFC of schizophrenia patients after an initial investigation of 667 candidate miRNAs were analysed using a real-time PCR based Taqman Low Density Array (TLDA). Further study of miR-34a, miR-132, and miR-212, from the 7 initially identified, revealed a significant negative correlation between miR-132 and miR-212 with tyrosine hydroxylase (TH) and phosphogluconate dehydrogenase (PGD) (Kim et al., 2010). A genome wide association study (GWAS) investigating 21,856 subjects with European ancestry and a further 29,839 independent subjects revealed seven mi-RNA related SNPs, five of which were new findings (REF). The strongest correlation was with a SNP, rs1625579, found in the transcript of miR-137. miR-137 is found on chromosome 1p22 in a non-protein-coding RNA gene *AK094607* that is known for its importance to neuronal development (Smrt et al., 2010). Of the other SNPs found, an additional four of them were shown to target the same gene (Schizophrenia Psychiatric Genome-Wide Association Study, 2011). Finally, another recent post-mortem investigation miRNAs in prefrontal cortices of patients with schizophrenia and bipolar disorder revealed that two miRNAs, miR-497 and miR-29c were significantly increased in patients with bipolar disorder vs. controls (Banigan et al., 2013). Changes in expression of these two miRNAs may reflect differences between schizophrenia and bipolar disorder, however, given that this investigation failed to replicate previous miRNA findings for the dlPFC brings into question the consistency in miRNA dysregulation signatures across cohorts. As the miRNA field progresses attention to treatment related changes in these markers will prove dividend, with a recent study demonstrating that some implicated miRNAs for neuropsychiatric disease like, miR-34a above are regulated significantly by the mood stabilizers lithium and sodium valproate (Hunsberger et al., 2013).

BLOOD BASED DYSREGULATION OF miRNAs IN SCHIZOPHRENIA

Recently, a blood-based miRNA study using PBMCs reported the significant down-regulation of 83 miRNA among individuals with schizophrenia compared to controls. Of those 83 miRNA showing down-regulation, 20% were transcribed from a single maternally expressed, imprinted locus, DLK1-DIO3 (Beveridge and Cairns, 2012). Another study employed mononuclear leukocytes as the cells of interest and completed a genome wide miRNA expression profile of 365 miRNA. An miRNA signature comprising seven miRNA for schizophrenia arose, six up-regulated and one down-regulated. Although these initial results are promising further work is required to determine the if these observed effects are a results of antipsychotic, alcohol, and/or illicit drug exposure rather than underling pathophysiology of schizophrenia (Lai et al., 2011). In relation to biomarker investigations in other living tissues, a recent study has demonstrated that miR-382 was elevated in the olfactory epithelium taken from patients with schizophrenia (Mor et al., 2013), this is of significance as miR-382 regulates expression of fibroblast growth factor receptor-1 (*FGFR1)* that has been previously linked to schizophrenia (Jungerius et al., 2008).

CONCLUSION

Despite some overlap between structural, cellular, and molecular data generated from post-mortem investigations, functional neuroimaging studies and blood-based biomarker investigations, the full contribution and impact of past post-mortem literature on biomarker discovery remains to be unearthed. This is largely due to advantages and disadvantages in these different modalities to assess alterations in candidate gene and proteins. For post-mortem investigations, they have the distinct advantage of being able to assess the macro- and microstructure of the brain in a very detailed way in order to map pathways that might be affected in schizophrenia. To date this same resolution cannot be achieved for neuroimaging studies, although the field is technologically evolving rapidly. Given the growing evidence for glutamatergic dysregulation as being a potential driver of schizophrenia and psychosis related pathways, an increase in the number of functional neuroimaging studies to investigate glutamatergic receptor populations is likely. A significant disadvantage of post-mortem investigations is that they are confounded by a number of artifacts, including fixation times, post-mortem interval, and alterations in pH, not to mention trying to account

for medication effects. Controlling for these variables in post-mortem studies is challenging, however, with well-characterized brain cohorts this can be achieved. Nevertheless, differences in these variables that can influence both gene and protein expression may account for some discrepancies between post-mortem and functional neuroimaging studies. With regards to blood based biomarker research utilizing high throughput technologies such as microarrays for genotyping and gene expression are relatively new fields, and therefore time is required for the full impact of such investigations to providing biomarkers for diagnosis, prognosis and intervention. While many future biomarker investigations for schizophrenia and psychosis may still largely be hypothesis-free, the growing literature and relative consistency in the data implicating genes such as *NRG1* and the glutamatergic system as a whole are providing researchers within neuropsychiatric genetics with more mechanistic correlates of these biomarkers. In this way, post-mortem literature can act to drive blood-based biomarker investigations by looking for correlates of gene and protein dysfunction within the blood and also via functional changes using neuroimaging. Although blood-based biomarker investigations are exciting and have a realistic potential in uncovering candidate genes and/or proteins relating to schizophrenia, care must be taken in the interpretation of initial preliminary findings to prevent generation of spurious paths of research. By implementing careful study design many of these potential confounders can be reduced or accounted for. In this regard, problems exist in standardization of diagnosis, collection and processing of samples, platform design, and evolution in consistency, laboratory techniques, and tools used for analysis (Chow et al., 2012) Other important potential confounding variables relating to sampling of mixed proportions of PBMCs, and differences in gene expression that exist between these (Palmer et al., 2006) as well as treatment related effects need to be addressed. Investigation of gene expression changes within separate PBMC subpopulations and treatment related gene expression via *in vitro* exposure to various antipsychotic medications may provide one way to address these concerns. Nonetheless as the field of biomarker discovery evolves, links with previous post-mortem data and also current investigations will be made and will potentially add a level of strength to the validity of such candidates. This will be aided technologically by more sophisticated and accurate ways of measuring gene expression such as RNAseq and more sensitive and stringent validation approached such as digital PCR. A point to bear in mind, and that was already mentioned within the introduction is that biomarkers need not necessarily reflect directly brain related changes in patients with psychosis but instead may be purely associative and related to symptoms without a clear pathogenic mechanism of involvement. The strength of these biomarkers lies within the predictive capability in deducing susceptibility, diagnosis or potentially as prognostic indicators, and while a link with brain related changes may exist and be preferential it may not be evidenced straight away. Establishing this link between a biomarker and neuropathophysiology will be one of the ways that future post-mortem investigations will contribute to resolving biomarkers for schizophrenia and psychosis. This approach will be bolstered by investigations of factors such as miRNAs in both blood and brain compartments and the corroboration of these regulatory elements.

REFERENCES

Abadie, P., Boulenger, J. P., Benali, K., Barre, L., Zarifian, E., and Baron, J. C. (1999). Relationships between trait and state anxiety and the central benzodiazepine receptor: a PET study. *Eur. J. Neurosci.* 11, 1470–1478. doi: 10.1046/j.1460-9568.1999.00556.x

Abi-Dargham, A., Gil, R., Krystal, J., Baldwin, R. M., Seibyl, J. P., Bowers, M., et al. (1998). Increased striatal dopamine transmission in schizophrenia: confirmation in a second cohort. *Am. J. Psychiatry* 155, 761–767.

Abi-Dargham, A., Laruelle, M., Krystal, J., D'souza, C., Zoghbi, S., Baldwin, R. M., et al. (1999). No evidence of altered *in vivo* benzodiazepine receptor binding in schizophrenia. *Neuropsychopharmacology* 20, 650–661. doi: 10.1016/S0893-133X(98)00107-9

Abi-Dargham, A., van de Giessen, E., Slifstein, M., Kegeles, L. S., and Laruelle, M. (2009). Baseline and amphetamine-stimulated dopamine activity are related in drug-naive schizophrenic subjects. *Biol. Psychiatry* 65, 1091–1093. doi: 10.1016/j.biopsych.2008.12.007

Abi-Dargham, A., Xu, X., Thompson, J. L., Gil, R., Kegeles, L. S., Urban, N., et al. (2012). Increased prefrontal cortical D(1) receptors in drug naive patients with schizophrenia: a PET study with [(1)(1)C]NNC112. *J. Psychopharmacol.* 26, 794–805. doi: 10.1177/0269881111409265

Addington, A. M., Gornick, M., Sporn, A. L., Gogtay, N., Greenstein, D., Lenane, M., et al. (2004). Polymorphisms in the 13q33.2 gene G72/G30 are associated with childhood-onset schizophrenia and psychosis not otherwise specified. *Biol. Psychiatry* 55, 976–980. doi: 10.1016/j.biopsych.2004.01.024

Akbarian, S., and Huang, H. S. (2006). Molecular and cellular mechanisms of altered GAD1/GAD67 expression in schizophrenia and related disorders. *Brain Res. Rev.* 52, 293–304. doi: 10.1016/j.brainresrev.2006.04.001

Akbarian, S., Kim, J. J., Potkin, S. G., Hagman, J. O., Tafazzoli, A., Bunney, W. E. Jr., et al. (1995). Gene expression for glutamic acid decarboxylase is reduced without loss of neurons in prefrontal cortex of schizophrenics. *Arch. Gen. Psychiatry* 52, 258–266. doi: 10.1001/archpsyc.1995.03950160008002

Altar, C. A., Jurata, L. W., Charles, V., Lemire, A., Liu, P., Bukhman, Y., et al. (2005). Deficient hippocampal neuron expression of proteasome, ubiquitin, and mitochondrial genes in multiple schizophrenia cohorts. *Biol. Psychiatry* 58, 85–96. doi: 10.1016/j.biopsych.2005.03.031

Amato, D., Natesan, S., Yavich, L., Kapur, S., and Muller, C. P. (2011). Dynamic regulation of dopamine and serotonin responses to salient stimuli during chronic haloperidol treatment. *Int. J. Neuropsychopharmacol.* 14, 1327–1339. doi: 10.1017/S1461145711000010

Arranz, B., Eriksson, A., Mellerup, E., Plenge, P., and Marcusson, J. (1994). Brain 5-HT1A, 5-HT1D, and 5-HT2 receptors in suicide victims. *Biol. Psychiatry* 35, 457–463. doi: 10.1016/0006-3223(94)90044-2

Aston, C., Jiang, L., and Sokolov, B. P. (2004). Microarray analysis of postmortem temporal cortex from patients with schizophrenia. *J. Neurosci. Res.* 77, 858–866. doi: 10.1002/jnr.20208

Aston, C., Jiang, L., and Sokolov, B. P. (2005). Transcriptional profiling reveals evidence for signaling and oligodendroglial abnormalities in the temporal cortex from patients with major depressive disorder. *Mol. Psychiatry* 10, 309–322. doi: 10.1038/sj.mp.4001565

Banigan, M. G., Kao, P. F., Kozubek, J. A., Winslow, A. R., Medina, J., Costa, J., et al. (2013). Differential expression of exosomal microRNAs in prefrontal cortices of schizophrenia and bipolar disorder patients. *PLoS ONE* 8:e48814. doi: 10.1371/journal.pone.0048814

Bartzokis, G., Lu, P. H., Raven, E. P., Amar, C. P., Detore, N. R., Couvrette, A. J., et al. (2012). Impact on intracortical myelination trajectory of long acting injection versus oral risperidone in first-episode schizophrenia. *Schizophr. Res.* 140, 122–128. doi: 10.1016/j.schres.2012.06.036

Bassett, A. S., and Chow, E. W. (2008). Schizophrenia and 22q11.2 deletion

syndrome. *Curr. Psychiatry Rep.* 10, 148–157. doi: 10.1007/s11920-008-0026-1

Bendikov, I., Nadri, C., Amar, S., Panizzutti, R., De Miranda, J., Wolosker, H., et al. (2007). A CSF and postmortem brain study of D-serine metabolic parameters in schizophrenia. *Schizophr. Res.* 90, 41–51. doi: 10.1016/j.schres.2006.10.010

Benes, F. M., Lim, B., Matzilevich, D., Walsh, J. P., Subburaju, S., and Minns, M. (2007). Regulation of the GABA cell phenotype in hippocampus of schizophrenics and bipolars. *Proc. Natl. Acad. Sci. U.S.A.* 104, 10164–10169. doi: 10.1073/pnas.0703806104

Bennett, A. O. M. (2008). Stress and anxiety in schizophrenia and depression: glucocorticoids, corticotropin-releasing hormone and synapse regression. *Aust. N. Z. J. Psychiatry* 42, 995–1002. doi: 10.1080/00048670802512073

Bennett, M. (2009). Positive and negative symptoms in schizophrenia: the NMDA receptor hypofunction hypothesis, neuregulin/ErbB4 and synapse regression. *Aust. N. Z. J. Psychiatry* 43, 711–721. doi: 10.1080/00048670903001943

Bertram, I., Bernstein, H. G., Lendeckel, U., Bukowska, A., Dobrowolny, H., Keilhoff, G., et al. (2007). Immunohistochemical evidence for impaired neuregulin-1 signaling in the prefrontal cortex in schizophrenia and in unipolar depression. *Ann. N. Y. Acad. Sci.* 1096, 147–156. doi: 10.1196/annals.1397.080

Beveridge, N. J., and Cairns, M. J. (2012). MicroRNA dysregulation in schizophrenia. *Neurobiol. Dis.* 46, 263–271. doi: 10.1016/j.nbd.2011.12.029

Beveridge, N. J., Gardiner, E., Carroll, A. P., Tooney, P. A., and Cairns, M. J. (2010). Schizophrenia is associated with an increase in cortical microRNA biogenesis. *Mol. Psychiatry* 15, 1176–1189. doi: 10.1038/mp.2009.84

Bousman, C. A., Chana, G., Glatt, S. J., Chandler, S. D., Lucero, G. R., Tatro, E., et al. (2010a). Preliminary evidence of ubiquitin proteasome system dysregulation in schizophrenia and bipolar disorder: convergent pathway analysis findings from two independent samples. *Am. J. Med. Genet. B Neuropsychiatr. Genet.* 153B, 494–502. doi: 10.1002/ajmg.b.31006

Bousman, C. A., Chana, G., Glatt, S. J., Chandler, S. D., May, T., Lohr, J., et al. (2010b). Positive symptoms of psychosis correlate with expression of ubiquitin proteasome genes in peripheral blood. *Am. J. Med. Genet. B Neuropsychiatr. Genet.* 153B, 1336–1341. doi: 10.1002/ajmg.b.31106

Bressan, R. A., Erlandsson, K., Mulligan, R. S., Gunn, R. N., Cunningham, V. J., Owens, J., et al. (2003). Evaluation of NMDA receptors *in vivo* in schizophrenic patients with [123I]CNS (1261). and SPET: preliminary findings. *Ann. N. Y. Acad. Sci.* 1003, 364–367. doi: 10.1196/annals.1300.027

Brzustowicz, L. M., Hodgkinson, K. A., Chow, E. W., Honer, W. G., and Bassett, A. S. (2000). Location of a major susceptibility locus for familial schizophrenia on chromosome 1q21-q22. *Science* 288, 678–682. doi: 10.1126/science.288.5466.678

Burnet, P. W., Eastwood, S. L., and Harrison, P. J. (1996). 5-HT1A and 5-HT2A receptor mRNAs and binding site densities are differentially altered in schizophrenia. *Neuropsychopharmacology* 15, 442–455. doi: 10.1016/S0893-133X(96)00053-X

Busatto, G. F., Pilowsky, L. S., Costa, D. C., Ell, P. J., David, A. S., Lucey, J. V., et al. (1997). Correlation between reduced *in vivo* benzodiazepine receptor binding and severity of psychotic symptoms in schizophrenia. *Am. J. Psychiatry* 154, 56–63.

Bymaster, F. P., Whitesitt, C. A., Shannon, H. E., Delapp, N., Ward, J. S., Calligaro, D. O., et al. (1997). Xanomeline: a selective muscarinic agonist for the treatment of Alzheimer's disease. *Drug Dev. Res.* 40, 158–170. doi: 10.1002/(SICI)1098-2299(199702)40:2<158::AID-DDR6>3.0.CO;2-K

Castano, Z., Gordon-Weeks, P. R., and Kypta, R. M. (2010). The neuron-specific isoform of glycogen synthase kinase-3beta is required for axon growth. *J. Neurochem.* 113, 117–130. doi: 10.1111/j.1471-4159.2010.06581.x

Chagnon, Y. C., Roy, M. A., Bureau, A., Merette, C., and Maziade, M. (2008). Differential RNA expression between schizophrenic patients and controls of the dystrobrevin binding protein 1 and neuregulin 1 genes in immortalized lymphocytes. *Schizophr. Res.* 100, 281–290. doi: 10.1016/j.schres.2007.12.471

Chana, G., Lucero, G., Salaria, S., Lozach, J., Du, P., Woelk, C., et al. (2009). Upregulation of NRG-1 and VAMP-1 in human brain aggregates exposed to clozapine. *Schizophr. Res.* 113, 273–276. doi: 10.1016/j.schres.2009.05.015

Charych, E. I., Liu, F., Moss, S. J., and Brandon, N. J. (2009). GABA(A) receptors and their associated proteins: implications in the etiology and treatment of schizophrenia and related disorders. *Neuropharmacology* 57, 481–495. doi: 10.1016/j.neuropharm.2009.07.027

Chen, C. K., Lin, S. K., Sham, P. C., Ball, D., Loh, E. W., Hsiao, C. C., et al. (2003). Pre-morbid characteristics and co-morbidity of methamphetamine users with and without psychosis. *Psychol. Med.* 33, 1407–1414. doi: 10.1017/S0033291703008353

Chen, J., Lipska, B. K., Halim, N., Ma, Q. D., Matsumoto, M., Melhem, S., et al. (2004). Functional analysis of genetic variation in catechol-O-methyltransferase (COMT): effects on mRNA, protein, and enzyme activity in postmortem human brain. *Am. J. Hum. Genet.* 75, 807–821. doi: 10.1086/425589

Chiesa, A., Pae, C. U., Porcelli, S., Han, C., Lee, S. J., Patkar, A. A., et al. (2011). DAOA variants and schizophrenia: Influence on diagnosis and treatment outcomes. *Int. J. Psychiatry Clin. Pract.* 15, 303–310. doi: 10.3109/13651501.2011.589518

Choi, K. H., Elashoff, M., Higgs, B. W., Song, J., Kim, S., Sabunciyan, S., et al. (2008). Putative psychosis genes in the prefrontal cortex: combined analysis of gene expression microarrays. *BMC psychiatry* 8:87. doi: 10.1186/1471-244X-8-87

Chow, M. L., Pramparo, T., Winn, M. E., Barnes, C. C., Li, H. R., Weiss, L., et al. (2012). Age-dependent brain gene expression and copy number anomalies in autism suggest distinct pathological processes at young versus mature ages. *PLoS Genet.* 8:e1002592. doi: 10.1371/journal.pgen.1002592

Craddock, N., O'donovan, M. C., and Owen, M. J. (2005). The genetics of schizophrenia and bipolar disorder: dissecting psychosis. *J. Med. Genet.* 42, 193–204. doi: 10.1136/jmg.2005.030718

Crook, J. M., Dean, B., Pavey, G., and Copolov, D. (1999). The binding of H-3 AF-DX 384 is reduced in the caudate-putamen of subjects with schizophrenia. *Life Sci.* 64, 1761–1771. doi: 10.1016/S0024-3205(99)00114-9

Crook, J. M., Tomaskovic-Crook, E., Copolov, D. L., and Dean, B. (2000). Decreased muscarinic receptor binding in subjects with schizophrenia: a study of the human hippocampal formation. *Biol. Psychiatry* 48, 381–388. doi: 10.1016/S0006-3223(00)00918-5

Dean, B. (2012). Neurochemistry of schizophrenia: the contribution of neuroimaging postmortem pathology and neurochemistry in schizophrenia. *Curr. Top. Med. Chem.* 12, 2375–2392. doi: 10.2174/156802612805289935

Dean, B., Hayes, W., Opeskin, K., Naylor, L., Pavey, G., Hill, C., et al. (1996). Serotonin2 receptors and the serotonin transporter in the schizophrenic brain. *Behav. Brain Res.* 73, 169–175. doi: 10.1016/0166-4328(96)00091-5

Dean, B., Keriakous, D., Scarr, E., and Thomas, E. A. (2007). Gene expression profiling in Brodmann's area 46 from subjects with schizophrenia. *Aust. N. Z. J. Psychiatry* 41, 308–320. doi: 10.1080/00048670701213245

Dean, B., Pavey, G., and Opeskin, K. (1997). [3H]raclopride binding to brain tissue from subjects with schizophrenia: methodological aspects. *Neuropharmacology* 36, 779–786. doi: 10.1016/S0028-3908(97)00067-1

Dinan, T. G. (2010). MicroRNAs as a target for novel antipsychotics: a systematic review of an emerging field. *Int. J. Neuropsychopharmacol.* 13, 395–404. doi: 10.1017/S1461145709990800

Duan, J., Martinez, M., Sanders, A. R., Hou, C., Krasner, A. J., Schwartz, D. B., et al. (2005). Neuregulin 1 (NRG1) and schizophrenia: analysis of a US family sample and the evidence in the balance. *Psychol. Med.* 35, 1599–1610. doi: 10.1017/S0033291705005428

Eastwood, S. L., Salih, T., and Harrison, P. J. (2005). Differential expression of calcineurin A subunit mRNA isoforms during rat hippocampal and cerebellar development. *Eur. J. Neurosci.* 22, 3017–3024. doi: 10.1111/j.1460-9568.2005.04518.x

Emamian, E. S., Hall, D., Birnbaum, M. J., Karayiorgou, M., and Gogos, J. A. (2004). Convergent evidence for impaired AKT1-GSK3beta signaling in schizophrenia. *Nat. Genet.* 36, 131–137. doi: 10.1038/ng1296

Erritzoe, D., Rasmussen, H., Kristiansen, K. T., Frokjaer, V. G., Haugbol, S., Pinborg, L., et al. (2008). Cortical and subcortical 5-HT2A receptor binding in neuroleptic-naive first-episode schizophrenic patients. *Neuropsychopharmacology* 33, 2435–2441. doi: 10.1038/sj.npp.1301656

Feldcamp, L. A., Souza, R. P., Romano-Silva, M., Kennedy, J. L., and Wong, A. H. (2008). Reduced prefrontal cortex DARPP-32 mRNA in completed suicide victims with schizophrenia. *Schizophr. Res.* 103, 192–200. doi: 10.1016/j.schres.2008.05.014

Flynn, S. W., Lang, D. J., Mackay, A. L., Goghari, V., Vavasour, I. M., Whittall, K. P., et al. (2003). Abnormalities of myelination in schizophrenia detected *in vivo* with MRI, and post-mortem with analysis of oligodendrocyte proteins. *Mol. Psychiatry* 8, 811–820. doi: 10.1038/sj.mp.4001337

Frederiksen, S. O., Ekman, R., Gottfries, C. G., Widerlov, E., and Jonsson, S. (1991). Reduced concentrations of galanin, arginine vasopressin, neuropeptide Y and peptide YY in the temporal cortex but not in the hypothalamus of brains from schizophrenics. *Acta Psychiatr. Scand.* 83, 273–277. doi: 10.1111/j.1600-0447.1991.tb05539.x

Gao, X. M., Sakai, K., Roberts, R. C., Conley, R. R., Dean, B., and Tamminga, C. A. (2000). Ionotropic glutamate receptors and expression of N-methyl-D-aspartate receptor subunits in subregions of human hippocampus: effects of schizophrenia. *Am. J. Psychiatry* 157, 1141–1149. doi: 10.1176/appi.ajp.157.7.1141

Gardiner, E. J., Cairns, M. J., Liu, B., Beveridge, N. J., Carr, V., Kelly, B., et al. (2013). Gene expression analysis reveals schizophrenia-associated dysregulation of immune pathways in peripheral blood mononuclear cells. *J. Psychiatr. Res.* 47, 425–437. doi: 10.1016/j.jpsychires.2012.11.007.

Garver, D. L., Holcomb, J. A., and Christensen, J. D. (2008). Compromised myelin integrity during psychosis with repair during remission in drug-responding schizophrenia. *Int. J. Neuropsychopharmacol.* 11, 49–61. doi: 10.1017/S1461145707007730

Gibbons, A. S., Scarr, E., Boer, S., Money, T., Won-Je, F., Felder, C., et al. (2013). Widespread decreases in cortical muscarinic receptors in a subset of people with schizophrenia. *Int. J. Neuropsychopharmacol.* 16, 37–46. doi: 10.1017/S1461145712000028

Gibbons, A. S., Scarr, E., McLean, C., Sundram, S., and Dean, B. (2009). Decreased muscarinic receptor binding in the frontal cortex of bipolar disorder and major depressive disorder subjects. *J. Affect. Disord.* 116, 184–191. doi: 10.1016/j.jad.2008.11.015

Gladkevich, A., Kauffman, H. F., and Korf, J. (2004). Lymphocytes as a neural probe: potential for studying psychiatric disorders. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 28, 559–576. doi: 10.1016/j.pnpbp.2004.01.009

Glatt, S. J., Everall, I. P., Kremen, W. S., Corbeil, J., Sasik, R., Khanlou, N., et al. (2005). Comparative gene expression analysis of blood and brain provides concurrent validation of SELENBP1 up-regulation in schizophrenia. *Proc. Natl. Acad. Sci. U.S.A.* 102, 15533–15538. doi: 10.1073/pnas.0507666102

Glatt, S. J., Faraone, S. V., and Tsuang, M. T. (2003). Association between a functional catechol O-methyltransferase gene polymorphism and schizophrenia: meta-analysis of case-control and family-based studies. *Am. J. Psychiatry* 160, 469–476. doi: 10.1176/appi.ajp.160.3.469

Glatt, S. J., Stone, W. S., Nossova, N., Liew, C. C., Seidman, L. J., and Tsuang, M. T. (2011). Similarities and differences in peripheral blood gene-expression signatures of individuals with schizophrenia and their first-degree biological relatives. *Am. J. Med. Genet. B Neuropsychiatr. Genet.* 156B, 869–887. doi: 10.1002/ajmg.b.31239

Gonzalez-Maeso, J., Ang, R. L., Yuen, T., Chan, P., Weisstaub, N. V., Lopez-Gimenez, J. F., et al. (2008). Identification of a serotonin/glutamate receptor complex implicated in psychosis. *Nature* 452, 93–97. doi: 10.1038/nature06612

Guidotti, A., Auta, J., Davis, J. M., Di-Giorgi-Gerevini, V., Dwivedi, Y., Grayson, D. R., et al. (2000). Decrease in reelin and glutamic acid decarboxylase67 (GAD67) expression in schizophrenia and bipolar disorder: a post-mortem brain study. *Arch. Gen. Psychiatry* 57, 1061–1069. doi: 10.1001/archpsyc.57.11.1061

Gutierrez-Fernandez, A., Gonzalez-Pinto, A., Vega, P., Barbeito, S., and Matute, C. (2010). Expression of oligodendrocyte and myelin genes is not altered in peripheral blood cells of patients with first-episode schizophrenia and bipolar disorder. *Bipolar. Disord.* 12, 107–109. doi: 10.1111/j.1399-5618.2009.00776.x

Habl, G., Zink, M., Petroianu, G., Bauer, M., Schneider-Axmann, T., Von Wilmsdorff, M., et al. (2009). Increased D: -amino acid oxidase expression in the bilateral hippocampal CA4 of schizophrenic patients: a post-mortem study. *J. Neural Transm.* 116, 1657–1665. doi: 10.1007/s00702-009-0312-z

Hakak, Y., Walker, J. R., Li, C., Wong, W. H., Davis, K. L., Buxbaum, J. D., et al. (2001). Genome-wide expression analysis reveals dysregulation of myelination-related genes in chronic schizophrenia. *Proc. Natl. Acad. Sci. U.S.A.* 98, 4746–4751. doi: 10.1073/pnas.081071198

Hansen, T., Olsen, L., Lindow, M., Jakobsen, K. D., Ullum, H., Jonsson, E., et al. (2007). Brain expressed microRNAs implicated in schizophrenia etiology. *PLoS ONE* 2:e873. doi: 10.1371/journal.pone.0000873

Hashimoto, R., Straub, R. E., Weickert, C. S., Hyde, T. M., Kleinman, J. E., and Weinberger, D. R. (2004). Expression analysis of neuregulin-1 in the dorsolateral prefrontal cortex in schizophrenia. *Mol. Psychiatry* 9, 299–307. doi: 10.1038/sj.mp.4001434

Hattori, K., Fukuzako, H., Hashiguchi, T., Hamada, S., Murata, Y., Isosaka, T., et al. (2009). Decreased expression of Fyn protein and disbalanced alternative splicing patterns in platelets from patients with schizophrenia. *Psychiatry Res.* 168, 119–128. doi: 10.1016/j.psychres.2008.04.014

Heckers, S., Stone, D., Walsh, J., Shick, J., Koul, P., and Benes, F. M. (2002). Differential hippocampal expression of glutamic acid decarboxylase 65 and 67 messenger RNA in bipolar disorder and schizophrenia. *Arch. Gen. Psychiatry* 59, 521–529. doi: 10.1001/archpsyc.59.6.521

Hietala, J., Syvalahti, E., Vilkman, H., Vuorio, K., Rakkolainen, V., Bergman, J., et al. (1999). Depressive symptoms and presynaptic dopamine function in neuroleptic-naive schizophrenia. *Schizophr. Res.* 35, 41–50. doi: 10.1016/S0920-9964(98)00113-3

Hietala, J., Syvalahti, E., Vuorio, K., Nagren, K., Lehikoinen, P., Ruotsalainen, U., et al. (1994). Striatal D2 dopamine receptor characteristics in neuroleptic-naive schizophrenic patients studied with positron emission tomography. *Arch. Gen. Psychiatry* 51, 116–123. doi: 10.1001/archpsyc.1994.03950020040004

Hietala, J., Syvalahti, E., Vuorio, K., Rakkolainen, V., Bergman, J., Haaparanta, M., et al. (1995). Presynaptic dopamine function in striatum of neuroleptic-naive schizophrenic patients. *Lancet* 346, 1130–1131. doi: 10.1016/S0140-6736(95)91801-9

Hollingshead, D., Lewis, D. A., and Mirnics, K. (2005). Platform influence on DNA microarray data in postmortem brain research. *Neurobiol. Dis.* 18, 649–655. doi: 10.1016/j.nbd.2004.10.020

Hunsberger, J. G., Austin, D. R., Chen, G., and Manji, H. K. (2009). MicroRNAs in mental health: from biological underpinnings to potential therapies. *Neuromolecular Med.* 11, 173–182. doi: 10.1007/s12017-009-8070-5

Hunsberger, J. G., Fessler, E. B., Chibane, F. L., Leng, Y., Maric, D., Elkahloun, A. G., et al. (2013). Mood stabilizer-regulated miRNAs in neuropsychiatric and neurodegenerative diseases: identifying associations and functions. *Am. J. Transl. Res.* 5, 450–464.

Huot, P., Johnston, T. H., Darr, T., Hazrati, L. N., Visanji, N. P., Pires, D., et al. (2010). Increased 5-HT2A receptors in the temporal cortex of parkinsonian patients with visual hallucinations. *Mov. Disord.* 25, 1399–1408. doi: 10.1002/mds.23083

Hur, E. M., and Zhou, F. Q. (2010). GSK3 signalling in neural development. *Nat. Rev. Neurosci.* 11, 539–551. doi: 10.1038/nrn2870

Hurlemann, R., Matusch, A., Kuhn, K. U., Berning, J., Elmenhorst, D., Winz, O., et al. (2008). 5-HT2A receptor density is decreased in the at-risk mental state. *Psychopharmacology (Berl.)* 195, 579–590. doi: 10.1007/s00213-007-0921-x

Impagnatiello, F., Guidotti, A. R., Pesold, C., Dwivedi, Y., Caruncho, H., Pisu, M. G., et al. (1998). A decrease of reelin expression as a putative vulnerability factor in schizophrenia. *Proc. Natl. Acad. Sci. U.S.A.* 95, 15718–15723. doi: 10.1073/pnas.95.26.15718

Iritani, S., Kuroki, N., Niizato, K., and Ikeda, K. (2000). Morphological changes in neuropeptide Y-positive fiber in the hippocampal formation of schizophrenics. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 24, 241–249. doi: 10.1016/S0278-5846(99)00102-5

Iwamoto, K., Bundo, M., and Kato, T. (2005). Altered expression of mitochondria-related genes in postmortem brains of patients with bipolar disorder or schizophrenia, as revealed by large-scale DNA microarray analysis. *Hum. Mol. Genet.* 14, 241–253. doi: 10.1093/hmg/ddi022

Janowsky, D. S., and Risch, C. (1979). Amphetamine psychosis and psychotic symptoms.

Psychopharmacology (Berl.) 65, 73–77. doi: 10.1007/BF00491982

Jarskog, L. F., Miyamoto, S., and Lieberman, J. A. (2007). Schizophrenia: new pathological insights and therapies. *Annu. Rev. Med.* 58, 49–61. doi: 10.1146/annurev.med.58.060904.084114

Jeong, J. Y., Wang, Y., and Sytkowski, A. J. (2009). Human selenium binding protein-1 (hSP56) interacts with VDU1 in a selenium-dependent manner. *Biochem. Biophys. Res. Commun.* 379, 583–588. doi: 10.1016/j.bbrc.2008.12.110

Jungerius, B. J., Hoogendoorn, M. L., Bakker, S. C., Van't Slot, R., Bardoel, A. F., Ophoff, R. A., et al. (2008). An association screen of myelin-related genes implicates the chromosome 22q11 PIK4CA gene in schizophrenia. *Mol. Psychiatry* 13, 1060–1068. doi: 10.1038/sj.mp.4002080

Kakiuchi, C., Ishiwata, M., Hayashi, A., and Kato, T. (2006). XBP1 induces WFS1 through an endoplasmic reticulum stress response element-like motif in SH-SY5Y cells. *J. Neurochem.* 97, 545–555. doi: 10.1111/j.1471-4159.2006.03772.x

Kakiuchi, C., Iwamoto, K., Ishiwata, M., Bundo, M., Kasahara, T., Kusumi, I., et al. (2003). Impaired feedback regulation of XBP1 as a genetic risk factor for bipolar disorder. *Nat. Genet.* 35, 171–175. doi: 10.1038/ng1235

Kanazawa, T., Chana, G., Glatt, S. J., Mizuno, H., Masliah, E., Yoneda, H., et al. (2008). The utility of SELENBP1 gene expression as a biomarker for major psychotic disorders: replication in schizophrenia and extension to bipolar disorder with psychosis. *Am. J. Med. Genet. B Neuropsychiatr. Genet.* 147B, 686–689. doi: 10.1002/ajmg.b.30664

Katsel, P., Davis, K. L., Gorman, J. M., and Haroutunian, V. (2005). Variations in differential gene expression patterns across multiple brain regions in schizophrenia. *Schizophr. Res.* 77, 241–252. doi: 10.1016/j.schres.2005.03.020

Kaufer, D., Friedman, A., Seidman, S., and Soreq, H. (1998). Acute stress facilitates long-lasting changes in cholinergic gene expression. *Nature* 393, 373–377. doi: 10.1038/30741

Kerns, D., Vong, G. S., Barley, K., Dracheva, S., Katsel, P., Casaccia, P., et al. (2010). Gene expression abnormalities and oligodendrocyte deficits in the internal capsule in schizophrenia. *Schizophr. Res.* 120, 150–158. doi: 10.1016/j.schres.2010.04.012

Kim, A. H., Reimers, M., Maher, B., Williamson, V., McMichael, O., McClay, J. L., et al. (2010). MicroRNA expression profiling in the prefrontal cortex of individuals affected with schizophrenia and bipolar disorders. *Schizophr. Res.* 124, 183–191. doi: 10.1016/j.schres.2010.07.002

Kim, S., and Webster, M. J. (2011). Integrative genome-wide association analysis of cytoarchitectural abnormalities in the prefrontal cortex of psychiatric disorders. *Mol. Psychiatry* 16, 452–461. doi: 10.1038/mp.2010.23

Knable, M. B., Hyde, T. M., Herman, M. M., Carter, J. M., Bigelow, L., and Kleinman, J. E. (1994). Quantitative autoradiography of dopamine-D1 receptors, D2 receptors, and dopamine uptake sites in postmortem striatal specimens from schizophrenic patients. *Biol. Psychiatry* 36, 827–835. doi: 10.1016/0006-3223(94)90593-2

Konradi, C. (2005). Gene expression microarray studies in polygenic psychiatric disorders: applications and data analysis. *Brain Res. Brain Res. Rev.* 50, 142–155. doi: 10.1016/j.brainresrev.2005.05.004

Kornhuber, J., Riederer, P., Reynolds, G. P., Beckmann, H., Jellinger, K., and Gabriel, E. (1989). 3H-spiperone binding sites in post-mortem brains from schizophrenic patients: relationship to neuroleptic drug treatment, abnormal movements, and positive symptoms. *J. Neural Transm.* 75, 1–10. doi: 10.1007/BF01250639

Kosaka, J., Takahashi, H., Ito, H., Takano, A., Fujimura, Y., Matsumoto, R., et al. (2010). Decreased binding of [11C]NNC112 and [11C]SCH23390 in patients with chronic schizophrenia. *Life Sci.* 86, 814–818. doi: 10.1016/j.lfs.2010.03.018

Kotaka, T., Ujike, H., Okahisa, Y., Takaki, M., Nakata, K., Kodama, M., et al. (2009). G72 gene is associated with susceptibility to methamphetamine psychosis. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 33, 1046–1049. doi: 10.1016/j.pnpbp.2009.05.017

Kouzmenko, A. P., Hayes, W. L., Pereira, A. M., Dean, B., Burnet, P. W., and Harrison, P. J. (1997). 5-HT2A receptor polymorphism and steady state receptor expression in schizophrenia. *Lancet* 349, 1815. doi: 10.1016/S0140-6736(05)61695-9

Kuepper, R., Skinbjerg, M., and Abi-Dargham, A. (2012). The dopamine dysfunction in schizophrenia revisited: new insights into topography and course. *Handb. Exp. Pharmacol.* 212, 1–26. doi: 10.1007/978-3-642-25761-2_1

Kumarasinghe, N., Beveridge, N. J., Gardiner, E., Scott, R. J., Yasawardene, S., Perera, A., et al. (2013). Gene expression profiling in treatment-naive schizophrenia patients identifies abnormalities in biological pathways involving AKT1 that are corrected by antipsychotic medication. *Int. J. Neuropsychopharmacol.* doi: 10.1017/S1461145713000035. [Epub ahead of print].

Kunii, Y., Hyde, T. M., Ye, T., Li, C., Kolachana, B., Dickinson, D., et al. (2013). Revisiting DARPP-32 in postmortem human brain: changes in schizophrenia and bipolar disorder and genetic associations with t-DARPP-32 expression. *Mol. Psychiatry*. doi: 10.1038/mp.2012.174. [Epub ahead of print].

Kurian, S. M., Le-Niculescu, H., Patel, S. D., Bertram, D., Davis, J., Dike, C., et al. (2011). Identification of blood biomarkers for psychosis using convergent functional genomics. *Mol. Psychiatry* 16, 37–58. doi: 10.1038/mp.2009.117

Kuromitsu, J., Yokoi, A., Kawai, T., Nagasu, T., Aizawa, T., Haga, S., et al. (2001). Reduced neuropeptide Y mRNA levels in the frontal cortex of people with schizophrenia and bipolar disorder. *Brain Res. Gene Expr. Patterns* 1, 17–21. doi: 10.1016/S1567-133X(01)00003-5

Laakso, A., Bergman, J., Haaparanta, M., Vilkman, H., Solin, O., Syvalahti, E., et al. (2001). Decreased striatal dopamine transporter binding *in vivo* in chronic schizophrenia. *Schizophr. Res.* 52, 115–120. doi: 10.1016/S0920-9964(00)00095-5

Lai, C. Y., Yu, S. L., Hsieh, M. H., Chen, C. H., Chen, H. Y., Wen, C. C., et al. (2011). MicroRNA expression aberration as potential peripheral blood biomarkers for schizophrenia. *PLoS ONE* 6:e21635. doi: 10.1371/journal.pone.0021635

Lai, M. K. P., Lai, O. F., Keene, J., Esiri, M. M., Francis, P. T., Hope, T., et al. (2001). Psychosis of Alzheimer's disease is associated with elevated muscarinic M-2 binding in the cortex. *Neurology* 57, 805–811. doi: 10.1212/WNL.57.5.805

Laruelle, M., Abi-Dargham, A., Van Dyck, C. H., Gil, R., D'souza, C. D., Erdos, J., et al. (1996). Single photon emission computerized tomography imaging of amphetamine-induced dopamine release in drug-free schizophrenic subjects. *Proc. Natl. Acad. Sci. U.S.A.* 93, 9235–9240. doi: 10.1073/pnas.93.17.9235

Li, D., Collier, D. A., and He, L. (2006). Meta-analysis shows strong positive association of the neuregulin 1 (NRG1) gene with schizophrenia. *Hum. Mol. Genet.* 15, 1995–2002. doi: 10.1093/hmg/ddl122

Law, A. J., Lipska, B. K., Weickert, C. S., Hyde, T. M., Straub, R. E., Hashimoto, R., et al. (2006). Neuregulin 1 transcripts are differentially expressed in schizophrenia and regulated by 5′ SNPs associated with the disease. *Proc. Natl. Acad. Sci. U. S. A.* 6747–6752. doi: 10.1073/pnas.0602002103

Law, A. J., Wang, Y., Sei, Y., O'donnell, P., Piantadosi, P., Papaleo, F., et al. (2012). Neuregulin 1-ErbB4-PI3K signaling in schizophrenia and phosphoinositide 3-kinase-p110delta inhibition as a potential therapeutic strategy. *Proc. Natl. Acad. Sci. U.S.A.* 109, 12165–12170. doi: 10.1073/pnas.1206118109

Lipska, B. K., Mitkus, S., Caruso, M., Hyde, T. M., Chen, J., Vakkalanka, R., et al. (2006). RGS4 mRNA expression in postmortem human cortex is associated with COMT Val158Met genotype and COMT enzyme activity. *Hum. Mol. Genet.* 15, 2804–2812. doi: 10.1093/hmg/ddl222

Liu, L., Chen, L., Xu, Y., Li, R., and Du, X. (2010). microRNA-195 promotes apoptosis and suppresses tumorigenicity of human colorectal cancer cells. *Biochem. Biophys. Res. Commun.* 400, 236–240. doi: 10.1016/j.bbrc.2010.08.046

Luby, E. D., Gottlieb, J. S., Cohen, B. D., Rosenbaum, G., and Domino, E. F. (1962). Model psychoses and schizophrenia. *Am. J. Psychiatry* 119, 61–67.

MacKay, A. V., Iversen, L. L., Rossor, M., Spokes, E., Bird, E., Arregui, A., et al. (1982). Increased brain dopamine and dopamine receptors in schizophrenia. *Arch. Gen. Psychiatry* 39, 991–997. doi: 10.1001/archpsyc.1982.04290090001001

Madeira, C., Freitas, M. E., Vargas-Lopes, C., Wolosker, H., and Panizzutti, R. (2008). Increased brain D-amino acid oxidase (DAAO) activity in schizophrenia. *Schizophr. Res.* 101, 76–83. doi: 10.1016/j.schres.2008.02.002

Marballi, K., Cruz, D., Thompson, P., and Walss-Bass, C. (2012). Differential neuregulin 1 cleavage in the prefrontal cortex and

hippocampus in schizophrenia and bipolar disorder: preliminary findings. *PLoS ONE* 7:e36431. doi: 10.1371/journal.pone.0036431

Mateos, J. J., Lomena, F., Parellada, E., Mireia, F., Fernandez-Egea, E., Pavia, J., et al. (2007). Lower striatal dopamine transporter binding in neuroleptic-naive schizophrenic patients is not related to antipsychotic treatment but it suggests an illness trait. *Psychopharmacology (Berl.)* 191, 805–811. doi: 10.1007/s00213-006-0570-5

Mathew, S. V., Law, A. J., Lipska, B. K., Davila-Garcia, M. I., Zamora, E. D., Mitkus, S. N., et al. (2007). Alpha7 nicotinic acetylcholine receptor mRNA expression and binding in postmortem human brain are associated with genetic variation in neuregulin 1. *Hum. Mol. Genet.* 16, 2921–2932. doi: 10.1093/hmg/ddm253

Matthews, P. R., Eastwood, S. L., and Harrison, P. J. (2012). Reduced myelin basic protein and actin-related gene expression in visual cortex in schizophrenia. *PLoS ONE* 7:e38211. doi: 10.1371/journal.pone.0038211

Mellios, N., Huang, H. S., Baker, S. P., Galdzicka, M., Ginns, E., and Akbarian, S. (2009). Molecular determinants of dysregulated GABAergic gene expression in the prefrontal cortex of subjects with schizophrenia. *Biol. Psychiatry* 65, 1006–1014. doi: 10.1016/j.biopsych.2008.11.019

Mellios, N., and Sur, M. (2012). The emerging role of microRNAs in schizophrenia and autism spectrum disorders. *Front. Psychiatry* 3:39. doi: 10.3389/fpsyt.2012.00039

Meltzer, H. Y. (1992). The importance of serotonin-dopamine interactions in the action of clozapine. *Br. J. Psychiatry. Suppl.* 17, 22–29.

Meltzer, H. Y., and Stahl, S. M. (1976). The dopamine hypothesis of schizophrenia: a review. *Schizophr. Bull.* 2, 19–76. doi: 10.1093/schbul/2.1.19

Meza-Sosa, K. F., Valle-Garcia, D., Pedraza-Alva, G., and Perez-Martinez, L. (2012). Role of microRNAs in central nervous system development and pathology. *J. Neurosci. Res.* 90, 1–12. doi: 10.1002/jnr.22701

Middleton, F. A., Mirnics, K., Pierri, J. N., Lewis, D. A., and Levitt, P. (2002). Gene expression profiling reveals alterations of specific metabolic pathways in schizophrenia. *J. Neurosci.* 22, 2718–2729.

Mineur, Y. S., Obayemi, A., Wigestrand, M. B., Fote, G. M., Calarco, C. A., Li, A. M., et al. (2013). Cholinergic signaling in the hippocampus regulates social stress resilience and anxiety- and depression-like behavior. *Proc. Natl. Acad. Sci. U.S.A.* 110, 3573–3578. doi: 10.1073/pnas.1219731110

Miyaguchi, K. (2004). Localization of selenium-binding protein at the tips of rapidly extending protrusions. *Histochem. Cell Biol.* 121, 371–376. doi: 10.1007/s00418-004-0623-y

Miyake, N., Thompson, J., Skinbjerg, M., and Abi-Dargham, A. (2011). Presynaptic dopamine in schizophrenia. *CNS Neurosci. Ther.* 17, 104–109. doi: 10.1111/j.1755-5949.2010.00230.x

Mor, E., Kano, S., Colantuoni, C., Sawa, A., Navon, R., and Shomron, N. (2013). MicroRNA-382 expression is elevated in the olfactory neuroepithelium of schizophrenia patients. *Neurobiol. Dis.* 55, 1–10. doi: 10.1016/j.nbd.2013.03.011

Mossner, R., Schuhmacher, A., Wagner, M., Quednow, B. B., Frommann, I., Kuhn, K. U., et al. (2010). DAOA/G72 predicts the progression of prodromal syndromes to first episode psychosis. *Eur. Arch. Psychiatry Clin. Neurosci.* 260, 209–215. doi: 10.1007/s00406-009-0044-y

Munafo, M. R., Thiselton, D. L., Clark, T. G., and Flint, J. (2006). Association of the NRG1 gene and schizophrenia: a meta-analysis. *Mol. Psychiatry* 11, 539–546. doi: 10.1038/sj.mp.4001817

Murata, M., Tsunoda, M., Sumiyoshi, T., Sumiyoshi, C., Matsuoka, T., Suzuki, M., et al. (2008). Calcineurin A gamma and B gene expressions in the whole blood in Japanese patients with schizophrenia. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 32, 1000–1004. doi: 10.1016/j.pnpbp.2008.01.013

Nakata, K., Lipska, B. K., Hyde, T. M., Ye, T., Newburn, E. N., Morita, Y., et al. (2009). DISC1 splice variants are upregulated in schizophrenia and associated with risk polymorphisms. *Proc. Natl. Acad. Sci. U.S.A.* 106, 15873–15878. doi: 10.1073/pnas.0903413106

Narayan, S., Tang, B., Head, S. R., Gilmartin, T. J., Sutcliffe, J. G., Dean, B., et al. (2008). Molecular profiles of schizophrenia in the CNS at different stages of illness. *Brain Res.* 1239, 235–248. doi: 10.1016/j.brainres.2008.08.023

Nave, K. A. (2010). Myelination and support of axonal integrity by glia. *Nature* 468, 244–252. doi: 10.1038/nature09614

Ngan, E. T., Yatham, L. N., Ruth, T. J., and Liddle, P. F. (2000). Decreased serotonin 2A receptor densities in neuroleptic-naive patients with schizophrenia: a PET study using [(18)F]setoperone. *Am. J. Psychiatry* 157, 1016–1018. doi: 10.1176/appi.ajp.157.6.1016

Nih. (2001). Biomarkers and surrogate endpoints: preferred definitions and conceptual framework. *Clin. Pharmacol. Ther.* 69, 89–95. doi: 10.1067/mcp.2001.113989

Nord, M., and Farde, L. (2011). Antipsychotic occupancy of dopamine receptors in schizophrenia. *CNS Neurosci. Ther.* 17, 97–103. doi: 10.1111/j.1755-5949.2010.00222.x

Nordstrom, A. L., Farde, L., Eriksson, L., and Halldin, C. (1995). No elevated D2 dopamine receptors in neuroleptic-naive schizophrenic patients revealed by positron emission tomography and [11C]N-methylspiperone. *Psychiatry Res.* 61, 67–83. doi: 10.1016/0925-4927(95)02732-D

Okochi, T., Ikeda, M., Kishi, T., Kawashima, K., Kinoshita, Y., Kitajima, T., et al. (2009). Meta-analysis of association between genetic variants in COMT and schizophrenia: an update. *Schizophr. Res.* 110, 140–148. doi: 10.1016/j.schres.2009.02.019

Okubo, Y., Suhara, T., Suzuki, K., Kobayashi, K., Inoue, O., Terasaki, O., et al. (1997). Decreased prefrontal dopamine D1 receptors in schizophrenia revealed by PET. *Nature* 385, 634–636. doi: 10.1038/385634a0

Oliet, S. H., and Mothet, J. P. (2009). Regulation of N-methyl-D-aspartate receptors by astrocytic D-serine. *Neuroscience* 158, 275–283. doi: 10.1016/j.neuroscience.2008.01.071

Olney, J. W., and Farber, N. B. (1995). Glutamate receptor dysfunction and schizophrenia. *Arch. Gen. Psychiatry* 52, 998–1007. doi: 10.1001/archpsyc.1995.03950240016004

Palmer, B. A., Pankratz, V. S., and Bostwick, J. M. (2005). The lifetime risk of suicide in schizophrenia: a reexamination. *Arch. Gen. Psychiatry* 62, 247–253. doi: 10.1001/archpsyc.62.3.247

Palmer, C., Diehn, M., Alizadeh, A. A., and Brown, P. O. (2006). Cell-type specific gene expression profiles of leukocytes in human peripheral blood. *BMC Genomics* 7:115. doi: 10.1186/1471-2164-7-115

Parlapani, E., Schmitt, A., Wirths, O., Bauer, M., Sommer, C., Rueb, U., Skowronek, M. H., et al. (2010). Gene expression of neuregulin-1 isoforms in different brain regions of elderly schizophrenia patients. *World J. Biol. Psychiatry* 11, 243–250. doi: 10.3109/15622970802022376

Pearlson, G. D., Wong, D. F., Tune, L. E., Ross, C. A., Chase, G. A., Links, J. M., et al. (1995). *In vivo* D2 dopamine receptor density in psychotic and nonpsychotic patients with bipolar disorder. *Arch. Gen. Psychiatry* 52, 471–477. doi: 10.1001/archpsyc.1995.03950180057008

Perkins, D. O., Jeffries, C. D., Jarskog, L. F., Thomson, J. M., Woods, K., Newman, M. A., et al. (2007). microRNA expression in the prefrontal cortex of individuals with schizophrenia and schizoaffective disorder. *Genome Biol.* 8, R27. doi: 10.1186/gb-2007-8-2-r27

Perl, O., Ilani, T., Strous, R. D., Lapidus, R., and Fuchs, S. (2003). The alpha7 nicotinic acetylcholine receptor in schizophrenia: decreased mRNA levels in peripheral blood lymphocytes. *FASEB J.* 17, 1948–1950. doi: 10.1096/fj.03-0104fje

Perl, O., Strous, R. D., Dranikov, A., Chen, R., and Fuchs, S. (2006). Low levels of alpha7-nicotinic acetylcholine receptor mRNA on peripheral blood lymphocytes in schizophrenia and its association with illness severity. *Neuropsychobiology* 53, 88–93. doi: 10.1159/000091725

Petryshen, T. L., Middleton, F. A., Kirby, A., Aldinger, K. A., Purcell, S., Tahl, A. R., et al. (2005). Support for involvement of neuregulin 1 in schizophrenia pathophysiology. *Mol. Psychiatry* 10, 366–374, 328. doi: 10.1038/sj.mp.4001661

Pilowsky, L. S., Bressan, R. A., Stone, J. M., Erlandsson, K., Mulligan, R. S., Krystal, J. H., et al. (2006). First *in vivo* evidence of an NMDA receptor deficit in medication-free schizophrenic patients. *Mol. Psychiatry* 11, 118–119. doi: 10.1038/sj.mp.4001751

Pomarol-Clotet, E., Honey, G. D., Murray, G. K., Corlett, P. R., Absalom, A. R., Lee, M., et al. (2006). Psychological effects of ketamine in healthy volunteers. Phenomenological study. *Br. J. Psychiatry* 189, 173–179. doi: 10.1192/bjp.bp.105.015263

Pongrac, J., Middleton, F. A., Lewis, D. A., Levitt, P., and Mirnics, K. (2002). Gene expression profiling with DNA microarrays: advancing our understanding of psychiatric disorders. *Neurochem. Res.* 27, 1049–1063. doi: 10.1023/A:1020904821237

Prabakaran, S., Swatton, J. E., Ryan, M. M., Huffaker, S. J., Huang, J. T., Griffin, J. L., et al. (2004). Mitochondrial dysfunction in schizophrenia: evidence for compromised brain metabolism and oxidative stress. *Mol. Psychiatry* 9, 684–697, 643. doi: 10.1038/sj.mp.4001511

Radich, J. P., Mao, M., Stepaniants, S., Biery, M., Castle, J., Ward, T., et al. (2004). Individual-specific variation of gene expression in peripheral blood leukocytes. *Genomics* 83, 980–988. doi: 10.1016/j.ygeno.2003.12.013

Raedler, T. J., Knable, M. B., Jones, D. W., Urbina, R. A., Gorey, J. G., Lee, K. S., et al. (2003). *In vivo* determination of muscarinic acetylcholine receptor availability in schizophrenia. *Am. J. Psychiatry* 160, 118–127. doi: 10.1176/appi.ajp.160.1.118

Rao, J. S., Kellom, M., Reese, E. A., Rapoport, S. I., and Kim, H. W. (2012). Dysregulated glutamate and dopamine transporters in postmortem frontal cortex from bipolar and schizophrenic patients. *J. Affect. Disord.* 136, 63–71. doi: 10.1016/j.jad.2011.08.017

Rasmussen, H., Erritzoe, D., Andersen, R., Ebdrup, B. H., Aggernaes, B., Oranje, B., et al. (2010). Decreased frontal serotonin2A receptor binding in antipsychotic-naive patients with first-episode schizophrenia. *Arch. Gen. Psychiatry* 67, 9–16. doi: 10.1001/archgenpsychiatry.2009.176

Ren, Y., Wang, H., and Xiao, L. (2013). Improving myelin/oligodendrocyte-related dysfunction: a new mechanism of antipsychotics in the treatment of schizophrenia. *Int. J. Neuropsychopharmacol.* 16, 691–700. doi: 10.1017/S1461145712001095

Reynolds, G. P., Riederer, P., Jellinger, K., and Gabriel, E. (1981). Dopamine receptors and schizophrenia: the neuroleptic drug problem. *Neuropharmacology* 20, 1319–1320.

Rinne, J. O., Hietala, J., Ruotsalainen, U., Sako, E., Laihinen, A., Nagren, K., et al. (1993). Decrease in human striatal dopamine D2 receptor density with age: a PET study with [11C]raclopride. *J. Cereb. Blood Flow Metab.* 13, 310–314. doi: 10.1038/jcbfm.1993.39

Rosa, A., Gardner, M., Cuesta, M. J., Peralta, V., Fatjo-Vilas, M., Miret, S., et al. (2007). Family-based association study of neuregulin-1 gene and psychosis in a Spanish sample. *Am. J. Med. Genet. B Neuropsychiatr. Genet.* 144B, 954–957. doi: 10.1002/ajmg.b.30511

Santarelli, D. M., Beveridge, N. J., Tooney, P. A., and Cairns, M. J. (2011). Upregulation of dicer and microRNA expression in the dorsolateral prefrontal cortex Brodmann area 46 in schizophrenia. *Biol. Psychiatry* 69, 180–187. doi: 10.1016/j.biopsych.2010.09.030

Sarras, H., Semeralul, M. O., Fadel, M. P., Feldcamp, L. A., Labrie, V., and Wong, A. H. (2010). Elevated PICK1 mRNA in schizophrenia increased SRR mRNA in suicide. *Schizophr. Res.* 120, 236–237. doi: 10.1016/j.schres.2010.03.002

Scarr, E., Beneyto, M., Meador-Woodruff, J. H., and Dean, B. (2005). Cortical glutamatergic markers in schizophrenia. *Neuropsychopharmacology* 30, 1521–1531. doi: 10.1038/sj.npp.1300758

Scarr, E., Cowie, T. F., Kanellakis, S., Sundram, S., Pantelis, C., and Dean, B. (2009). Decreased cortical muscarinic receptors define a subgroup of subjects with schizophrenia. *Mol. Psychiatry* 14, 1017–1023. doi: 10.1038/mp.2008.28

Scarr, E., Sundrarn, S., Keriakous, D., and Dean, B. (2007). Altered hippocampal muscarinic M4, but not M1, receptor expression from subjects with schizophrenia. *Biol. Psychiatry* 61, 1161–1170. doi: 10.1016/j.biopsych.2006.08.050

Schizophrenia Psychiatric Genome-Wide Association Study, Consortium. (2011). Genome-wide association study identifies five new schizophrenia loci. *Nat. Genet.* 43, 969–976. doi: 10.1038/ng.940

Schreiber, S., Bernstein, H. G., Fendrich, R., Stauch, R., Ketzler, B., Dobrowolny, H., et al. (2011). Increased density of GAD65/67 immunoreactive neurons in the posterior subiculum and parahippocampal gyrus in treated patients with chronic schizophrenia. *World J. Biol. Psychiatry* 12, 57–65. doi: 10.3109/15622975.2010.539270

Seeman, P., Bzowej, N. H., Guan, H. C., Bergeron, C., Reynolds, G. P., Bird, E. D., et al. (1987). Human brain D1 and D2 dopamine receptors in schizophrenia, Alzheimer's, Parkinson's, and Huntington's diseases. *Neuropsychopharmacology* 1, 5–15. doi: 10.1016/0893-133X(87)90004-2

Sei, Y., Ren-Patterson, R., Li, Z., Tunbridge, E. M., Egan, M. F., Kolachana, B. S., et al. (2007). Neuregulin1-induced cell migration is impaired in schizophrenia: association with neuregulin1 and catechol-o-methyltransferase gene polymorphisms. *Mol. Psychiatry* 12, 946–957. doi: 10.1038/sj.mp.4001994

Shekhar, A., Potter, W. Z., Lightfoot, J., Lienemann, J., Dube, S., Mallinckrodt, C., et al. (2008). Selective muscarinic receptor agonist xanomeline as a novel treatment approach for schizophrenia. *Am. J. Psychiatry* 165, 1033–1039. doi: 10.1176/appi.ajp.2008.06091591

Shifman, S., Bronstein, M., Sternfeld, M., Pisante-Shalom, A., Lev-Lehman, E., Weizman, A., et al. (2002). A highly significant association between a COMT haplotype and schizophrenia. *Am. J. Hum. Genet.* 71, 1296–1302. doi: 10.1086/344514

Shiohama, A., Sasaki, T., Noda, S., Minoshima, S., and Shimizu, N. (2003). Molecular cloning and expression analysis of a novel gene DGCR8 located in the DiGeorge syndrome chromosomal region. *Biochem. Biophys. Res. Commun.* 304, 184–190. doi: 10.1016/S0006-291X(03)00554-0

Smrt, R. D., Szulwach, K. E., Pfeiffer, R. L., Li, X., Guo, W., Pathania, M., et al. (2010). MicroRNA miR-137 regulates neuronal maturation by targeting ubiquitin ligase mind bomb-1. *Stem Cells* 28, 1060–1070. doi: 10.1002/stem.431

Stanley, M., and Mann, J. J. (1983). Increased serotonin-2 binding sites in frontal cortex of suicide victims. *Lancet* 1, 214–216. doi: 10.1016/S0140-6736(83)92590-4

Steffek, A. E., Haroutunian, V., and Meador-Woodruff, J. H. (2006). Serine racemase protein expression in cortex and hippocampus in schizophrenia. *Neuroreport* 17, 1181–1185. doi: 10.1097/01.wnr.0000230512.01339.72

Sun, X., Wang, J. F., Tseng, M., and Young, L. T. (2006). Downregulation in components of the mitochondrial electron transport chain in the postmortem frontal cortex of subjects with bipolar disorder. *J. Psychiatry Neurosci.* 31, 189–196.

Tan, P. K., Downey, T. J., Spitznagel, E. L. Jr., Xu, P., Fu, D., Dimitrov, D. S., et al. (2003). Evaluation of gene expression measurements from commercial microarray platforms. *Nucleic Acids Res.* 31, 5676–5684. doi: 10.1093/nar/gkg763

Tang, B., Capitao, C., Dean, B., and Thomas, E. A. (2012). Differential age- and disease-related effects on the expression of genes related to the arachidonic acid signaling pathway in schizophrenia. *Psychiatry Res.* 196, 201–206. doi: 10.1016/j.psychres.2011.09.026

Tang, B., Chang, W. L., Lanigan, C. M., Dean, B., Sutcliffe, J. G., and Thomas, E. A. (2009). Normal human aging and early-stage schizophrenia share common molecular profiles. *Aging Cell* 8, 339–342. doi: 10.1111/j.1474-9726.2009.00468.x

Tateno, A., Arakawa, R., Okumura, M., Fukuta, H., Honjo, K., Ishihara, K., et al. (2013). Striatal and extrastriatal dopamine D2 receptor occupancy by a novel antipsychotic, blonanserin: a PET study with [11C]Raclopride and [11C]FLB 457 in schizophrenia. *J. Clin. Psychopharmacol.* 33, 162–169. doi: 10.1097/JCP.0b013e3182825bce

Teaktong, T., Piggott, M. A., McKeith, I. G., Perry, R. H., Ballard, C. G., and Perry, E. K. (2005). Muscarinic M2 and M4 receptors in anterior cingulate cortex: relation to neuropsychiatric symptoms in dementia with Lewy bodies. *Behav. Brain Res.* 161, 299–305. doi: 10.1016/j.bbr.2005.02.019

Teyssier, J. R., Ragot, S., Chauvet-Gelinier, J. C., Trojak, B., and Bonin, B. (2011). Psychotic features in major depressive disorder are not associated with changes in expression of psychosis related genes in the prefrontal cortex. *Psychiatry Res.* 186, 156–157. doi: 10.1016/j.psychres.2010.06.028

Thiselton, D. L., Webb, B. T., Neale, B. M., Ribble, R. C., O'neill, F. A., Walsh, D., et al. (2004). No evidence for linkage or association of neuregulin-1 (NRG1) with disease in the Irish study of high-density schizophrenia families (ISHDSF). *Mol. Psychiatry* 9, 777–783; image 729.

Thompson, M., Weickert, C. S., Wyatt, E., and Webster, M. J. (2011). Decreased BDNF, trkB-TK+ and GAD(67) mRNA expression in the hippocampus of individuals with schizophrenia and mood disorders. *J. Psychiatry Neurosci.* 36, 195–203. doi: 10.1503/jpn.100048

Tkachev, D., Mimmack, M. L., Huffaker, S. J., Ryan, M., and Bahn, S. (2007). Further evidence for altered myelin biosynthesis and glutamatergic dysfunction in schizophrenia. *Int. J. Neuropsychopharmacol.* 10, 557–563. doi: 10.1017/S1461145706007334

Tkachev, D., Mimmack, M. L., Ryan, M. M., Wayland, M., Freeman, T., Jones, P. B., et al. (2003).

Oligodendrocyte dysfunction in schizophrenia and bipolar disorder. *Lancet* 362, 798–805. doi: 10.1016/S0140-6736(03)14289-4

Torres, K. C., Souza, B. R., Miranda, D. M., Nicolato, R., Neves, F. S., Barros, A. G., et al. (2009). The leukocytes expressing DARPP-32 are reduced in patients with schizophrenia and bipolar disorder. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 33, 214–219. doi: 10.1016/j.pnpbp.2008.10.020

Treutlein, J., Muhleisen, T. W., Frank, J., Mattheisen, M., Herms, S., Ludwig, K. U., et al. (2009). Dissection of phenotype reveals possible association between schizophrenia and Glutamate Receptor Delta 1 (GRID1) gene promoter. *Schizophr. Res.* 111, 123–130. doi: 10.1016/j.schres.2009.03.011

Tsai, G., Passani, L. A., Slusher, B. S., Carter, R., Baer, L., Kleinman, J. E., et al. (1995). Abnormal excitatory neurotransmitter metabolism in schizophrenic brains. *Arch. Gen. Psychiatry* 52, 829–836. doi: 10.1001/archpsyc.1995.03950220039008

Tsang, S. W. Y., Francis, P. T., Esiri, M. M., Wong, P. T. H., Chen, C., and Lai, M. K. P. (2008). Loss of (3)H 4-DAMP binding to muscarinic receptors in the orbitofrontal cortex of Alzheimer's disease patients with psychosis. *Psychopharmacology* 198, 251–259. doi: 10.1007/s00213-008-1124-9

Tunbridge, E. M., Harrison, P. J., and Weinberger, D. R. (2006). Catechol-o-methyltransferase, cognition, and psychosis: Val158Met and beyond. *Biol. Psychiatry* 60, 141–151. doi: 10.1016/j.biopsych.2005.10.024

Tune, L. E., Wong, D. F., Pearlson, G., Strauss, M., Young, T., Shaya, E. K., et al. (1993). Dopamine D2 receptor density estimates in schizophrenia: a positron emission tomography study with 11C-N-methylspiperone. *Psychiatry Res.* 49, 219–237. doi: 10.1016/0165-1781(93)90063-M

Tune, L., Barta, P., Wong, D., Powers, R. E., Pearlson, G., Tien, A. Y., et al. (1996). Striatal dopamine D2 receptor quantification and superior temporal gyrus: volume determination in 14 chronic schizophrenic subjects. *Psychiatry Res.* 67, 155–158. doi: 10.1016/0925-4927(96)02728-X

Turecki, G., Briere, R., Dewar, K., Antonetti, T., Lesage, A. D., Seguin, M., et al. (1999). Prediction of level of serotonin 2A receptor binding by serotonin receptor 2A genetic variation in postmortem brain samples from subjects who did or did not commit suicide. *Am. J. Psychiatry* 156, 1456–1458.

Uranova, N. A., Vikhreva, O. V., Rachmanova, V. I., and Orlovskaya, D. D. (2011). Ultrastructural alterations of myelinated fibers and oligodendrocytes in the prefrontal cortex in schizophrenia: a postmortem morphometric study. *Schizophr. Res. Treatment* 2011, 325789. doi: 10.1155/2011/325789

van Beveren, N. J., Buitendijk, G. H., Swagemakers, S., Krab, L. C., Roder, C., De Haan, L., et al. (2012). Marked reduction of AKT1 expression and deregulation of AKT1-associated pathways in peripheral blood mononuclear cells of schizophrenia patients. *PLoS ONE* 7:e32618. doi: 10.1371/journal.pone.0032618

Vawter, M. P., Ferran, E., Galke, B., Cooper, K., Bunney, W. E., and Byerley, W. (2004). Microarray screening of lymphocyte gene expression differences in a multiplex schizophrenia pedigree. *Schizophr. Res.* 67, 41–52. doi: 10.1016/S0920-9964(03)00151-8

Veldic, M., Guidotti, A., Maloku, E., Davis, J. M., and Costa, E. (2005). In psychosis, cortical interneurons overexpress DNA-methyltransferase 1. *Proc. Natl. Acad. Sci. U.S.A.* 102, 2152–2157. doi: 10.1073/pnas.0409665102

Verhoeff, N. P., Soares, J. C., D'souza, C. D., Gil, R., Degen, K., Abi-Dargham, A., et al. (1999). [123I]Iomazenil SPECT benzodiazepine receptor imaging in schizophrenia. *Psychiatry Res.* 91, 163–173. doi: 10.1016/S0925-4927(99)00027-X

Verrall, L., Walker, M., Rawlings, N., Benzel, I., Kew, J. N., Harrison, P. J., et al. (2007). d-Amino acid oxidase and serine racemase in human brain: normal distribution and altered expression in schizophrenia. *Eur. J. Neurosci.* 26, 1657–1669. doi: 10.1111/j.1460-9568.2007.05769.x

Vik-Mo, A. O., Birkenaes, A. B., Ferno, J., Jonsdottir, H., Andreassen, O. A., and Steen, V. M. (2008). Increased expression of lipid biosynthesis genes in peripheral blood cells of olanzapine-treated patients. *Int. J. Neuropsychopharmacol.* 11, 679–684. doi: 10.1017/S1461145708008468

Volk, D. W., Austin, M. C., Pierri, J. N., Sampson, A. R., and Lewis, D. A. (2000). Decreased glutamic acid decarboxylase67 messenger RNA expression in a subset of prefrontal cortical gamma-aminobutyric acid neurons in subjects with schizophrenia. *Arch. Gen. Psychiatry* 57, 237–245. doi: 10.1001/archpsyc.57.3.237

Vollenweider, F. X., and Geyer, M. A. (2001). A systems model of altered consciousness: integrating natural and drug-induced psychoses. *Brain Res. Bull.* 56, 495–507. doi: 10.1016/S0361-9230(01)00646-3

Walterfang, M., Velakoulis, D., Whitford, T. J., and Pantelis, C. (2011). Understanding aberrant white matter development in schizophrenia: an avenue for therapy. *Expert Rev. Neurother.* 11, 971–987. doi: 10.1586/ern.11.76

Wang, J. C., Hinrichs, A. L., Stock, H., Budde, J., Allen, R., Bertelsen, S., et al. (2004). Evidence of common and specific genetic effects: association of the muscarinic acetylcholine receptor M2 (CHRM2) gene with alcohol dependence and major depressive syndrome. *Hum. Mol. Genet.* 13, 1903–1911. doi: 10.1093/hmg/ddh194

Weickert, C. S., Straub, R. E., Mcclintock, B. W., Matsumoto, M., Hashimoto, R., Hyde, T. M., et al. (2004). Human dysbindin (DTNBP1) gene expression in normal brain and in schizophrenic prefrontal cortex and midbrain. *Arch. Gen. Psychiatry* 61, 544–555. doi: 10.1001/archpsyc.61.6.544

Williams, H. J., Owen, M. J., and O'donovan, M. C. (2007). Is COMT a susceptibility gene for schizophrenia. *Schizophr. Bull.* 33, 635–641. doi: 10.1093/schbul/sbm019

Woo, T. U., Kim, A. M., and Viscidi, E. (2008). Disease-specific alterations in glutamatergic neurotransmission on inhibitory interneurons in the prefrontal cortex in schizophrenia. *Brain Res.* 1218, 267–277. doi: 10.1016/j.brainres.2008.03.092

Woo, T. U., Walsh, J. P., and Benes, F. M. (2004). Density of glutamic acid decarboxylase 67 messenger RNA-containing neurons that express the N-methyl-D-aspartate receptor subunit NR2A in the anterior cingulate cortex in schizophrenia and bipolar disorder. *Arch. Gen. Psychiatry* 61, 649–657. doi: 10.1001/archpsyc.61.7.649

Woolley, M. L., Carter, H. J., Gartlon, J. E., Watson, J. M., and Dawson, L. A. (2009). Attenuation of amphetamine-induced activity by the non-selective muscarinic receptor agonist, xanomeline, is absent in muscarinic M-4 receptor knockout mice and attenuated in muscarinic M-1 receptor knockout mice. *Eur. J. Pharmacol.* 603, 147–149. doi: 10.1016/j.ejphar.2008.12.020

Xu, B., Roos, J. L., Levy, S., Van Rensburg, E. J., Gogos, J. A., and Karayiorgou, M. (2008). Strong association of de novo copy number mutations with sporadic schizophrenia. *Nat. Genet.* 40, 880–885. doi: 10.1038/ng.162

Xu, J., Sun, J., Chen, J., Wang, L., Li, A., Helm, M., et al. (2012). RNA-Seq analysis implicates dysregulation of the immune system in schizophrenia. *BMC Genomics* 13(Suppl. 8):S2. doi: 10.1186/1471-2164-13-S8-S2

Yamamori, H., Hashimoto, R., Verrall, L., Yasuda, Y., Ohi, K., Fukumoto, M., et al. (2011). Dysbindin-1 and NRG-1 gene expression in immortalized lymphocytes from patients with schizophrenia. *J. Hum. Genet.* 56, 478–483. doi: 10.1038/jhg.2011.40

Yao, Y., Schroder, J., and Karlsson, H. (2008). Verification of proposed peripheral biomarkers in mononuclear cells of individuals with schizophrenia. *J. Psychiatr. Res.* 42, 639–643. doi: 10.1016/j.jpsychires.2007.07.011

Zavitsanou, K., Katsifis, A., Yu, Y. H., and Huang, X. F. (2005). M2/M4 muscarinic receptor binding in the anterior cingulate cortex in schizophrenia and mood disorders. *Brain Res. Bull.* 65, 397–403. doi: 10.1016/j.brainresbull.2005.02.007

Zhan, L., Kerr, J. R., Lafuente, M. J., Maclean, A., Chibalina, M. V., Liu, B., et al. (2011). Altered expression and coregulation of dopamine signalling genes in schizophrenia and bipolar disorder. *Neuropathol. Appl. Neurobiol.* 37, 206–219. doi: 10.1111/j.1365-2990.2010.01128.x

Zhang, H. X., Zhao, J. P., Lv, L. X., Li, W. Q., Xu, L., Ouyang, X., et al. (2008). Explorative study on the expression of neuregulin-1 gene in peripheral blood of schizophrenia. *Neurosci. Lett.* 438, 1–5. doi: 10.1016/j.neulet.2007.09.051

Zhang, W. L., Basile, A. S., Gomeza, J., Volpicelli, L. A., Levey, A. I., and Wess, J. (2002). Characterization of central inhibitory muscarinic autoreceptors by the use of muscarinic acetylcholine receptor knock-out mice. *J. Neurosci.* 22, 1709–1717.

Zhang, H. X., Zhao, J. P., Lv, L. X., Li, W. Q., Xu, L., Ouyang, X., et al. (2008). Explorative study on the expression of neuregulin-1 gene in peripheral blood of schizophrenia. *Neurosci. Lett.* 438, 1–5. doi: 10.1016/j.neulet.2007.09.051

Zhao, Z., Nair, S. M., Chou, D. K., Tobet, S. A., and Jungalwala, F. B. (2000). Expression and role of

sulfoglucuronyl (HNK-1) carbohydrate and its binding protein SBP-1 in developing rat cerebral cortex. *J. Neurosci. Res.* 62, 186–205. doi: 10.1002/1097-4547(20001015)62:2<186::AID-JNR4>3.0.CO;2-C

Zhu, Y., Kalbfleisch, T., Brennan, M. D., and Li, Y. (2009). A MicroRNA gene is hosted in an intron of a schizophrenia-susceptibility gene. *Schizophr. Res.* 109, 86–89. doi: 10.1016/j.schres.2009.01.022

Zvara, A., Szekeres, G., Janka, Z., Kelemen, J. Z., Cimmer, C., Santha, M., et al. (2005). Over-expression of dopamine D2 receptor and inwardly rectifying potassium channel genes in drug-naive schizophrenic peripheral blood lymphocytes as potential diagnostic markers. *Dis Markers* 21, 61–69.

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Impact of structural aberrancy of polysialic acid and its synthetic enzyme ST8SIA2 in schizophrenia

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Psychiatric disorders are a group of human diseases that impair higher cognitive functions. Whole-genomic analyses have recently identified susceptibility genes for several psychiatric disorders, including schizophrenia. Among the genes reported to be involved in psychiatric disorders, a gene encoding a polysialyltransferase involved in the biosynthesis of polysialic acid (polySia or PSA) on cell surfaces has attracted attention for its potential role in emotion, learning, memory, circadian rhythm, and behaviors. PolySia is a unique polymer that spatio-temporally modifies neural cell adhesion molecule (NCAM) and is predominantly found in embryonic brains, although it persists in areas of the adult brain where neural plasticity, remodeling of neural connections, or neural generation is ongoing, such as the hippocampus, subventricular zone (SVZ), thalamus, prefrontal cortex, and amygdala. PolySia is thought to be involved in the regulation of cell-cell interactions; however, recent evidence suggests that it is also involved in the functional regulation of ion channels and neurologically active molecules, such as Brain-derived neurotrophic factor (BDNF), FGF2, and dopamine (DA) that are deeply involved in psychiatric disorders. In this review, the possible involvement of polysialyltransferase (ST8SIA2/ST8SiaII/STX/Siat8B) and its enzymatic product, polySia, in schizophrenia is discussed.

Keywords: schizophrenia, psychiatric disorder, polysialic acid, polysialyltransferase, ST8SIA2, NCAM, BDNF, dopamine

INTRODUCTION

Schizophrenia is a severe and persistent psychiatric disorder that affects approximately one percent of the population worldwide. Although several factors are associated with an increased risk of developing schizophrenia, the underlying mechanism for this disorder remains unclear. The overall risk profile is mainly determined by the presence of causative genes, such as those encoding disrupted-in-schizophrenia 1 (DISC1) (Millar et al., 2001), Neuregulin 1 (Stefansson et al., 2002), COMP (catechol-*o*-methyltransferase) (Strous et al., 1997), and dopamine (DA)-receptors (Allen et al., 2008). Schizophrenia is also deeply related to neurodevelopmental and neurodegenerative diseases involving disconnectivity and disorder of synapses (Woods, 1998; Ashe et al., 2001), which may occur at a restricted stage during early brain development. Despite the identification of these schizophrenia risk factors, studies investigating the specific relationships between genes and phenotypes are needed. In this regard, the contribution of the posttranslational modification of proteins and the presence of membrane glycans, such as glycoproteins, gangliosides, and proteoglycans, are important to consider. However, these factors are often overlooked, even though nearly all cell-surface and extracellular proteins are glycosylated.

Animal cells are covered by the glycocalyx, a dense sugar coat composed of glycoproteins, glycolipids, and proteoglycans that provide hot spots for interaction with other cells and extracellular space components. The glycocalyx has a unique and characteristic structure, particularly in the peripheral region, which is the dominant region for communication with other cells and extracellular factors. Among glycans in the glycocalyx, polysialic acid (polySia, PSA), HNK-1, and sulfated glycans share spatio-temporally regulated expression. In particular, polySia is of considerable recent interest in the field of schizophrenia research, and has been studied using histochemical, genome-wide, and biochemical approaches.

In this review, we describe: (1) the unique features of polySia and its biosynthesis enzyme, ST8SIA2; (2) the newly discovered functions of polySia; and (3) the relationship between polySia and psychiatric disorders, particularly schizophrenia, which have been reported to date.

STRUCTURE AND DISTRIBUTION OF polySia AND ST8SIA2

PolySia is a linear homopolymer of sialic acid with a degree of polymerization (DP) ranging from 8 to 400 (**Figure 1A**) (Troy, 1996) and was first found as part of polysaccharide chains on the cell surface of neuroinvasive bacteria. PolySia is thought to adopt a helical structure (Evans et al., 1995) (**Figure 1B**) and can be identified with specific probes, such as antibodies (mAb. 735 and mAb. 12E3) and endo-*N*-acylneuraminidase (Endo-N) (Troy, 1996; Rutishauser, 2008; Sato, 2013). The expression of polySia in vertebrates is spacio-temporally regulated (Rutishauser, 2008) and is highly restricted to the brain during embryonic and post-neonatal development. In the adult brain, polySia is typically found at very low levels; however, it persists in distinct regions where neural plasticity, remodeling of neural connections, or neural generation is ongoing, such as the hippocampus, subventricular zone (SVZ), thalamus, prefrontal cortex, and amygdala. It is

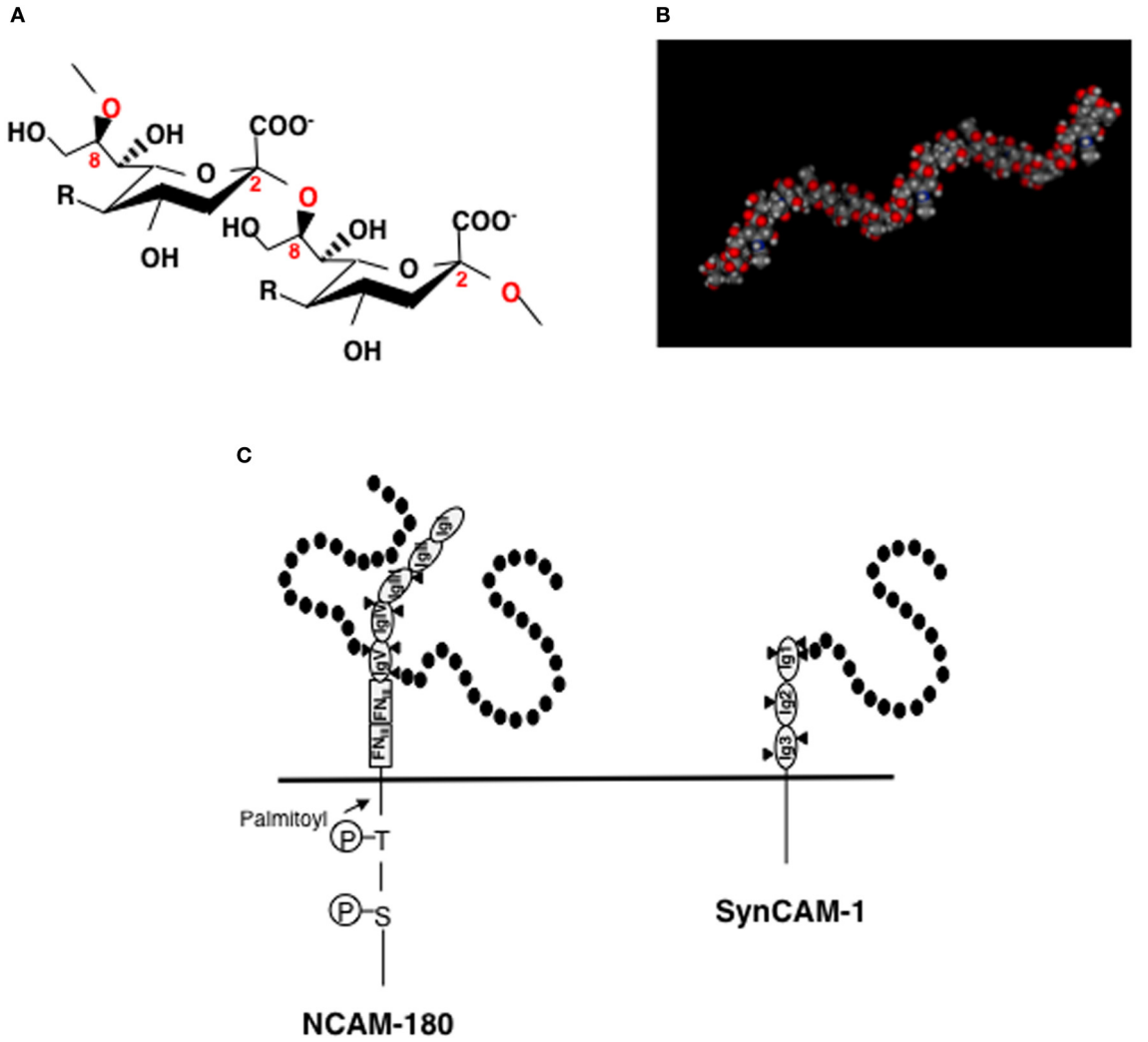

FIGURE 1 | Structures of polySia, polySia-NCAM, and polySia-synCAM. **(A)** α2-8linked polySia structure. R = -NHCOCH$_3$, Neu5Ac; -NHCOCH$_2$OH, Neu5Gc, -OH, KDN. In the brain, polySia is polyNeu5Ac. **(B)** Molecular modeling of polySia (DP = 12). The polySia structure is linked to Gal at the C3-position. Calculated α2,8-linked dodecaNeu5Ac-Gal structure is shown as space-filling models. The MOE program was used for the construction and calculation of the energies of α2,8-linked polySia. **(C)** polySia-NCAM-180 and polySia-synCAM-1. **Left panel**, NCAM-180. NCAM has five immunoglobulin domains (IgI∼IgV) and two fibronectin type-III (FN$_{III}$) domains in its extracellular domain. In the IgV domain, two of the three *N*-glycosylation sites (triangle) are polysialylated and are indicated by black circles (sialic acid). **Right panel**, SynCAM-1. SynCAM-1 has three Ig domains. In the IgI domain, one of the three *N*-glycosylation sites (triangle) is occupied by polySia which is indicated by black circles (sialic acid).

also interesting that polySia expression is restricted to interneurons in different cortical regions, such as the prefrontal cortex and amygdala (Gómez-Climent et al., 2011; Nacher et al., 2013). There are several precise reviews on polySia and polysialylated NCAM (polySia-NCAM) expression in brains (Bonfanti, 2006; Nacher et al., 2013; Sato, 2013). The major carrier protein of polySia in vertebrate brains is neural cell adhesion molecule (NCAM) (Finne et al., 1983). As NCAM expression levels remain relatively unchanged throughout normal development, it is speculated that polySia expression is tightly correlated with that of the polySia biosynthetic enzymes, particularly the polysialyltransferases ST8SIA2 and/or ST8SIA4 (Mühlenhoff et al., 2009). Recently, ST8SIA2 was also demonstrated to modify synaptic cell adhesion molecule 1 (synCAM-1) (also known as Cadm1 or TSLC1) (Rollenhagen et al., 2012). The polysialylation sites on NCAM and synCAM have been well studied. NCAM consists of five immunoglobulin (Ig)-like domains with six *N*-glycosylation sites and two fibronectin type-III (FN$_{III}$)-like -domains in the extracellular region. PolySia chains are linked to the tri- or tetra-antennary *N*-linked glycan chains on Ig domain V of NCAM (**Figure 1C**, left panel). First FN$_{III}$ domain is important for the polysialylation of NCAM (Close et al., 2003). In SynCAM, which consists of three extracellular Ig domains, polySia is linked to a *N*-linked glycan chain on Ig domain I (**Figure 1C**, right panel).

Both ST8SIA2 and ST8SIA4 catalyze the transfer of sialic acid through α2,8-linkages onto sialic acid residues and cooperatively elongate the polySia chain using CMP-Sia as a donor substrate (**Figure 2A**). ST8SIA2 and ST8SIA4, which belong to a family of

sialyltransferases that are part of the glycosyltransferase family, are type II membrane proteins that localize in the Golgi apparatus. The protein structure of ST8SIA2 and ST8SIA4 consists of a short cytoplasmic region connected to a transmembrane (TM) region that is joined to an intra-luminal region (**Figure 2B**). The intra-luminal region consists of a stem region and a catalytic domain, and sialyl motifs L (Large), S (Small), III, and VS (Very Short), which are a common feature of α2,3-, α2,6-, and α2,8-sialyltransferases (Angata and Fukuda, 2003; Takashima, 2008). Sialyl motif L is positioned in the center of the enzyme and is characterized by a 55-amino-acid region that serves as a donor substrate (CMP-Sia) binding site. Sialyl motif S is located at the C-terminal region of the enzyme and consists of 28 amino acid residues that are involved in the binding of both donor and acceptor substrates. Sialyl motif VS (HXXXXEX) is also located in the C-terminal region and is reported to be involved in catalytic activity. Histidine (H) and glutamic acid (E) residues in this motif are highly conserved between sialyltransferases. Motif III (YHYYD) is located between sialyl motif S and VS and is also involved in the catalytic activity of ST8SIA2 and ST8SIA4. In addition, a novel polybasic polysialyltransferase domain (PSTD; 32 amino acids) identified next to the motif S in both sialyltransferases was demonstrated to be involved in the polysialylation activity of ST8SIA4 (Nakata et al., 2006). More recently, a second conserved polybasic motif, named polybasic region (PBR), was identified close to sialyl motif L in ST8SIA2 and ST8SIA4 (Foley et al., 2009). The PBR consists of 35 amino acids, of which seven are the basic amino acids arginine (R) and lysine (K), and is involved in NCAM-specific polysialylation. These basic amino acids are considered to be important for IgV specific polysialylation of NCAM through binding via acidic patch of first FNIII domain.

The human ST8SIA2 gene is located on chromosome 15 (Angata et al., 1997) and the corresponding enzyme consists of six exons (Takashima, 2008). Although the promoter region of the ST8SIA2 gene has not been well examined *in vitro*, the schizophrenia-associated haplotype block of ST8SIA2 appears to localize in the putative promoter region based on database searches (TFSEARCH, http://mbs.cbrc.jp/research/db/TFSEARCHJ.html), which identified several putative consensus motifs and transcriptional factor binding sites, including those for CCAAT, MZF1, CREB, GATA, TATA, and SP1 (Arai et al., 2006). In the mouse genome, ST8SIA2 expression is driven by SP1-binding motifs present in a TATA-less GC-rich domain (Yoshida et al., 1996) and by the cAMP-CREB cascade (Nakagawa et al., 2002). It is also reported that ST8SIA2 is under the control of Pax3 (Mayanil et al., 2000, 2001), a member of a paired homeobox

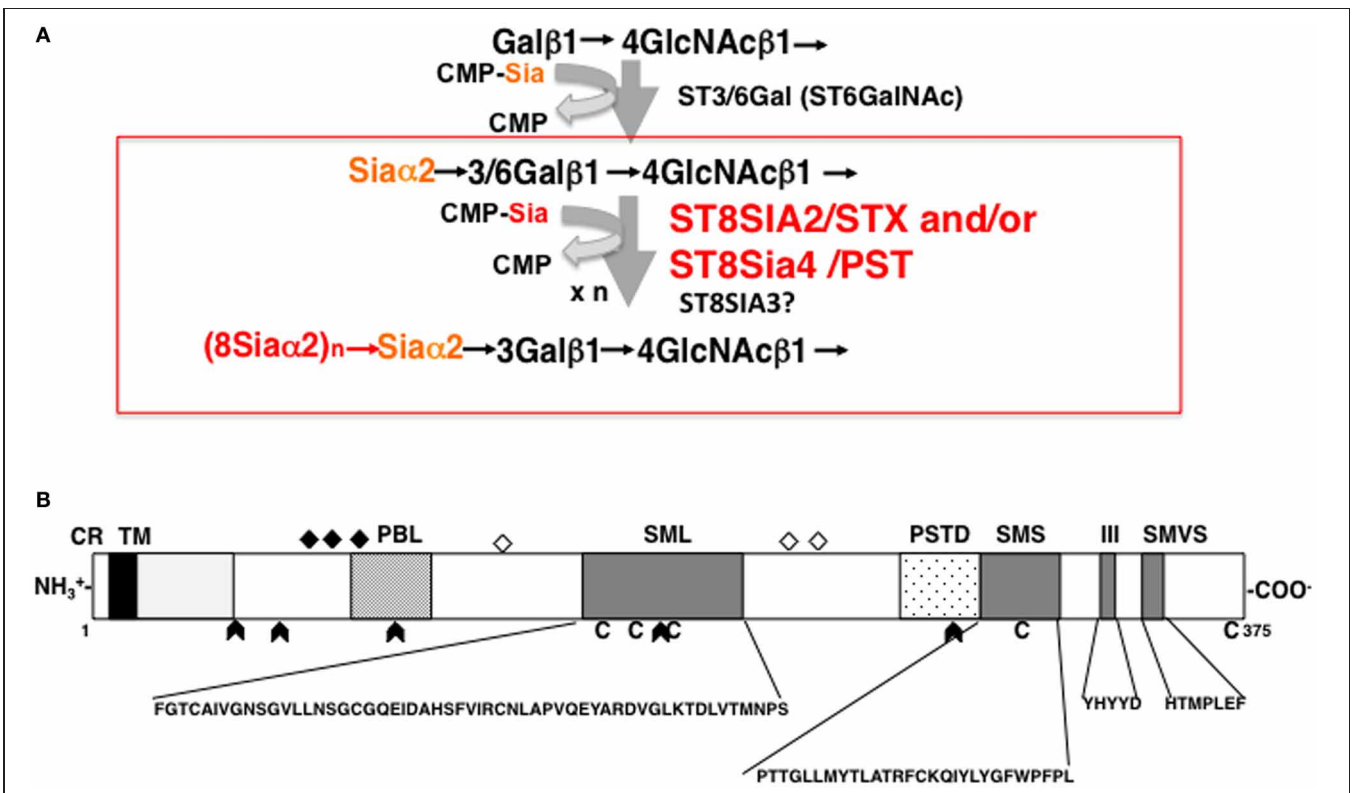

FIGURE 2 | Biosynthesis of polySia and schematic structure of ST8SIA2. **(A)** Mono and di/oligo/polysialylation pathway on the terminal Gal residue of N-glycan chains. Asialo-glycoprotein is mono-sialylated by the action of ST3/6GAL. ST8SIA2, ST8SIA4, and/or ST8SIA2/4 can synthesize polySia chains on the monosialyl residue, particularly on NCAM. **(B)** Schematic representation of the protein structure of ST8SIA2. ST8SIA2 consists of a short cytoplasmic region (CR) connected to a transmembrane (TM) region, which is followed by an intraluminal region consisting of a stem region and catalytic domain. In the catalytic domain, SML (Sialyl Motif Large), SMS (Small), motif III, and SMVS (Very Short) are present. Two additional regions have been newly characterized: a polybasic polysialyltransferase domain (PSTD) and a polybasic motif named the polybasic region (PBR). C, cysteine residues; diamonds, *N*-glycosylation site; open diamonds, autopolysialylation sites (Asn 89, 219, and 234) of six *N*-linked glycans; arrowheads, exon junction.

family of evolutionary conserved transcription factors that are important for brain development.

PHENOTYPES OF polySia-IMPAIRED MICE

To understand the function of polySia, a NCAM-deficient mouse line was established (Cremer et al., 1994), because NCAM is the major carrier of polySia in the brain. Based on the observed phenotype of NCAM knockout (KO) mice, polySia-NCAM was shown to be required for cell migration, neuronal path finding, and synaptic plasticity necessary for memory formation. As polySia-NCAM is highly expressed in suprachiasmatic nuclei (SCN), the effect of polySia on SCN-mediated circadian clock function was analyzed in adult mice. The removal of polySia from SCN by the microinjection of endoneuraminidase (endo-N; polySia specific endo-sialidase) shortened the free-running period to a similar extent as in the NCAM KO mutant, demonstrating that NCAM and polySia are involved in the development and physiology of the mammalian SCN circadian clock (Shen et al., 1997). NCAM-KO mice also exhibit increased anxiety, which is thought to be due to altered serotoninergic transmission (Stork et al., 1999).

To investigate the function of polySia in more detail, ST8SIA2 single KO (SKO) (Angata et al., 2004) and ST8SIA4 SKO mice (Eckhardt et al., 2000) were established. ST8SIA2 SKO mice exhibit misguided infrapyramidal mossy fibers and form ectopic synapses in the hippocampus. Quantification of the myelinated axons in ST8SIA2 SKO mice revealed the number and size of regenerated fibers are significantly decreased, although remyelination is not impaired. In addition, ST8SIA2 SKO mice also exhibit higher exploratory drive and reduced behavioral responses to Pavlovian fear conditioning. The phenotype of ST8SIA4 SKO mice was characterized by a marked decrease of polySia in the CA1 region of Ammon's horn, indicating that ST8SIA4 is involved in hippocampal synaptic plasticity, particularly in long-term potentiation (LTP) and long-term depression (LTD) in the hippocampal CA1 region. Interestingly, both ST8SIA2 and ST8SIA4 SKO mice show a profound impairment in social behavior (Calandreau et al., 2010), including decreased motivation to interact socially. ST8SIA2 SKO mice exhibit a behavioral profile that combines increased aggressive behavior and hyperactivity with reduced anxiety-like behavior, which is similar to certain attention-deficit hyperactivity disorder-related pathologies. In contrast, ST8SIA4 SKO mice are predominantly characterized by decreased motivation in social interaction. This behavior is the result of olfactory deficits and is associated with a clear decrease in polySia-NCAM expression in all brain regions. Recently, precise observations of the polySia-NCAM expression with adult SKO mice especially in cerebral cortex were reported. The facts that ST8SIA4 is a responsible for polySia expression in mature interneurons and in most regions of cortical neuropili and that ST8SIA2 is the main polysialyltransferase in immature neurons of the paleocortex layer II and the hippocampal subgranular zone (Nacher et al., 2010) are important information to understand biological meaning of polysialyltransferases.

As polySia can be biosynthesized by either one of the two polysialyltransferases, SKO mice still contain a large amount of residual polySia in the brain (Oltmann-Norden et al., 2008). Thus, to completely remove the background levels polySia, ST8SIA2, and ST8SIA4 double KO (DKO) mice were established (Weinhold et al., 2005). DKO mice show a severe phenotype, characterized by postnatal growth retardation, precocious death, high incidence of hydrocephalus and agenesis, and hypoplasia of major brain fiber tracts. Because almost all DKO mice die soon after birth (80% die before the age of 4 weeks), it appears that the presence of polySia on not only NCAM, but also other polySia-containing glycoproteins, plays a direct and important role in both brain and other unknown tissue functions. Interestingly, in NCAM, ST8SIA2, and ST8SIA4 triple-KO (TKO) mice, the severe phenotype of the DKO mice is rescued, suggesting that an uncontrolled type of NCAM-mediated cell adhesion is followed by increased signal transduction events. In TKO mice, improved signaling via increased cell-cell interactions in the polySia-deficient brain is likely to result from the reduced levels of cell adhesion molecules resulting from the NCAM deficiency. Thus, the reduction of NCAM leads to the recovery of normal physiological interactions and to the rescue of the severe phenotype of polySia-DKO mice (Hildebrandt et al., 2007).

KNOWN FUNCTIONS OF polySia

It is well known that polySia is involved in numerous important neurological functions, including neural outgrowth, cell migration, axonal guidance, and branching, neuronal pathfinding, lamination of mossy fibers, LTP, and LTD in the hippocampus, synapse formation, and plasticity. The expression of polySia is spatio-temporally regulated and intimately influences neurogenesis and neural circuit development. Thus, the anomalous expression of polySia impairs learning, memory, behavior (fear and social behavior), and circadian clock rhythm. There are several excellent reviews on the role of polySia in different aspects of neural development, plasticity, and repair (Bonfanti, 2006; Gascon et al., 2007; Hildebrandt et al., 2007; Rutishauser, 2008). Although the underlying mechanisms for how aberrant expression of polySia leads to these phenotypic abnormalities remain to be elucidated, several properties, and functions of polySia may shed light on this issue.

REGULATOR OF CELL ADHESION

The function of polySia as a regulator of cell adhesion has been well studied in relation to NCAM. NCAM mediates not only homophilic binding, but also the heterophilic binding of other CAMs, receptors, and ECMs, including L1, Tag-1, FGF-receptor (FGFR), GFR1, collagen, HSPG, and CSPG (Gascon et al., 2007). The binding of these counterpart molecules by NCAM affects many downstream signaling pathways, including those that regulate neurite outgrowth, cell migration, fasciculation, axonal guidance and branching, and synaptogenesis. As polySia contains extremely large exclusion volumes due to its bulky polyanionic nature, polySia modification physically inhibits the homophilic binding of NCAM. Moreover, polySia-NCAM increases the intercellular space between cells, thereby inhibiting cell-cell interactions by hampering the binding between other cell adhesion molecules, as well as the docking between ligands and receptors on cell surfaces (Rutishauser, 2008). In addition to inhibiting trans-interactions, the polysialylation of NCAM may also inhibit

cis-interactions with other NCAM-associated molecules on the same membrane (Gascon et al., 2007). Together, the inhibitory effects of polySia on cell adhesion are termed the "anti-adhesive effect" (**Figure 3A**). In addition, polySia can function as an insulating molecule because it displays the repulsive field due to the large exclusion volume.

As described above, polySia-deficient mice (ST8SIA2- and 4-DKO mice) do not survive past the early stages of development, whereas NCAM- and polySia-deficient mice (TKO mice) can typically reach adulthood. PolySia-deficiency may greatly enhance NCAM-mediated cell adhesion, which is typically suppressed by polySia during early development, leading to stronger cell adhesion and abnormal signaling. In TKO mice, the anomalous cell adhesion found in polySia-deficient DKO mice is compensated by marked reductions in cell adhesion and signal transduction due to the deficiency in NCAM. Therefore, polySia is thought to regulate cell signaling events by influencing the strength of cell adhesion (Hildebrandt et al., 2007, 2009).

To date, 27 isoforms of NCAM generated by RNA splicing have been identified, among which four major isoforms, NCAM-180, -140, -120, and sNCAM (soluble NCAM), have been characterized. NCAM-180 is the major NCAM isoform involved in the transmission of signals into cells. Among the known molecules that interact with NCAM, spectrin, Fyn, and FAK bind to the cytosolic region of NCAM-180, which contains a palmitoylation and two phosphorylation sites, while NCAM interaction with spectrin, and PKCβ2 leads to neurite outgrowth. Homophilic interactions between non-polySia-NCAMs accelerate FAK phosphorylation, recruits adaptor proteins such as Grb2, Cas, and Shc, and leads to activation of the MAP kinase pathway through Ras and Raf. In addition, non-polySia-NCAMs interact heterophilically through their extracellular domains with other CAMs (L1 and TAG1), proteoglycan (HSPG, CSPG), and FGFRs as described above. Through these heterophilic interactions, signal transduction events are not only directly regulated by NCAM, but are also indirectly regulated via other CAMs and/or growth factor receptors. All of these cell signal pathways are thus influenced by polysialylation of NCAM.

REGULATION OF ION CHANNELS

Several reports have investigated the relationship between polySia and memory. For example, NCAM-KO and ST8SIA4-KO mice have impaired memory, as described above and studies on the synaptic functions through glutamate receptors have been examined. The results of *in-vitro* studies demonstrate that polySia on NCAM modulates the activity

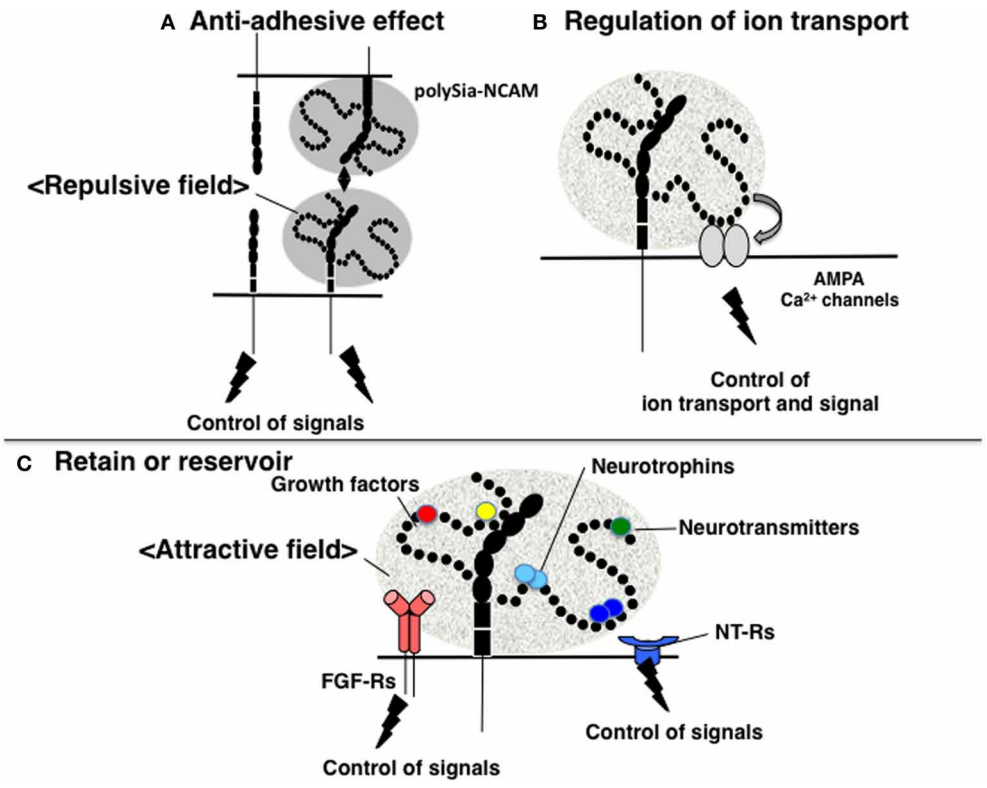

FIGURE 3 | Functions of polySia. (A) Anti-adhesive effect. PolySia-NCAM imparts a repulsive field on the cell surface due to the large volume of polySia (shown in gray) to negatively regulate cell-cell interactions. **(B)** Regulation of ion transport. The influx and efflux of ions, such as Ca^{2+} are regulated by polySia. PolySia on NCAM interacts with Ca^{2+} channels, such as AMPA receptors, and regulates their opening and closing, by which polySia controls ion transport. **(C)** Retention or reservoir of biologically active molecules. PolySia on NCAM directly binds to various types of biologically active molecules involved in neural function, such as neurotrophins, neurotransmitters, and growth factors. PolySia-NCAM provides attractive fields that regulate their extracellular concentrations and signaling modes.

of the α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptors (AMPA-Rs) in immature pyramidal neurons isolated from the CA1 region of the hippocampus (Vaithianathan et al., 2004). Specifically, polySia prolongs the open channel time of AMPA-R-mediated currents and alters the bursting pattern of the receptor channels, although polySia does not modify AMPA-R single-channel conductance (Vaithianathan et al., 2004). In this case, polySia likely directly interacts with AMPA-R (**Figure 3B**). In addition, there are several reports on the relationship between polySia and *N*-methyl-D-aspartate (NMDA) receptors. Impaired CA1 LTP in hippocampal slices is rescued by the addition of polySia or polySia-NCAM but not NCAM alone (Senkov et al., 2006), and polySia alone or polySia-NCAM inhibits the activation of GluN2B-containing NMDA-Rs by low micromolar concentrations of glutamate (Hammond et al., 2006). PolySia reduces the open probability, but not the conductance, of NR2B-containing NMDA-Rs in a polySia- and glutamate concentration-dependent manner by inhibiting NR2B subunit-containing NMDA-Rs through Ras-GRF1-p38 MAPK signaling cascade that is deeply involved in LTP. These findings suggest that polySia-NCAM is involved in synaptic function in the hippocampus, where it regulates different types of channels in a specific manner.

Interestingly, a unique α2,9-linked polySia structure, which was identified on the surface of sperm cells of sea urchin (Miyata et al., 2006), is involved in sperm motility through the regulation of intracellular calcium (Ca^{2+}) concentrations. The regulation seems to be dependent on the binding of α2,9-linked polySia with of Ca^{2+}transporters, suNCKX (K^+-dependent Na^+/Ca^{2+} exchanger), and suPMCA (Ca^{2+} ATPase), which are involved in regulating the influx and efflux of Ca^{2+} in sperm. Consistent with this finding, the polySia epitope has also been demonstrated to regulate sperm motility (Kambara et al., 2011). In addition to the regulation of Ca^{2+} channels, it is notable that polySia has the ability to restore Ca^{2+} ions (Shimoda et al., 1994).

REGULATOR OF NEUROLOGICALLY ACTIVE MOLECULES

Recently, polySia has been shown to directly bind and regulate the function of a number of soluble bioactive factors (Sato, 2013). Thus, polySia appears to retain and regulate the function of specific bioactive factors involved in neural function in the intercellular spaces, and clearly indicates that polySia is involved in not only neurogenesis, but also in the regulation of neural function. In this case, polySia has an attractive field toward these bioactive molecules (**Figure 3C**). Notably, the bioactive molecules that bind to polySia have been well characterized in relation to schizophrenia and other psychiatric disorders. This novel function of polySia completely differs from its anti-adhesive effect on cell-cell and cell-extracellular matrix interactions that is mediated by its bulky nature and large exclusion volume (i.e., repulsive field), and is of particular importance to the field of the psychiatry.

Neurotrophins—BDNF, NT3, and NGF

Brain-derived neurotrophic factor (BDNF), which is a member of the neurotrophins, displays 50% homology with nerve growth factor (NGF) and is most abundant in brain tissue. BDNF promotes the growth and development of immature neurons, and also enhances the survival and functional maintenance of adult neurons via binding to a low-affinity receptor, p75NTR, and a high-affinity receptor, TrkB. This neurotrophic factor also has an important role in the neural plasticity that is integral to memory and learning. Notably, the analyses of NCAM-KO mice has also demonstrated that polySia-NCAM is involved in memory and learning. For example, the additions of BDNF to hippocampal slices derived from NCAM-KO mice rescued the reduction of LTP resulting from the disappearance of polySia-NCAM, indicating that polySia-NCAM is involved in signal transduction mediated by BDNF receptors (Muller et al., 2000).

Biochemically, the direct binding between polySia and BDNF was first demonstrated using gel filtration, horizontal native-PAGE, and surface plasmon resonance (SPR) methods (Kanato et al., 2008). These solid-based approaches were the first to demonstrate that BDNF dimers directly bind to polySia and that the minimum DP required for this interaction is 12 or greater. The resulting complex between polySia and BDNF is extremely large (approximately 2500 kDa) was shown to consist of 14 mol BDNF dimer molecules and 28 mol polySia (mean DP = 43) based on titration and gel filtration experiments. The binding of polySia is also observed with the neurotrophins NT-3 and NGF most likely through C-terminal basic regions. BDNF and polySia do not form ternary complexes with BDNF receptors and BDNF easily migrate toward receptors after forming a complex with polySia. The migration can be explained by the affinities of BDNF toward polySia and BDNF receptors (**Figure 4**, left panel). The K_D of BDNF toward polySia, as calculated by SPR, is approximately 10^{-9} M (Sato et al., 2010; Hane et al., 2012). In contrast, the K_D of BDNF toward TrkB and p75NTR is 10^{-12} and 10^{-10} M, respectively. Based on these results, BDNF in BDNF-polySia complexes would move toward BDNF receptors because BDNF has one to three orders of magnitude stronger affinity toward BDNF receptors than toward polySia. PolySia and polySia-BDNF complexes were also shown to increase the proliferation of neuroblastoma cells compared to untreated control cells. Recently, evidence was presented to show that ProBDNF processed outside the cell by tPA/plasmin is important for memory in the hippocampus (Pang et al., 2004). In this context, it is also important to consider the reservoir function of polySia, because proBDNF and BDNF, but not the pro-domain, are capable of binding to polySia (Sato, unpublished results). Taken together, the findings from these studies demonstrate that polySia is involved in several neurotrophin-mediated biological functions, including cell growth, neurogenesis, and memory.

Neurotransmitters—catecholamines

The specific binding between polySia and catecholamine neurotransmitters, particularly DA, has been demonstrated by the frontal affinity chromatography (FAC) analyses of numerous factors, including histamine, acetylcholine, serotonin, catecholamines (DA, epinephrine, and norepinephrine), and their precursors. As the binding is not observed with disialic acid (DP = 2), catecholamine binding appears specific to polySia, and it is speculated that these interactions occur between specific structures of polySia and the catechol backbone (Isomura et al., 2011). As the K_D of DA toward polySia changes depending

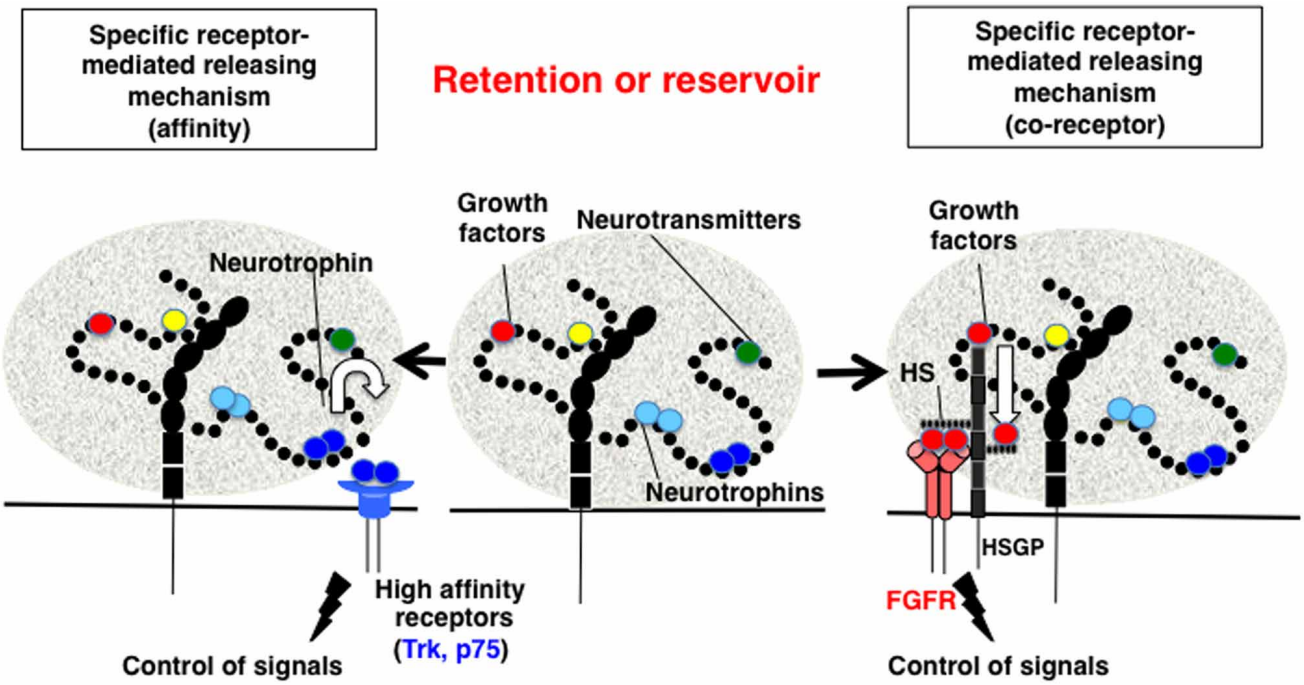

FIGURE 4 | Proposed mechanism for the retention and release of biologically active molecules by polySia. Middle panel, PolySia captures biologically active molecules by direct binding. The retained molecules are released by one of several ways, as shown in the **left** and **right panels**. **Left panel**, Specific receptor-mediated release mechanism-1 (Affinity). In this example, brain-derived neurotrophic factor (BDNF) in a complex with polySia migrates to the BDNF receptors, TrkB or p75NTR, based on differences in the affinity between BDNF, the receptors, and polySia. **Right panel**, Specific receptor-mediated release mechanism-1 (Co-receptor). In the case of fibroblast growth factor 2 (FGF2), polySia does not release FGF2 from the FGF2-polySia complex to the FGF receptor (FGFR). Notably, FGF2 in the FGF2-polySia complex can migrate to heparan sulfate (HS) to form a FGF2-HS complex, which can then bind to FGFR as a ternary complex to enhance FGF signaling. Therefore, polySia regulates FGF2 signaling by passing FGF2 to HS and finally to FGFR.

on the pH of the solution, the specific interaction between these molecules might be fine-tuned by subtle changes of the extracellular pH (Sato et al., 2010).

PolySia is also involved in Akt signaling in human neuroblastoma cells via DRD2 (Isomura et al., 2011). It is also reported that polySia is required for DRD2-mediated plasticity involving inhibitory circuits of the rat medial prefrontal cortex (Castillo-Gómez et al., 2011). Together, these results suggest that the polySia-NCAM localized on postsynaptic membranes directly interacts with catecholamine neurotransmitters, representing a novel function of polySia.

Growth factors—FGF2

FGF2 is a prototypical member of the FGF family that stimulates the growth of various cell types, from fibroblasts to tumor cells, and was first identified in the bovine pituitary gland as a factor with the potential to induce fibroblast cell proliferation. FGF2 is highly expressed in the brain during earlier stages of development, and is involved in brain formation. As recent studies have demonstrated that FGF2 is a potent modulator of proliferation and differentiation of multi-potent neural progenitor cells isolated from the adult SVZ, FGF2 also plays a pivotal role in adult neurogenesis (Mudò et al., 2009). Due to its importance in both brain development and function, it is not surprising that FGF2 has been implicated in a number of psychiatric disorders (Fumagalli et al., 2005; Gaughran et al., 2006; Turner et al., 2008, 2009; Perez et al., 2009; Graham and Richardson, 2010).

FGF2-FGFR signals are enhanced following the formation of ternary complexes with heparan sulfate (HS) on HSPG, which is a component of the ECM. However, the relationship between polySia and FGF2 was not identified until several recent biochemical analyses, including gel shift assays, gel filtration, and SPR, revealed that polySia binds to FGF2 directly (Ono et al., 2012). FGF2 monomers bind to polySia and form a large complex that does not migrate toward FGFR, even if the receptors are located next to the complex. The K_D of FGF2 toward polySia (1.47×10^{-8} M) is smaller than that toward HS (2.81×10^{-8} M). Consistent with these differences in affinity, FGF2-polySia and FGF2-HS complexes display unique physical and biochemical properties. For example, FGF2-polySia binds to HS- or polySia-coated surfaces, whereas HS-polySia cannot bind to either of these surfaces, indicating that the binding regions of FGF2 to polySia and HS differ. In addition, FGF2 complexed with polySia cannot migrate toward FGFRs, but do migrate toward HS (**Figure 4**, right panel). It is also demonstrated that Erk and Akt signaling is regulated by polySia and HS in polySia- and HS-expressing cells, respectively (Ono et al., 2012).

PolySia AND SCHIZOPHRENIA

Schizophrenia is a psychiatric disorder with a complex pathophysiology that is influenced by multiple factors. It is also considered that schizophrenia is deeply related to both neurodevelopmental and neurodegenerative disorders involving disconnectivity and disorder of synapses. Indeed, there are several hypotheses such as glutamate hypothesis, DA hypothesis, and neurodevelopmental hypothesis (Coyle et al., 2012). Thus, several factors are mutually involved in pathophysiology of schizophrenia in a complicated manner, which makes it difficult to understand the underlying mechanism of schizophrenia clearly. Among the factors, polySia might be an important molecule for understanding this disorder.

Recently, indirect evidence has been reported that suggests that polySia is involved in schizophrenia. For example, it was first reported that the degree of immunostaining for polySia-NCAM derived from the hippocampus of schizophrenic brains is decreased compared with that of normal brains (Barbeau et al., 1995). In addition, chromosome 15q26, which is the genomic region containing the gene encoding ST8SIA2, is related to schizophrenia and bipolar disorders among the population of Eastern Quebec (Maziade et al., 2005). Recently, a relationship between SNPs in the promoter region of the ST8SIA2 gene and schizophrenia was identified by genome-wide association studies in the Japanese (Arai et al., 2006) and Chinese Han populations (Tao et al., 2007). The ST8SIA2 gene is also reported to be a generalized susceptibility marker for psychotic and mood disorders on chromosome 15q25-26 (McAuley et al., 2012), and is associated with an increased risk of mental illness, such as autism (Anney et al., 2010). Interestingly, the mutation of synCAM, which is another substrate for ST8SIA2, is also related with autism spectrum disorders (Zhiling et al., 2008).

As described above, to determine the relationship between genes and phenotypes, it is necessary to biochemically characterize the target gene product, particularly if it is an enzyme, because the enzymatic reaction product has a biological role. In this context, the *in-vitro* and *in-vivo* enzymatic activity of ST8SIA2 (SNP-7; Glu141Lys) that was reported from a schizophrenic patient was measured and was shown to be markedly decreased under both conditions (Isomura et al., 2011). The mutated amino acid is localized near sialyl motif L that has clearly been shown to be required for the enzymatic activity of ST8SIA2. In addition, the amount and quality (DP) of the produced polySia were also impaired (Hane et al., 2012), a result that is consistent with the histochemical data, although it only represents a change in the amount of polySia. Because the polySia structure biosynthesized by the SNP-7 of ST8SIA2 was impaired compared with that by normal ST8SIA2, the bindings toward BDNF, FGF2, and DA were also impaired (Isomura et al., 2011; Hane et al., 2012). BDNF, FGF2, and DA are known to be key molecules for the causes and biomarker of schizophrenia (Terwisscha van Scheltinga et al., 2010; Buckley et al., 2011; Balaratnasingam and Janca, 2012; Eyles et al., 2012; Tritsch and Sabatini, 2012). Impairment of the new function of polySia as a regulator of neurological active molecules will thus lead to pathophysiology of schizophrenia. Especially, behavioral deficits in psychiatric disorders have been hypothesized to arise from the elevations in the cellular balance of excitation and inhibition within neural microcircuitry (Yizhar et al., 2011). Therefore, the impact on the glycocalyx such as polySia located in the region that generates neural microcircuitry might be important. Taken together, these results suggest that

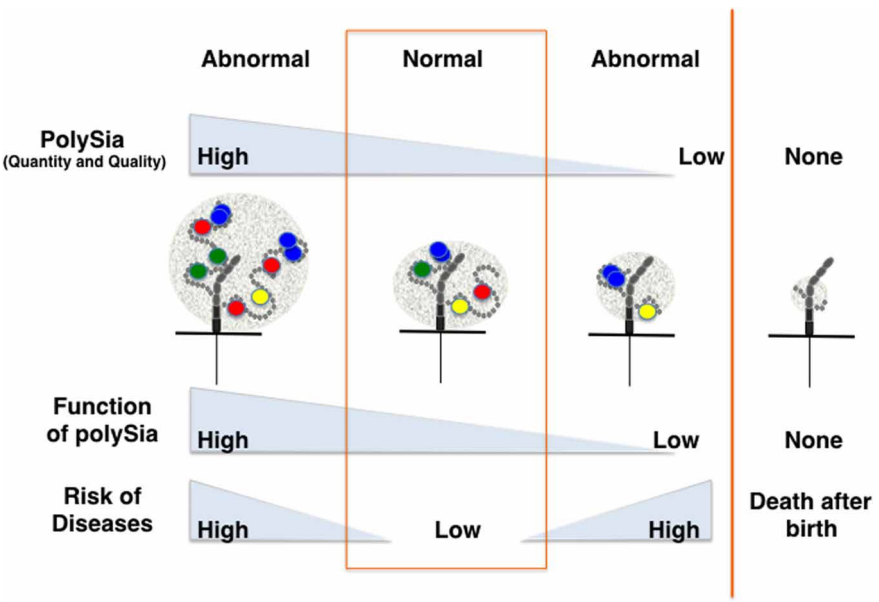

FIGURE 5 | Disease risk depends on the quantity and quality of polySia. The quantity and quality (degree of polymerization) of polySia may differ from person to person, particularly for patients suffering from psychiatric disorders or other neurodegenerative diseases. Direct and indirect lines of evidence show that quantity and quality of polySia on NCAM are critically important for normal brain functions. Hyper- or hypo-polysialylation of NCAM might lead to the impairment of brain function through the stimulation or repression of dopamine-, BDNF- and FGF2-mediated signaling.

changes in the quantity and quality, particularly DP, of polySia, which are closely related with the enzymatic activity of ST8SIA2, lead to an altered binding affinities toward BDNF, FGF2, and DA, may be one of the underlying causes of schizophrenia.

Anatomically, the volume of olfactory bulbs derived from schizophrenic brains is reduced (Turetsky et al., 2003), which is a similar phenotype to that of NCAM-KO mice (Cremer et al., 1994). The functional impairment and disturbed organization of the hippocampus are also involved in the etiology of schizophrenia (Harrison, 2004). In addition, a reduction of polySia-NCAM in dorsolateral prefrontal cortex of schizophrenic patients was reported (Gilabert-Juan et al., 2012). In this aspect, it is interesting that loss of ST8SIA2 or NCAM resulted in the misguidance of infrapyramidal mossy fibers and the formation of ectopic synapses in the hippocampus (Angata et al., 2004). In addition, several characteristic properties, such as brain structure, neural plasticity, and various morphological, cognitive, and emotional deficits related to schizophrenia have been observed in ST8SIA2- or ST8SIA4-SKO mice (Hildebrandt et al., 2007; Calandreau et al., 2010). Very recently, NCAM-KO mice were demonstrated that they are useful for studying specific endophenotypes with relevance to the schizophrenia although they do not display a typical schizophrenia-like phenotypes (Albrecht and Stork, 2012). As NCAM is not the only substrate for ST8SIA2 and the underlying biosynthetic mechanisms of polySia by ST8SIA2 and ST8SIA4 are not well understood, it is important to focus on the contribution of glycoepitopes, such as polySia, to schizophrenia.

CONCLUSION

As psychiatric disorders such as schizophrenia are complex diseases with multiple factors contributing to pathogenesis, the mechanisms by which polySia is involved in these disorders are also likely complex. However, it is clear that the quality and quantity of polySia-NCAM are strictly regulated in normal cells, and that the impairment of polySia has profound effects on various brain functions through increasing cell adhesion, modifying ion channel activity, and reducing binding affinity toward biologically active molecules. Such impairments might lead to psychiatric disorders or affect the prognosis of these diseases (**Figure 5**). Interestingly, imbalances in the quantity of polySia-NCAM are also found in patients suffering from Alzheimer's disease (Mikkonen et al., 1999), Parkinson's disease (Oizumi et al., 2008), and drug abuse (Murphy et al., 2006). As demonstrated by the study of polySia, exploring the structure and function of unique glycocalyx components on the cell surface is expected to give further insight into psychiatric diseases because the glycocalyx is a major, but often ignored, player for the communication between cells and the extracellular environment.

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REFERENCES

Albrecht, A., and Stork, O. (2012). Are NCAM deficient mice an animal model for schizophrenia? *Front. Behav. Neurosci.* 6:43. doi: 10.3389/fnbeh.2012.00043

Allen, N., Bagade, S., McQueen, M., Ioannidis, J., Kavvoura, F., Khoury, M., et al. (2008). Systematic meta-analyses and field synopsis of genetic association studies in schizophrenia: the SzGene database. *Nat. Genet.* 40, 827–834.

Angata, K., and Fukuda, M. (2003). Polysialyltransferases: major players in polysialic acid synthesis on the neural cell adhesion molecule. *Biochimie* 85, 195–206.

Angata, K., Long, J. M., Bukalo, O., Lee, W., Dityatev, A., Wynshaw-Boris, A., et al. (2004). Sialyltransferase ST8Sia-II assembles a subset of polysialic acid that directs hippocampal axonal targeting and promotes fear behavior. *J. Biol. Chem.* 279, 32603–32613.

Angata, K., Nakayama, J., Fredette, B., Chong, K., Ranscht, B., and Fukuda, M. (1997). Human STX polysialyltransferase forms the embryonic form of the neural cell adhesion molecule. Tissue-specific expression, neurite outgrowth, and chromosomal localization in comparison with another polysialyltransferase, PST. *J. Biol. Chem.* 272, 7182–7190.

Anney, R., Klei, L., Pinto, D., Regan, R., Conroy, J., Magalhaes, T. R., et al. (2010). A genome-wide scan for common alleles affecting risk for autism. *Hum. Mol. Genet.* 19, 4072–4082.

Arai, M., Yamada, K., Toyota, T., Obata, N., Haga, S., Yoshida, Y., et al. (2006). Association between polymorphisms in the promoter region of the sialyltransferase 8B (SIAT8B) gene and schizophrenia. *Biol. Psychiatry* 59, 652–659.

Ashe, P., Berry, M., and Boulton, A. (2001). Schizophrenia, a neurodegenerative disorder with neurodevelopmental antecedents. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 25, 691–707.

Balaratnasingam, S., and Janca, A. (2012). Brain derived neurotrophic factor: a novel neurotrophin involved in psychiatric and neurological disorders. *Pharmacol. Ther.* 134, 116–124.

Barbeau, D., Liang, J., Robitalille, Y., Quirion, R., and Srivastava, L. (1995). Decreased expression of the embryonic form of the neural cell adhesion molecule in schizophrenic brains. *Proc. Natl. Acad. Sci. U.S.A.* 92, 2785–2789.

Bonfanti, L. (2006). PSA-NCAM in mammalian structural plasticity and neurogenesis. *Prog. Neurobiol.* 80, 129–164.

Buckley, P. F., Pillai, A., and Howell, K. R. (2011). Brain-derived neurotrophic factor: findings in schizophrenia. *Curr. Opin. Psychiatry* 24, 122–127.

Calandreau, L., Márquez, C., Bisaz, R., Fantin, M., and Sandi, C. (2010). Differential impact of polysialyltransferase ST8SiaII and ST8SiaIV knockout on social interaction and aggression. *Genes Brain Behav.* 9, 958–967.

Castillo-Gómez, E., Varea, E., Blasco-Ibáñez, J. M., Crespo, C., and Nacher, J. (2011). Polysialic acid is required for dopamine D2 receptor-mediated plasticity involving inhibitory circuits of the rat medial prefrontal cortex. *PLoS ONE* 6:e29516. doi: 10.1371/journal.pone.0029516

Close, B., Mendiratta, S., Geiger, K., Broom, L., Ho, L., and Colley, K. (2003). The minimal structural domains required for neural cell adhesion molecule polysialylation by PST/ST8Sia IV and STX/ST8Sia, I. I. *J. Biol. Chem.* 278, 30796–30805.

Coyle, J. T., Basu, A., Benneyworth, M., Balu, D., and Konopaske, G. (2012). Glutamatergic synaptic dysregulation in schizophrenia: therapeutic implications. *Handb. Exp. Pharmacol.* 267–295.

Cremer, H., Lange, R., Christoph, A., Plomann, M., Vopper, G., Roes, J., et al. (1994). Inactivation of the N-CAM gene in mice results in size reduction of the olfactory bulb and deficits in spatial learning. *Nature* 367, 455–459.

Eckhardt, M., Bukalo, O., Chazal, G., Wang, L., Goridis, C., Schachner, M., et al. (2000). Mice deficient in the polysialyltransferase ST8SiaIV/PST-1 allow discrimination of the roles of neural cell adhesion molecule protein and polysialic acid in neural development and synaptic plasticity. *J. Neurosci.* 20, 5234–5244.

Evans, S., Sigurskjold, B., Jennings, H., Brisson, J., To, R., Tse, W., et al. (1995). Evidence for the extended helical nature of polysaccharide epitopes. The 2.8 A resolution structure and thermodynamics of ligand binding of an antigen binding fragment specific

for alpha-(2–>8)-polysialic acid. *Biochemistry* 34, 6737–6744.

Eyles, D., Feldon, J., and Meyer, U. (2012). Schizophrenia: do all roads to dopamine or is this where they start? *Transl. Psychiatry* 2, e81.

Finne, J., Finne, U., Deagostini-Bazin, H., and Goridis, C. (1983). Occurrence of alpha 2-8 linked polysialosyl units in a neural cell adhesion molecule. *Biochem. Biophys. Res. Commun.* 112, 482–487.

Foley, D. A., Swartzentruber, K. G., and Colley, K. J. (2009). Identification of sequences in the polysialyltransferases ST8Sia II and ST8Sia IV that are required for the protein-specific polysialylation of the neural cell adhesion molecule, NCAM. *J. Biol. Chem.* 284, 15505–15516.

Fumagalli, F., Bedogni, F., Slotkin, T., Racagni, G., and Riva, M. (2005). Prenatal stress elicits regionally selective changes in basal FGF-2 gene expression in adulthood and alters the adult response to acute or chronic stress. *Neurobiol. Dis.* 20, 731–737.

Gascon, E., Vutskits, L., and Kiss, J. (2007). Polysialic acid-neural cell adhesion molecule in brain plasticity: from synapses to integration of new neurons. *Brain Res. Rev.* 56, 101–118.

Gaughran, F., Payne, J., Sedgwick, P., Cotter, D., and Berry, M. (2006). Hippocampal FGF-2 and FGFR1 mRNA expression in major depression, schizophrenia and bipolar disorder. *Brain Res. Bull.* 70, 221–227.

Gilabert-Juan, J., Varea, E., Guirado, R., Blasco-Ibáñez, J. M., Crespo, C., and Nácher, J. (2012). Alterations in the expression of PSA-NCAM and synaptic proteins in the dorsolateral prefrontal cortex of psychiatric disorder patients. *Neurosci. Lett.* 530, 97–102.

Graham, B., and Richardson, R. (2010). Fibroblast growth factor-2 enhances extinction and reduces renewal of conditioned fear. *Neuropsychopharmacology* 35, 1348–1355.

Gómez-Climent, M., Guirado, R., Castillo-Gómez, E., Varea, E., Gutierrez-Mecinas, M., Gilabert-Juan, J., et al. (2011). The polysialylated form of the neural cell adhesion molecule (PSA-NCAM) is expressed in a subpopulation of mature cortical interneurons characterized by reduced structural features and connectivity. *Cereb. Cortex* 21, 1028–1041.

Hammond, M., Sims, C., Parameshwaran, K., Suppiramaniam, V., Schachner, M., and Dityatev, A. (2006). Neural cell adhesion molecule-associated polysialic acid inhibits NR2B-containing N-methyl-D-aspartate receptors and prevents glutamate-induced cell death. *J. Biol. Chem.* 281, 34859–34869.

Hane, M., Sumida, M., Kitajima, K., and Sato, C. (2012). Structural and functional impairments of polySia-NCAM synthesized by a mutated polysialyltransferase of a schizophrenic patient. *Pure Appl. Chem.* 84, 1895–1906.

Harrison, P. (2004). The hippocampus in schizophrenia: a review of the neuropathological evidence and its pathophysiological implications. *Psychopharmacology (Berl.)* 174, 151–162.

Hildebrandt, H., Mühlenhoff, M., Weinhold, B., Gerardy-Schahn, R. (2007). Dissecting polysialic acid and NCAM functions in brain development. *J. Neurochem.* 103, 56–64.

Hildebrandt, H., Mühlenhoff, M., Oltmann-Norden, I., Röckle, I., Burkhardt, H., Weinhold, B., et al. (2009). Imbalance of neural cell adhesion molecule and polysialyltransferase alleles causes defective brain connectivity. *Brain* 132, 2831–2838.

Isomura, R., Kitajima, K., and Sato, C. (2011). Structural and functional impairments of polysialic acid by a mutated polysialyltransferase found in schizophrenia. *J. Biol. Chem.* 286, 21535–21545.

Kambara, Y., Shiba, K., Yoshida, M., Sato, C., Kitajima, K., and Shingyoji, C. (2011). Mechanism regulating Ca2+-dependent mechanosensory behaviour in sea urchin spermatozoa. *Cell Struct. Funct.* 36, 69–82.

Kanato, Y., Kitajima, K., and Sato, C. (2008). Direct binding of polysialic acid to a brain-derived neurotrophic factor depends on the degree of polymerization. *Glycobiology* 18, 1044–1053.

Mayanil, C., George, D., Freilich, L., Miljan, E., Mania-Farnell, B., McLone, D., et al. (2001). Microarray analysis detects novel Pax3 downstream target genes. *J. Biol. Chem.* 276, 49299–49309.

Mayanil, C., George, D., Mania-Farnell, B., Bremer, C., McLone, D., and Bremer, E. (2000). Overexpression of murine Pax3 increases NCAM polysialylation in a human medulloblastoma cell line. *J. Biol. Chem.* 275, 23259–23266.

Maziade, M., Roy, M., Chagnon, Y., Cliche, D., Fournier, J., Montgrain, N., et al. (2005). Shared and specific susceptibility loci for schizophrenia and bipolar disorder: a dense genome scan in Eastern Quebec families. *Mol. Psychiatry* 10, 486–499.

McAuley, E. Z., Scimone, A., Tiwari, Y., Agahi, G., Mowry, B. J., Holliday, E. G., et al. (2012). Identification of sialyltransferase 8B as a generalized susceptibility gene for psychotic and mood disorders on chromosome 15q25–26. *PLoS ONE* 7:e38172. doi: 10.1371/journal.pone.0038172

Mikkonen, M., Soininen, H., Tapiola, T., Alafuzoff, I., and Miettinen, R. (1999). Hippocampal plasticity in Alzheimer's disease: changes in highly polysialylated NCAM immunoreactivity in the hippocampal formation. *Eur. J. Neurosci.* 11, 1754–1764.

Millar, J., Christie, S., Anderson, S., Lawson, D., Hsiao-Wei Loh, D., Devon, R., et al. (2001). Genomic structure and localisation within a linkage hotspot of Disrupted In Schizophrenia 1, a gene disrupted by a translocation segregating with schizophrenia. *Mol. Psychiatry* 6, 173–178.

Miyata, S., Sato, C., Kumita, H., Toriyama, M., Vacquier, V., and Kitajima, K. (2006). Flagellasialin: a novel sulfated alpha2, 9-linked polysialic acid glycoprotein of sea urchin sperm flagella. *Glycobiology* 16, 1229–1241.

Mudò, G., Bonomo, A., Di Liberto, V., Frinchi, M., Fuxe, K., and Belluardo, N. (2009). The FGF-2/FGFRs neurotrophic system promotes neurogenesis in the adult brain. *J. Neural Transm.* 116, 995–1005.

Mühlenhoff, M., Oltmann-Norden, I., Weinhold, B., Hildebrandt, H., and Gerardy-Schahn, R. (2009). Brain development needs sugar: the role of polysialic acid in controlling NCAM functions. *Biol. Chem.* 390, 567–574.

Muller, D., Djebbara-Hannas, Z., Jourdain, P., Vutskits, L., Durbec, P., Rougon, G., et al. (2000). Brain-derived neurotrophic factor restores long-term potentiation in polysialic acid-neural cell adhesion molecule-deficient hippocampus. *Proc. Natl. Acad. Sci. U.S.A.* 97, 4315–4320.

Murphy, K., Foley, A., O'Connell, A., and Regan, C. (2006). Chronic exposure of rats to cognition enhancing drugs produces a neuroplastic response identical to that obtained by complex environment rearing. *Neuropsychopharmacology* 31, 90–100.

Nacher, J., Guirado, R., and Castillo-Gómez, E. (2013). Structural plasticity of interneurons in the adult brain: role of PSA-NCAM and implications for psychiatric disorders. *Neurochem. Res.* doi: 10.1007/s11064-013-0977-4. [Epub ahead of print].

Nacher, J., Guirado, R., Varea, E., Alonso-Llosa, G., Röckle, I., and Hildebrandt, H. (2010). Divergent impact of the polysialyltransferases ST8SiaII and ST8SiaIV on polysialic acid expression in immature neurons and interneurons of the adult cerebral cortex. *Neuroscience* 167, 825–837.

Nakagawa, S., Kim, J., Lee, R., Chen, J., Fujioka, T., Malberg, J., et al. (2002). Localization of phosphorylated cAMP response element-binding protein in immature neurons of adult hippocampus. *J. Neurosci.* 22, 9868–9876.

Nakata, D., Zhang, L., and Troy, F. N. (2006). Molecular basis for polysialylation: a novel polybasic polysialyltransferase domain (PSTD) of 32 amino acids unique to the alpha 2, 8-polysialyltransferases is essential for polysialylation. *Glycoconj. J.* 23, 423–436.

Oizumi, H., Hayashita-Kinoh, H., Hayakawa, H., Arai, H., Furuya, T., Ren, Y., et al. (2008). Alteration in the differentiation-related molecular expression in the subventricular zone in a mouse model of Parkinson's disease. *Neurosci. Res.* 60, 15–21.

Oltmann-Norden, I., Galuska, S. P., Hildebrandt, H., Geyer, R., Gerardy-Schahn, R., Geyer, H., et al. (2008). Impact of the polysialyltransferases ST8SiaII and ST8SiaIV on polysialic acid synthesis during postnatal mouse brain development. *J. Biol. Chem.* 283, 1463–1471.

Ono, S., Hane, M., Kitajima, K., and Sato, C. (2012). Novel regulation of fibroblast growth factor 2 (FGF2)-mediated cell growth by polysialic acid. *J. Biol. Chem.* 287, 3710–3722.

Pang, P. T., Teng, H. K., Zaitsev, E., Woo, N. T., Sakata, K., Zhen, S., et al. (2004). Cleavage of proBDNF by tPA/plasmin is essential for long-term hippocampal plasticity. *Science* 306, 487–491.

Perez, J., Clinton, S., Turner, C., Watson, S., and Akil, H. (2009). A new role for FGF2 as an endogenous inhibitor of anxiety. *J. Neurosci.* 29, 6379–6387.

Rollenhagen, M., Kuckuck, S., Ulm, C., Hartmann, M., Galuska, S. P., Geyer, R., et al. (2012). Polysialylation of the synaptic cell adhesion molecule 1 (SynCAM 1) depends exclusively on the polysialyltransferase

ST8SiaII *in vivo*. *J. Biol. Chem.* 287, 35170–35180.

Rutishauser, U. (2008). Polysialic acid in the plasticity of the developing and adult vertebrate nervous system. *Nat. Rev. Neurosci.* 9, 26–35.

Sato, C. (2013). "Polysialic acids in sialobiology: structure, biosynthesis, and function," in *Sialic Acid Glycoconjugates in Health and Disease* eds J. Tiralongo and I. Martinez-Duncker (Bentham Science (UEA)), 33–75.

Sato, C., Yamakawa, N., and Kitajima, K. (2010). Measurement of glycan-based interactions by frontal affinity chromatography and surface plasmon resonance. *Methods Enzymol.* 478, 219–232.

Senkov, O., Sun, M., Weinhold, B., Gerardy-Schahn, R., Schachner, M., and Dityatev, A. (2006). Polysialylated neural cell adhesion molecule is involved in induction of long-term potentiation and memory acquisition and consolidation in a fear-conditioning paradigm. *J. Neurosci.* 26, 10888–109898.

Shen, H., Watanabe, M., Tomasiewicz, H., Rutishauser, U., Magnuson, T., and Glass, J. (1997). Role of neural cell adhesion molecule and polysialic acid in mouse circadian clock function. *J. Neurosci.* 17, 5221–5229.

Shimoda, Y., Kitajima, K., Inoue, S., and Inoue, Y. (1994). Calcium ion binding of three different types of oligo/polysialic acids as studied by equilibrium dialysis and circular dichroic methods. *Biochemistry* 33, 1202–1208.

Stefansson, H., Sigurdsson, E., Steinthorsdottir, V., Bjornsdottir, S., Sigmundsson, T., Ghosh, S., et al. (2002). Neuregulin 1 and susceptibility to schizophrenia. *Am. J. Hum. Genet.* 71, 877–892.

Stork, O., Welzl, H., Wotjak, C. T., Hoyer, D., Delling, M., Cremer, H., et al. (1999). Anxiety and increased 5-HT1A receptor response in NCAM null mutant mice. *J. Neurobiol.* 40, 343–355.

Strous, R., Bark, N., Parsia, S., Volavka, J., and Lachman, H. (1997). Analysis of a functional catechol-O-methyltransferase gene polymorphism in schizophrenia: evidence for association with aggressive and antisocial behavior. *Psychiatry Res.* 69, 71–77.

Takashima, S. (2008). Characterization of mouse sialyltransferase genes: their evolution and diversity. *Biosci. Biotechnol. Biochem.* 72, 1155–1167.

Tao, R., Li, C., Zheng, Y., Qin, W., Zhang, J., Li, X., et al. (2007). Positive association between SIAT8B and schizophrenia in the Chinese Han population. *Schizophr. Res.* 90, 108–114.

Terwisscha van Scheltinga, A. F., Bakker, S. C., and Kahn, R. S. (2010). Fibroblast growth factors in schizophrenia. *Schizophr. Bull.* 36, 1157–1166.

Troy, F. A. I. (1996). Sialobiology and the polysialic acid glycotope. *Biol. Sialic Acids* 95–144.

Tritsch, N. X., and Sabatini, B. J. (2012). Dopaminergic modulation of synaptic transmission in cortex and striatum. *Neuron* 76, 33–41.

Turetsky, B., Moberg, P., Roalf, D., Arnold, S., and Gur, R. (2003). Decrements in volume of anterior ventromedial temporal lobe and olfactory dysfunction in schizophrenia. *Arch. Gen. Psychiatry* 60, 1193–1200.

Turner, C., Capriles, N., Flagel, S., Perez, J., Clinton, S., Watson, S., et al. (2009). Neonatal FGF2 alters cocaine self-administration in the adult rat. *Pharmacol. Biochem. Behav.* 92, 100–104.

Turner, C., Gula, E., Taylor, L., Watson, S., and Akil, H. (2008). Antidepressant-like effects of intracerebroventricular FGF2 in rats. *Brain Res.* 1224, 63–68.

Vaithianathan, T., Matthias, K., Bahr, B., Schachner, M., Suppiramaniam, V., Dityatev, A., et al. (2004). Neural cell adhesion molecule-associated polysialic acid potentiates alpha-amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptor currents. *J. Biol. Chem.* 279, 47975–47984.

Weinhold, B., Seidenfaden, R., Röckle, I., Mühlenhoff, M., Schertzinger, F., Conzelmann, S., et al. (2005). Genetic ablation of polysialic acid causes severe neurodevelopmental defects rescued by deletion of the neural cell adhesion molecule. *J. Biol. Chem.* 280, 42971–42977.

Woods, B. (1998). Is schizophrenia a progressive neurodevelopmental disorder? Toward a unitary pathogenetic mechanism. *Am. J. Psychiatry* 155, 1661–1670.

Yizhar, O., Fenno, L. E., Prigge, M., Schneider, F., Davidson, T. J., O'Shea, D. J., et al. (2011). Neocortical excitation/inhibition balance in information processing and social dysfunction. *Nature* 477, 171–178.

Yoshida, Y., Kurosawa, N., Kanematsu, T., Kojima, N., and Tsuji, S. (1996). Genomic structure and promoter activity of the mouse polysialic acid synthase gene (mST8Sia II). Brain-specific expression from a TATA-less GC-rich sequence. *J. Biol. Chem.* 271, 30167–30173.

Zhiling, Y., Fujita, E., Tanabe, Y., Yamagata, T., Momoi, T., and Momoi, M. Y. (2008). Mutations in the gene encoding CADM1 are associated with autism spectrum disorder. *Biochem. Biophys. Res. Commun.* 377, 926–929.

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ErbB1-4-dependent EGF/neuregulin signals and their cross talk in the central nervous system: pathological implications in schizophrenia and Parkinson's disease

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Ligands for ErbB1-4 receptor tyrosine kinases, such as epidermal growth factor (EGF) and neuregulins, regulate brain development and function. Thus, abnormalities in their signaling are implicated in the etiology or pathology of schizophrenia and Parkinson's disease. Among the ErbB receptors, ErbB1, and ErbB4 are expressed in dopamine and GABA neurons, while ErbB1, 2, and/or 3 are mainly present in oligodendrocytes, astrocytes, and their precursors. Thus, deficits in ErbB signaling might contribute to the neurological and psychiatric diseases stemming from these cell types. By incorporating the latest cancer molecular biology as well as our recent progress, we discuss signal cross talk between the ErbB1-4 subunits and their neurobiological functions in each cell type. The potential contribution of virus-derived cytokines (virokines) that mimic EGF and neuregulin-1 in brain diseases are also discussed.

Keywords: ErbB1-4, dopamine, GABA, virokine, schizophrenia, Parkinson's disease

INTRODUCTION

ErbB molecules are membrane-spanning receptor tyrosine kinases that act on epidermal growth factor (EGF) and its derivatives. The ErbB family consists of four members, ErbB1, B2, B3, and B4, that were originally identified in vertebrates (mammals) and share significant structural homology with members of the ErbB family (Downward et al., 1984; Schechter et al., 1984; Semba et al., 1985; Kraus et al., 1989; Plowman et al., 1993). ErbB1 homolog is identified also in invertebrates, *C. elegans* (LET-23; Aroian et al., 1990) and Drosophila (DER; Schejter and Shilo, 1989). ErbB receptor family members are distributed in many organs and cell types originating from ectodermal and mesodermal tissues and have functions in various cellular processes/functions such as proliferation, growth, migration, and adhesion. Upon binding to its ligand such as EGF and neuregulin-1 (NRG1), the ErbB receptor undergoes tertiary structural alterations in the juxtamembrane region and increases its affinity for another an ErbB molecule, leading to homo- or heterodimerization (Olayioye et al., 2000). This dimerization allows the kinase domain to phosphorylate the ErbB partner. The phosphorylated tyrosine residues recruit adaptor/effecter molecules, such as phosphatidylinositol 3-kinase (PI3K) subunit p85, Src, and Shc, and transmit signals to these transducers. As overexpression of ErbB receptors results in ligand-independent dimerization and auto-phosphorylation, receptor dimerization, rather than the activation of the kinase domain, is thought to limit ErbB signaling (i.e., phosphorylation) (**Figure 1**).

The primary structure of ErbB1 (EGFR, HER1) was first elucidated among ErbBs. The oncogene *v-erbB* was identified in avian erythroblastic leukemia virus. The ortholog and proto-oncogene of *c-erbB* was determined to be the gene for EGF receptor, *erbB1* (Downward et al., 1984). Following this discovery, homologous gene cloning led to the identification of the other of ErbB family members, including ErbB2 (HER2, Neu), ErbB3 (HER3), and ErbB4 (HER4) (Schechter et al., 1984; Semba et al., 1985; Kraus et al., 1989; Plowman et al., 1993). This family shares 40–50% structural homology in the extracellular domains and 60–80% in the intracellular domains (**Figure 2**, **Table 1**).

THE MOLECULAR FEATURES OF THE ErbB FAMILY

Ligands for ErbB receptors can be classified into two groups: the EGF family and the NRG family (Falls, 2003; Higashiyama et al., 2008; Mei and Xiong, 2008). The EGF family consists of transforming growth factor alpha (TGFα; Derynck et al., 1984), heparin-binding EGF-like growth factor (HB-EGF; Higashiyama et al., 1991), amphiregulin (AR; Shoyab et al., 1989), epiregulin (EPR; Toyoda et al., 1995), betacellulin, (BTC; Sasada et al., 1993; Shing et al., 1993), and epigen (EPG; Strachan et al., 2001). The NRG family includes NRG1 (Brockes and Kintner, 1986; Holmes et al., 1992; Peles et al., 1992; Falls et al., 1993; Ho et al., 1995), NRG2 (NTAK; Higashiyama et al., 1997), NRG3, NRG4 (Hobbs et al., 2002), NRG5 (tomoregulin; Uchida et al., 1999), and NRG6 (neuroglycan C; Kinugasa et al., 2004). Most of these ligands are initially synthesized as large membrane-anchored precursors that are processed into secretable and soluble forms and then liberated into the extracellular space where they interact with ErbB receptors (**Figure 2**). In contrast to this process of "endocrine signaling," these precursors are also thought to mediate "juxtacrine signaling" during cell–cell communication; the membrane-anchored precursors directly bind to ErbB receptors on the other side of the cell surface (Ono et al., 1994). Please read the details of "juxtacrine signaling" in other reviews (Iwamoto and Mekada, 2000; Singh and Harris, 2005).

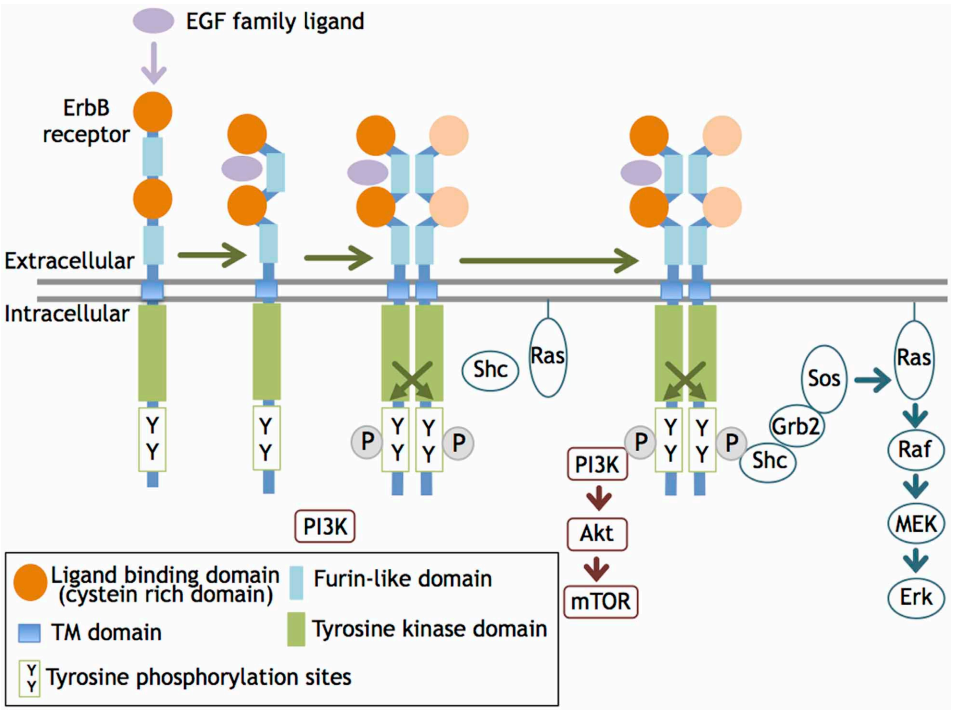

FIGURE 1 | ErbB receptor dimerization and activation. The ligand interaction with ErbB 1, 3, and 4 increases their affinity and induces homo- or heterodimerization of ErbB1-4. This dimerization activates the tyrosine kinase domain and allows it to phosphorylate the cytoplasmic region of the ErbB partner. The phosphorylated tyrosine residues recruit various adaptors/effectors that induce intracellular signals.

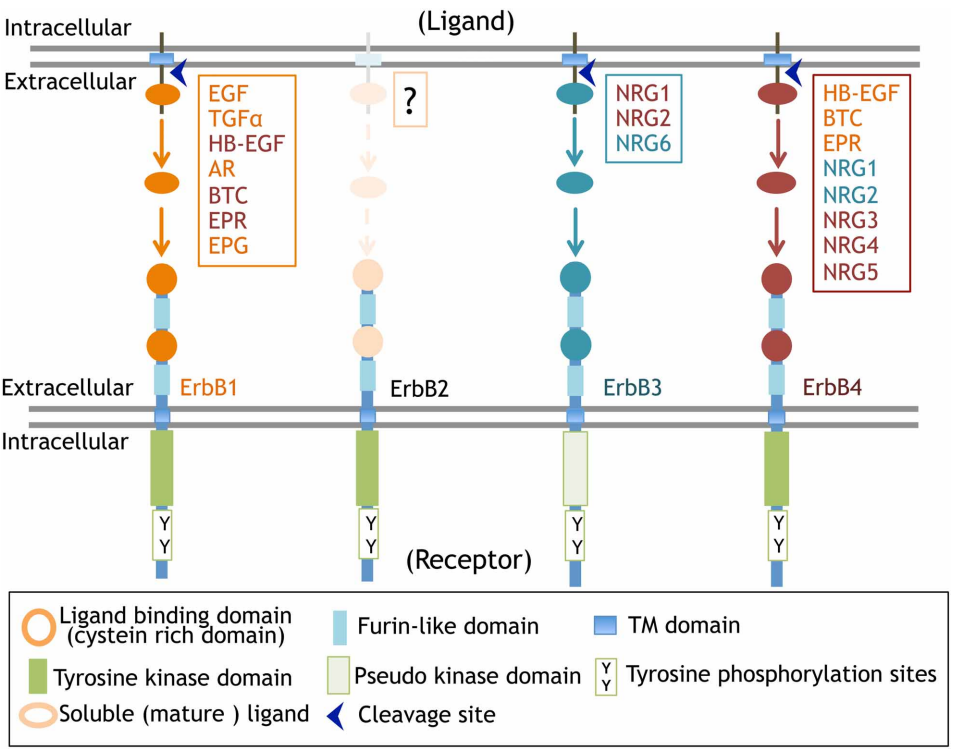

FIGURE 2 | ErbB receptors and their ligands. The molecular structure of ErbB receptors and proteolytic processing of their ligands are displayed.

Table 1 | ErbB molecules and their ligands and adaptors/effectors.

ErbB receptor	Nomenclature	Binding partner	Ligand		Major signaling molecules	Other binding proteins
ErbB1	EGF receptor (EGFR), HER1	ErbB1 ErbB2 ErbB3 ErbB4	EGF TGFα HB-EGF AR BTC	EPG EPR	PI3K-AKT Ras-MAPK PLCγ-PKC Crk FAK JAK Src PTEN	Cbl STAT3
ErbB2	Neu, HER2	ErbB1 ErbB3 ErbB4	Unknown		PI3K-AKT Ras-MAPK FAK Src	Erbin
ErbB3	HER3	ErbB1 ErbB2 ErbB4	NRG1 NRG2 NRG6		PI3K-AKT Ras-MAPK PLCγ-PKC Crk ITK JAK Lyn Src VAV	Cbl EBP1 TENC1
ErbB4	HER4	ErbB1 ErbB2 ErbB3 ErbB4	HB-EGF BTC EPG NRG1	NRG2 NRG3 NRG4 NRG5	PI3K-AKT Ras-MAPK JAK Src Ptprz	N-Cor PSD95 STAT5 TAB2

Among the ErbB receptors, ErbB3 lacks the active kinase domain and is unable to phosphorylate ErbB in this dimer complex. However, upon ligand binding, ErbB3 associates with the heterodimer complex containing the other ErbB and is phosphorylated by the partner ErbB kinase, leading to signal transduction by ErbB3 (Sierke et al., 1997). Conversely, ErbB2 harbors an active kinase domain, but its high-affinity ligands remain unknown (Cho et al., 2003; Garrett et al., 2003). The ErbB1 and ErbB2 genes are often amplified and overexpressed in various cancer cells, resulting in self-dimerization and auto-phosphorylation in a ligand-independent manner. For instance, ErbB2 is amplified in 3% of lung cancers, 30% of breast cancers, 20% of gastric cancers, and 60% of ovarian cancers. The combination of the ErbB1-4 subunits during heterodimerization does not appear to be limited; NRG1-bound ErbB4 can associate with ErbB1 to form a heterodimer, even though ErbB1 is not activated by EGF (Liu et al., 2012). NRG1 mimics EGF signaling through ErbB1 phosphorylation in ErbB4:ErbB1 heterodimer complexes. In the ErbB4:ErbB1 heterodimer, NRG promotes more threonine phosphorylation and less tyrosine phosphorylation of ErbB1, which results in Shc/Grb2 recruitment, than does EGF (Olayioye et al., 1998). Therefore, even within the context of the same heterodimer, distinct ligands can differentially impact receptor signaling (Moghal and Sternberg, 1999). Of note, external stimuli can also affect partnership during ErbB heterodimerization. Glucocorticoids can interfere with organized ErbB receptor dimerization in lung cells, leading to a switch from ErbB1:ErbB4 to ErbB2:ErbB4 heterodimer expression (**Table 1**; Dammann et al., 2006). In addition to the signaling complexity and diversity of ErbB heterodimerization, the individual *erbB* genes or their products are subjected to alternative splicing or proteolytic processing, resulting in truncated isoforms lacking the kinase domain or ligand-binding domain. These truncated isoforms function as an enhancer of tumorigenesis, a receptor decoy or a transcription factor (see below; Yamazaki et al., 1988; Lee et al., 2001; Vidal et al., 2005; Sundvall et al., 2007; Lin et al., 2008; Xia et al., 2011; Ward et al., 2012).

CELL SIGNALING AND FUNCTIONS OF INDIVIDUAL ErbB MOLECULES

All ErbB molecules are expressed not only in peripheral tissues but also in various neural cells (**Table 2**). In the view of their functionality in the brain, we need to consider which ErbB subtype is expressed, where it is expressed, and with which ErbB molecule it colocalizes or dimerizes. As discussed above, the phosphorylated ErbB partner determines the functional nature of signaling, irrespective of the ErbB ligand. In this context, it is not true that NRG only evokes the signals of its receptor, ErbB3 and/or ErbB4.

ErbB1 (EGFR, HER1)

ErbB1 signaling links to a large variety of cellular functions, such as cell survival and proliferation. The down-stream signals linked to ErbB include the phospholipase Cγ (PLCγ)- protein kinase C (PKC), Ras- mitogen-activated protein kinase (MAPK), PI3K-Akt, and janus kinase 2 (JAK2)-STAT3 pathways (**Figure 3**). The Ras-MAPK pathway is implicated in cell proliferation and differentiation, while the PI3K-Akt pathway is involved in cell growth and anti-apoptotic processes and the PLCγ-PKC pathway contributes to cell migration and division. The subcellular localization and protein levels of these adaptors/effectors appear to determine the distinct features of ErbB1 down-stream signals. ErbB1 has a deletion mutant, EGFRvIII (also known as ΔEGFR, type3 EGFR, de 2–7 EGFR, EGFR*), which lacks extracellular domain of EGFR (Yamazaki et al., 1988). In addition, alternative splicing and protein processing produce soluble EGFR isoform (sEGFR). sEGFR lacks intracellular domain (Flickinger et al., 1992; Rose-John and Heinrich, 1994; Perez-Torres et al., 2008). These truncated ErbB1 contribute to tumorigenesis, but their role in brain is not fully understood (Baron et al., 2003; Gan et al., 2009).

Table 2 | Brain distribution and functions of ErbB1-4.

ErbB receptor	Tissue	Cell type	Function
ErbB1	Subventrucular zone	Neural stem cell	Proliferation/ migration
	Midbrain	Dopaminergic neuron	Survival/ development
	Cortex, hippocampus	GABAergic neuron Astrocyte	Regulation of synaptic function Proliferation/ differentiation
	Cerebellum	Purkinje cell Granule cell Astrocyte	Development/ proliferation
	Pituitary grand	Lactotroph	Production/release of cortisol and prolactin
ErbB2	Cerebellum, cortex, hippocampus, midbrain, etc.	Oligodendrocyte Astrocyte Radial glia	Proliferation/ differentiation
ErbB3	Cortex, hippocampus, etc.	Oligodendrocyte	Maturation/ myelination
ErbB4	Cortex, hippocampus,	GABAergic neuron Astrocyte Oligodendrocyte	Attenuates synaptic function Proliferation/ differentiation
	Cerebellum	Granule cell	Regulation of synaptic function
	Midbrain	Dopaminergic neuron	Survival, attenuates synaptic function

In the central nervous system, ErbB1 protein levels are the highest during the gestational stages and gradually decline during development. Consistent with this expression pattern, neural stem cells in the subventricular zone (SVZ) are highly enriched with ErbB1 (Abe et al., 2009a). ErbB1 activation triggers the proliferation and migration of neural stem cells and their immediate decedents (Aguirre et al., 2005, 2010). In addition to these undifferentiated neural cells, several types of differentiated neurons also maintain expression at postnatal stages. In the midbrain region, the nigra-striatal dopamine neurons express ErbB1 together with ErbB4 (Abe et al., 2009a). ErbB1 activation contributes to the survival and postnatal development of dopaminergic neurons, although the molecular nature of the endogenous ErbB1 ligands has not been fully identified (Iwakura et al., 2005, 2011; Namba et al., 2009). Various types of GABAergic neurons also carry ErbB1 receptors. Interneurons in the hippocampus and neocortex as well as cerebella Purkinje cells express ErbB1 (Werner et al., 1988; Seroogy et al., 1995; Namba et al., 2006; Nagano et al., 2007; Abe et al., 2009b). In contrast to its action on dopaminergic neurons, the activation of ErbB1 in GABAergic neurons induces their de-differentiation, as seen in peripheral cancer cells (Namba et al., 2006; Nagano et al., 2007). The differences in the biological activities of EGF/ErbB1 signaling between these two neuronal populations are presumably attributed to differences in the ErbB partner. The signal pathways of ErbB1:ErbB1 homodimers and ErbB1:ErbB4 heterodimers differ significantly as discussed above. In addition to these neurons, astrocytes and their precursors express ErbB1, which is markedly enhanced in response to injury-associated astrogliosis (Liu et al., 2006). ErbB1 is also expressed in the pituitary and regulates the production and release of cortisol and prolactin (Cooper et al., 2011; Dahlhoff et al., 2011). ErbB1 in the hypothalamus reacts with TGFα, which is produced in the suprachiasmatic nucleus, and regulates circadian rhythm (Kramer et al., 2001; Snodgrass-Belt et al., 2005).

ErbB2 (HER2, Neu)

ErbB2 signals mainly link to the Ras-MAPK pathway and the PI3K-Akt pathway, leading to cell proliferation. Therefore, at the postnatal stages, ErbB2 levels are limited to postmitotic neurons or glial cells (Abe et al., 2009a). There are a variety of truncated ErbB2 isoforms that are produced by alternative splicing and metalloprotease digestion (Cappuzzo et al., 2012; Tse et al., 2012; Ward et al., 2012). p110ErbB2 (611CTF) and p95ErbB2 (CTF648) both lack the extracellular domain and contribute to cancer progression and metastasis (Xia et al., 2011; Ward et al., 2012), although their roles in the brain are poorly understood. The carboxyl terminal of ErbB2 carries a PDZ-binding motif and associates with a leucine-rich molecule, Erbin (Huang et al., 2001). As Erbin attenuates the activation of Ras-MAPK signaling linked to cell proliferation, its interaction with ErbB2 is implicated in oligodendrocyte differentiation and myelination (Tao et al., 2009; Dan et al., 2010; Liang et al., 2012).

Proliferating neural stem cells or precursors express high levels of ErbB2 (Abe et al., 2009a) in addition to ErbB1. Oligodendrocyte precursors express ErbB2 together with ErbB3, and ErbB2 activation contributes to the proliferation and differentiation of these cells via ErbB3 phosphorylation (Flores et al., 2000). A recent study revealed that signals from ErbB2:ErbB3 complexes in the neocortex regulate the expression of disrupted schizophrenia 1 (DISC1), which has been implicated in the genetics of schizophrenia (Seshadri et al., 2010). In hippocampal neurons, ErbB2:ErbB4 heterodimers influence the morphological differentiation of these cells (Gerecke et al., 2004).

ErbB3 (HER3)

ErbB3 displays ligand preference for some members of the NRG family; ErbB3 has a high affinity interaction with NRG1, NRG2, and NRG6. Indeed, the intracellular domain of ErbB3 harbors more tyrosine residues that accept various adaptor/effecter molecules (**Table 1**). As its kinase activity is impaired, heterodimer formation with ErbB2 or the other ErbBs is essential to evoke signal transduction cascades. Alternative splicing of the *erbB3* gene produces soluble isoforms of ErbB3 (sErbB3) as well as isoforms with truncations in the intracellular domain (Lee and Maihle, 1998). Among these isoforms, p45 and p85 sErbB3s bind

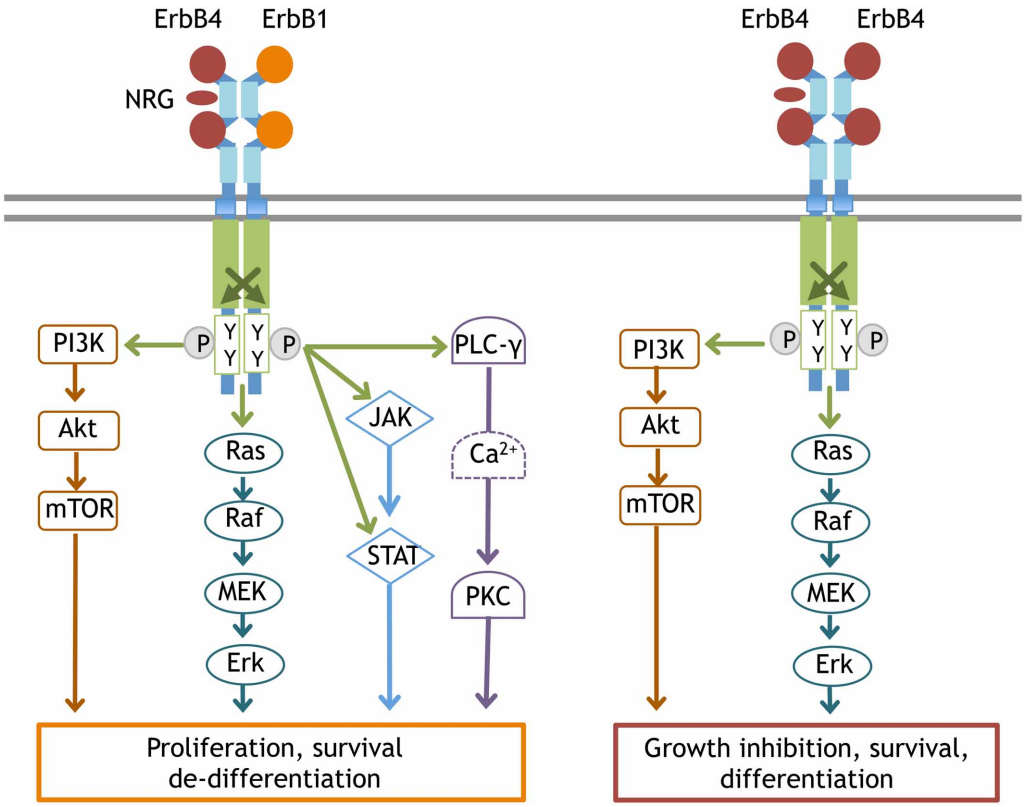

FIGURE 3 | Typical signal transduction from the ErbB4:ErbB1 and ErbB4:ErbB4 complex. Once ErbB1 is phosphorylated by the partner ErbB, the following signal cascades are activated; **(1)** In the PLCγ-PKC pathway, phosphorylated ErbB1 recruits and associates with PLCγ. As a result, PLCγ itself is phosphorylated to activate DAG/IP3 signaling (Chen et al., 1996). **(2)** In the Ras-MAPK pathway, phosphorylated ErbB1 associates with Shc and interacts with Grb2/Sos1. Activated Sos1 triggers GDP/GTP exchange in Ras and activates Ras, driving the sequential kinase reactions of Raf(MAPKKK), MEK(MAPKK), and Erk(MAPK). **(3)** In the PI3K-Akt pathway, the activated ErbB dimer interacts with Grb2/Gab1 and forms complexes with activated PI3Kinase, leading to the conversion of PIP2 to PIP3 and Akt activation. **(4)** In the JAK-STAT pathway, ErbB kinase phosphorylates and induces JAK to bind to ErbB1. Activated JAK phosphorylates STAT and allows STAT to homodimerize and translocate into the nucleus. Once ErbB4 is phosphorylated by ErbB4, the signaling cascades linked to differentiation become activated, notably the PI3K-Akt pathway and the Ras-MAPK pathway with longer durations.

to NRG and decrease the effective concentrations of NRG1 in the extracellular space (Lee et al., 2001; Lin et al., 2008). The truncated isoforms of ErbB3 are found in cortical astrocytes and might be involved in attenuating NRG signaling (Citri and Yarden, 2006; Sharif and Prevot, 2010).

In the brain, high ErbB3 expression is only found in oligodendrocytes and their precursors. ErbB3 activation is involved in their propagation and differentiation (Makinodan et al., 2012). ErbB3 expression is also observed in neural precursor cells in the adult hippocampus and contributes to their proliferation, although ErbB3 expression is modest (Mahar et al., 2011). In human astrocytes, ErbB3, and ErbB1 form heterodimers that transduce NRG-dependent signals (Sharif et al., 2009). Again, EGF evokes NRG-like signaling through the dimerization of ErbB3 and ErbB1.

ErbB4 (HER4)

ErbB4 mainly links to the Ras-MAPK and PI3K-Akt pathways. In contrast to ErbB1 signaling, ErbB4 phosphorylation induces sustained activation of the Ras-MAPK pathway, leading to cell cycle cessation and differentiation (Muraoka-Cook et al., 2008; Ortega et al., 2012). In the *erbB4* genome, alternative splicing of exon 15/16 and exon 26 produces ErbB4 variants, JM-a/b/c/d and CYT-1/2, respectively (**Figure 4**; Zeng et al., 2009; Veikkolainen et al., 2011). The phosphorylation of CYT-1 can recruit the p85 adaptor to activate PI3K-Akt signaling (Kainulainen et al., 2000). The CYT-1 sequence is susceptible to proteolytic cleavage by TNF-alpha converting enzyme (TACE) and γ-secretase (Vidal et al., 2005; Sundvall et al., 2010). Thus, ErbB4 proteolysis produces an 80 kD intracellular fragment (ErbB4-ICD) and liberates it into the cytoplasmic space. ErbB4-ICD interacts with the transcription factor STAT5 and migrates into the nucleus as a molecular chaperone (Vidal et al., 2005; Sundvall et al., 2010).

ErbB4 also contains a PDZ-binding motif at the carboxyl terminal and is anchored to postsynaptic density protein 95 (PSD95) in neurons (Huang et al., 2000). Even when proteolytic cleavage produces ErbB4-ICD or when ErbB4 is phosphorylated with the ErbB partner, the signal is only minimally transported to the soma or translocated into the nucleus. Rather, the interaction with the scaffold protein PSD95 allows NMDA receptors to

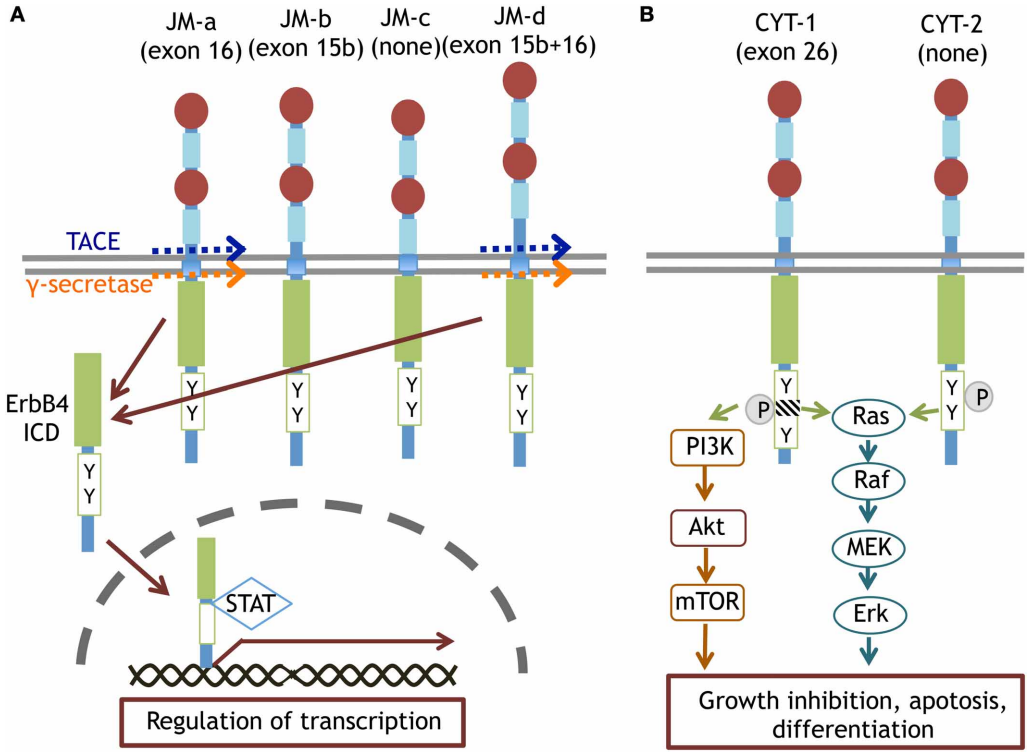

FIGURE 4 | Splice variants of ErbB4 proteins and their specific signaling. The JM-a and JM-d isoforms of ErbB4 are cleaved by TACE or γ-secretase and converted to ErbB4-ICD. ErbB4-ICD is translocated into the nucleus with STAT and regulates gene transcription **(A)**. The CYT-1 and CYT-2 isoforms of ErbB4 differentially trigger Ras-MAPK signaling and/or PI3K-Akt signaling **(B)**.

interact with ErbB4 and restrict ErbB4 signaling to the postsynaptic compartments (Garcia et al., 2000). Accordingly, impaired NRG1/ErbB4 signal is thought to underlie NMDA receptor dysfunction found in brain diseases such as schizophrenia (Hahn et al., 2006; Pitcher et al., 2011). ErbB4 also can form molecular complexes with the receptor-type tyrosine phosphatase (Ptprz) via its interaction with PSD95 (Fujikawa et al., 2007). In this complex, Ptprz interacts with ErbB4 as its substrate and dephosphorylates ErbB4. Given the ligands for Ptprz (i.e., midkines and pleiotropins), NRG/ErbB4 signals can be disrupted by other cytokines through this receptor-type tyrosine phosphatase (**Figure 5**).

ErbB4 expression gradually increases in the brain and becomes pronounced in postmitotic neurons such as GABAergic interneurons, dopamine neurons, and cerebellar granule cells (**Table 2**; Elenius et al., 1997; Abe et al., 2009a; Vullhorst et al., 2009). The developmental pattern of ErbB4 expression is the opposite of that of ErbB1 (Abe et al., 2009a). ErbB4 signals may accelerate neural differentiation in these cell populations, potentially attenuating ErbB1 signaling (Woo et al., 2007; Fazzari et al., 2010). The NRG-driven up-regulation of glutamate receptor functions may represent the typical phenotypic responses of this cell population; ErbB4 activation regulates the activity and/or expression of both AMPA-type and NMDA-type glutamate receptors in GABAergic neurons (Gajendran et al., 2009; Abe et al., 2011; Ting et al., 2011). ErbB4 in midbrain dopaminergic neurons regulates the enzyme activity and expression of tyrosine hydroxylase *in vivo* as well as dopamine synthesis and release (Kato et al., 2011). Although ErbB4 is suggested to contribute to the migration and differentiation of immature GABAergic interneurons, these processes also involve ErbB1, and thus, the interplay between ErbB4 and ErbB1 needs to be characterized to reveal the full mechanism (Mahar et al., 2011; Li et al., 2012). In our previous studies, these phenotypic actions of NRG1/ErbB4 signals appear to be more modest than those of EGF/ErbB1 signals in neural cultures. Consistent with these findings, the gross brain structures and function of ErbB4-null knockout mice appear to be modest compared with ErbB1-null knockout mice (Sibilia and Wagner, 1995; Sibilia et al., 1998; Thuret et al., 2004). In this context, the crucial functions and/or biological significance of ErbB4 in the brain might not be fully characterized.

IMPLICATIONS OF ABNORMAL ErbB SIGNALING IN BRAIN DISEASES

ErbB signaling contributes to the development and maintenance of various cell populations in the central nervous system and is therefore implicated in the etiology or neuropathology of various brain diseases such as schizophrenia and Parkinson's disease, which involve cell dysfunction of GABAergic and/or dopaminergic neurons. Here we would like to discuss the potential pathological links between ErbB signaling and these brain diseases.

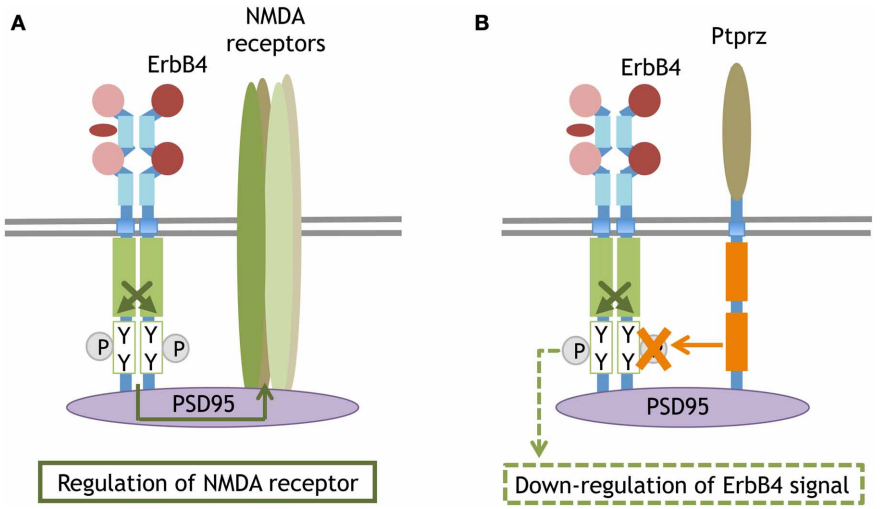

FIGURE 5 | Synaptic compartment of ErbB4 that binds to PSD95 or interacts with the phosphatase. The scaffolding protein PSD95 anchors ErbB4 and the NMDA receptor at postsynaptic sites. The molecular interaction between ErbB4 influences NMDA receptor activity and function **(A)**. The PSD95-mediated interaction between ErbB4 and a membrane attached phosphatase, Ptprz. Ptprz eliminates the phosphate from ErbB4 and attenuates its signaling **(B)**.

PARKINSON'S DISEASE

Parkinson's disease is a progressive neurodegenerative disorder in which patients exhibit obvious symptoms of motor dysfunction, such as shaking and muscle rigidity. This disease progresses to neurodegeneration of the midbrain dopaminergic neurons. Consistent with the neurotrophic actions of EGF on this cell population, the protein levels of EGF and ErbB1 are diminished in the postmortem brains of patients with this disease (Iwakura et al., 2005). A neurotrophic disturbance in ErbB1 signaling is reproduced in animal models of the disease; rats receiving a dopaminergic neurotoxin exhibit decreased ErbB1 and dopaminergic cell loss but EGF ameliorates these deficits (Pezzoli et al., 1991; Ventrella, 1993; Iwakura et al., 2005). Similarly, the contribution of ErbB4 signals to this illness is under investigation. Because of the higher blood-brain permiability of type 1 NRG1 (Kato et al., 2011), NRG1 was peripherally administeration to a Parkison's disease model to induce the neuroprotection of dopamine neurons (Zhang et al., 2004; Carlsson et al., 2011; Depboylu et al., 2012).

The molecular neuropathology of Parkinson's disease involves not only ErbB1 but also ErbB-interacting molecules. For example, LINGO-1, which associates with the Nogo-receptors in the nervous system, directly binds to ErbB1 to attenuate cell survival signals (i.e., PI3K-Akt signaling) in dopamine neurons (Inoue et al., 2007). Consistent with this finding, LINGO-1 expression is elevated in the substantia nigra of patients with Parkinson's disease. The molecule parkin, which is the causative gene for inheritable Parkinson's disease (Kitada et al., 1998), maintains ErbB1 signaling under normal conditions. Parkin can promote the ubiquitination and dissociation of Eps15 from ErbB1 to attenuate the internalization and degradation of ErbB1 (Fallon et al., 2006). Conversely, mutations in the parkin gene result in accelerated ErbB1 degradation, leading to the loss of neurotrophic ErbB1 signals in this disease.

SCHIZOPHRENIA

ErbB1 as well as ErbB4 is distributed in all the cell populations that are implicated in schizophrenia neuropathology, including GABAergic neurons, dopaminergic neurons, and glial cells. Several studies have focused on the ErbB1 molecule. Postmortem studies revealed that the ErbB1 protein is up-regulated in the forebrain regions of schizophrenia patients (Futamura et al., 2002). Animal studies demonstrate that acute and subchronic brain activation of ErbB1 triggers dopamine release in the striatum or globus pallidus, leading to behavioral impairments relevant to schizophrenia (Futamura et al., 2003; Tohmi et al., 2005; Mizuno et al., 2008; Sotoyama et al., 2011). In contrast to the effects on dopamine neurons, ErbB1 ligands negatively regulate GABAergic development in the neocortex and attenuate the activity of glutamate receptor channels in these neurons (Namba et al., 2006; Nagano et al., 2007). Conversely, quianozoline ErbB1 inhibitors can ameliorate schizophrenia-related behaviors in various animal models for schizophrenia (Mizuno et al., 2008). Both types of model studies indicate a pathological link between ErbB1 hypersignaling and schizophrenia. Given that ErbB1 and ErbB4 colocalization within the same neurons, it is likely that ErbB1 competes with NRG/ErbB4 signals, as was suggested in cancer studies (Moghal and Sternberg, 1999; Pitfield et al., 2006; Das et al., 2010).

Genetic studies have also demonstrated that schizophrenia is associated not only with the ligand NRG but also with its receptor ErbB4 (Stefansson et al., 2002). In 2006, SNP analysis revealed a genetic association between the *erbB4* gene and a particular type of splicing pattern associated with this illness (Norton et al., 2006; Silberberg et al., 2006). The risk of *erbB4* SNPs appears

to correlate with the disease-specific spicing pattern (i.e., JM-a and CYT-1) in the prefrontal cortex and hippocampus of patients (Law et al., 2007; Tan et al., 2010). A postmortem study also found an increase in phosphorylated ErbB4 protein and its ability to form complexes with PSD95 but failed to detect a difference in total ErbB4 levels in schizophrenia patients (Hahn et al., 2006).

In addition to the neuropathology of GABAergic and dopaminergic neurons in schizophrenia, postmortem studies indicate the deficits in white matter and myelin structures are associated with this illness (Davis et al., 2003; Flynn et al., 2003). ErbB3 signals play crucial roles in oligodendrocyte myelination and saltatory conduction of nerve impulses (Stewart and Davis, 2004). Thus, several schizophrenia studies have focused on ErbB3 function. Aston et al. (2004) found that mRNA levels of genes related to myelin and oligodendrocytes, including *erbB3* mRNA, are down-regulated in the middle temporal gyrus of schizophrenia patients (Aston et al., 2004). However, the genetic association between *erbB3* SNPs and schizophrenia remains controversial (Kanazawa et al., 2007; Watanabe et al., 2007). In addition to the genetic association between *erbB* SNPs and schizophrenia, viral infection also directly triggers ErbB signaling and potentially contributes to brain mal-development.

IMPACT OF VIROKINES ON ErbB SIGNALING

Virokine is a general term for a cytokine produced by viruses. By producing virokines, many viruses perturb the immune defense system of host organisms to escape clearance or promote host cell proliferation to enhance viral propagation (Klouche et al., 2004). A variety of virokines have been identified, including those that act on ErbB receptors (**Table 3**). In fact, some virokines are suggested to impair brain development (Billings et al., 2004). Thus, virokine production following viral infection directly influences brain development and might support the schizophrenia hypothesis of viral infection (Waddington and Buckley, 1996; Brown and Derkits, 2010).

Vaccinia virus growth factor (VGF, Vaccinia virus 19-kilodalton protein) is encoded by the genome of vaccinia virus in the poxvirus family and has an amino acid sequence homologous to EGF (**Figure 6**). VGF is produced and secreted from its membrane-anchored precursor and binds to ErbB1 receptors. VGF activates the Ras-MAPK pathway of host cells and promotes cell proliferation (Eppstein et al., 1985; Twardzik et al., 1985). Cowpox virus growth factor (CGF) displays high homology to VGF and enhances host cell propagation (da Fonseca et al., 1999). Additional EGF-like virokines have been identified in other pox viruses, including smallpox virus growth factor (SPGF; Kim et al., 2004), myxoma virus growth factor (MGF; Opgenorth et al., 1993), and shope fibroma virus growth factor (SFGF; Chang et al., 1987; Ye et al., 1988). These virokines also carry an EGF-like sequence and interact with ErbB receptors. According to the schizophrenia hypothesis of maternal and perinatal viral infection, the infection of these viruses and their translocation to the brain may perturb the normal development of dopaminergic or GABAergic neurons, although this assumption is fully hypothetical.

In addition to soluble virokines, virus-derived effectors can affect intracellular ErbB signaling. E5 is one of the early gene products of human papillomaviruses. E5 protein is also a membrane-anchored molecule, like the EGF precursor, but lacks the EGF-like domain. For example, the E5 protein of human papillomavirus type 16(HPV-16) associates with the ATPase motif of the ErbB1 tyrosine kinase domain, attenuate its interaction with Cbl, and inhibits the internalization and degradation of ErbB1 (**Figure 6**). Accordingly, papillomavirus infection enhances EGF/ErbB1 signaling to promote host cell proliferation (Chang et al., 2001; Venuti et al., 2011; Ganguly, 2012). In addition to ErbB1, the E5 protein also binds to the JM-b/CYT-1 isoform of ErbB4 and promotes host cell survival (Chen et al., 2007). The E5 protein in other papillomaviruses (human papillomavirus type 6 and bovine papillomavirus type1) exerts similar modifications on ErbB signaling (Martin et al., 1989; Cohen et al., 1993; Conrad et al., 1994). It is noteworthy that the human uterus can be infected with human papillomavirus type 16. Assuming that a human embryo develops in a uterus harboring papillomavirus, the potential direct or indirect impact of the E5 protein on fetal brain development cannot be overlooked.

Table 3 | Virokines that bind to ErbB receptors.

Virokine (ErbB-binding proteins)		Receptors	Related proteins
VGF	VGF (Vaccinia virus growth factor, vaccinia 19kd protein)	ErbB1 homodimer	EGF, TGFα
	CGF (Cowpox growth factor)	ErbB1	VGF EGF family
	SPGF (Smallpox growth factor)	ErbB1	VGF EGF family
	SFGF (Shope fibroma virus growth factor)	ErbB1 containing dimer	VGF EGF family
	MGF (Myxoma virus growth factor)	ErbB2/ErbB3	VGF EGF family
E5 protein	Human papilloma virus type16 E5 protein	ErbB1 ErbB4 (JM-b CYT-1)	
	Human papillomavirus type6 E5 protein	ErbB1, ErbB2	
	Bovine papillomavirus type1 E5 protein	ErbB1	

PROVISIONAL CONCLUSION

Studies in cancer biology clearly indicate the pathologic powers of abnormal ErbB signaling and its contribution to oncogenesis, asthma, injury repair, and rheumatoid arthritis (Stoll and Elder, 1998; Davies et al., 1999; Satoh et al., 2001; Bersell et al., 2009; Calvo et al., 2010; Finigan et al., 2011; Yarden and Pines, 2012). In contrast to our knowledge of ErbB signaling in the periphery, the biological functions and regulation of ErbB signaling in the brain are still limited (Buonanno and Fischbach, 2001; Wong and Guillaud, 2004; Mei and Xiong, 2008). The ligand-bound ErbB

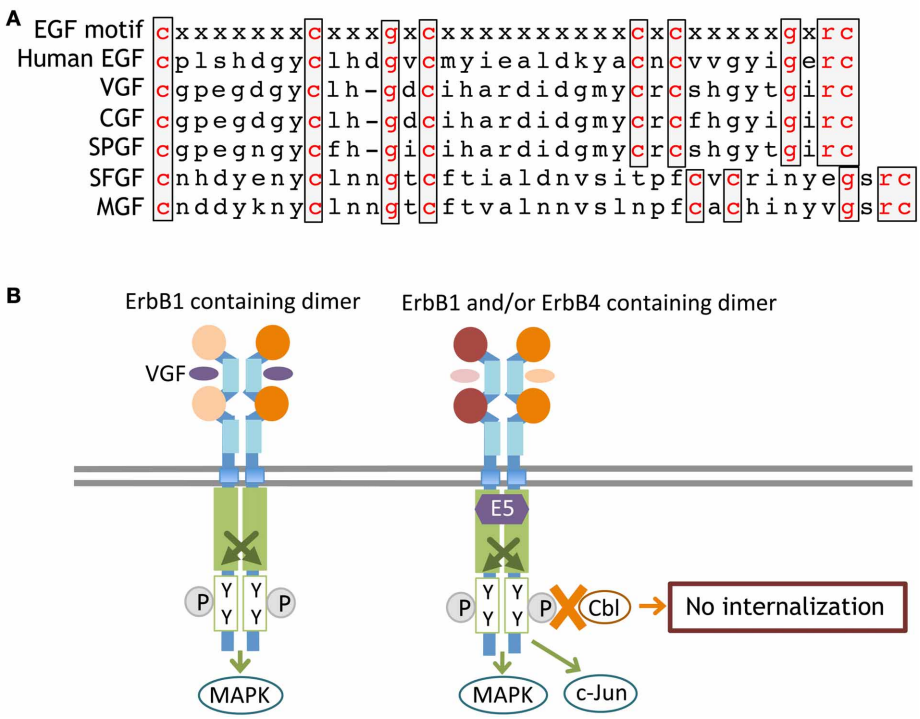

FIGURE 6 | Primary sequences of ErbB-interacting virokines and receptor interactions. The primary amino acid sequences of EGF and virokines in the poxvirus family are shown **(A)**. An ErbB adaptor, E5, which is produced by papillomaviruses, associates with the kinase domain of ErbB and inhibits its internalization and/or degradation **(B)**.

receptor does not transmit signals, and instead the ErbB partner acts as a kinase substrate to trigger intracellular signaling. In addition, we need to consider which individual ErbB splicing isoforms are expressed in individual neural cells because some ErbB isoforms have a dominant-active or -negative function. Currently, we only know that *erbB* gene products are present in certain neurons or glia and not the real structures of particular ErbB isoforms in various brain regions. In this context, a more elaborate analysis may be required to accurately discuss their functions in the nervous system.

Although there are a total of four ErbB molecules, their ligands have significantly more diversity. There are six endogenous ErbB ligands in the EGF family and six in the NRG family. Virus-derived ErbB ligands also need to be considered. In contrast to the investigations on EGF or NRG1, the contribution of the other ligands, such as HB-EGF and NRG6 (neuroglycan C), is poorly understood even though those are highly expressed in the brain (Kinugasa et al., 2004; Nakanishi et al., 2006; Oyagi et al., 2009, 2011). Together with the proteolytic regulation of ErbB proteins and ligand precursors, ErbB signaling is regulated at multiple levels, including SNPs, alternative splicing, proteolytic processing, intracellular translocation, and signal cross talks between ErbBs. We hope this review will hint at the biological importance of ErbB in the nervous system and drive readers to challenge biological or pathological questions regarding ErbB signaling.

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REFERENCES

Abe, Y., Namba, H., Kato, T., Iwakura, Y., and Nawa, H. (2011). Neuregulin-1 signals from the periphery regulate AMPA receptor sensitivity and expression in GABAergic interneurons in developing neocortex. *J. Neurosci.* 31, 5699–5709.

Abe, Y., Namba, H., Zheng, Y., and Nawa, H. (2009a). *In situ* hybridization reveals developmental regulation of ErbB1-4 mRNA expression in mouse midbrain: implication of ErbB receptors for dopaminergic neurons. *Neuroscience* 161, 95–110.

Abe, Y., Nawa, H., and Namba, H. (2009b). Activation of epidermal growth factor receptor ErbB1 attenuates inhibitory synaptic development in mouse dentate gyrus. *Neurosci. Res.* 63, 138–148.

Aguirre, A., Rizvi, T. A., Ratner, N., and Gallo, V. (2005). Overexpression of the epidermal growth factor receptor confers migratory properties to nonmigratory postnatal neural progenitors. *J. Neurosci.* 25, 11092–11106.

Aguirre, A., Rubio, M. E., and Gallo, V. (2010). Notch and EGFR pathway interaction regulates neural stem cell number and self-renewal. *Nature* 467, 323–327.

Aroian, R. V., Koga, M., Mendel, J. E., Ohshima, Y., and Sternberg, P. W. (1990). The let-23 gene necessary for *Caenorhabditis elegans* vulval induction encodes a tyrosine kinase of the EGF receptor subfamily. *Nature* 348, 693–699.

Aston, C., Jiang, L., and Sokolov, B. P. (2004). Microarray analysis of postmortem temporal cortex from patients with schizophrenia. *J. Neurosci. Res.* 77, 858–866.

Baron, A. T., Cora, E. M., Lafky, J. M., Boardman, C. H., Buenafe, M. C., Rademaker, A., et al. (2003). Soluble epidermal growth factor receptor (sEGFR/sErbB1) as a potential risk, screening, and diagnostic serum biomarker of epithelial ovarian cancer. *Cancer Epidemiol. Biomarkers Prev.* 12, 103–113.

Bersell, K., Arab, S., Haring, B., and Kuhn, B. (2009). Neuregulin1/ErbB4 signaling induces cardiomyocyte proliferation and repair of heart injury. *Cell* 138, 257–270.

Billings, B., Smith, S. A., Zhang, Z., Lahiri, D. K., and Kotwal, G. J. (2004). Lack of N1L gene expression results in a significant decrease of vaccinia virus replication in mouse brain. *Ann. N.Y. Acad. Sci.* 1030, 297–302.

Brockes, J. P., and Kintner, C. R. (1986). Glial growth factor and nerve-dependent proliferation in the regeneration. *Cell* 45, 301–306.

Brown, A. S., and Derkits, E. J. (2010). Prenatal infection and schizophrenia: a review of epidemiologic and translational studies. *Am. J. Psychiatry* 167, 261–280.

Buonanno, A., and Fischbach, G. D. (2001). Neuregulin and ErbB receptor signaling pathways in the nervous system. *Curr. Opin. Neurobiol.* 11, 287–296.

Calvo, M., Zhu, N., Tsantoulas, C., Ma, Z., Grist, J., Loeb, J. A., et al. (2010). Neuregulin-ErbB signaling promotes microglial proliferation and chemotaxis contributing to microgliosis and pain after peripheral nerve injury. *J. Neurosci.* 30, 5437–5450.

Cappuzzo, F., Cho, Y. G., Sacconi, A., Alì, G., Siclari, O., Incarbone, M., et al. (2012). p95HER2 truncated form in resected non-small cell lung cancer. *J. Thorac. Oncol.* 7, 520–527.

Carlsson, T., Schindler, F. R., Hollerhage, M., Depboylu, C., Arias-Carrión, O., Schnurrbusch, S., et al. (2011). Systemic administration of neuregulin-1beta1 protects dopaminergic neurons in a mouse model of Parkinson's disease. *J. Neurochem.* 117, 1066–1074.

Chang, J. L., Tsao, Y. P., Liu, D. W., Huang, S. J., Lee, W. H., and Chen, S. L. (2001). The expression of HPV-16 E5 protein in squamous neoplastic changes in the uterine cervix. *J. Biomed. Sci.* 8, 206–213.

Chang, W., Upton, C., Hu, S. L., Purchio, A. F., and McFadden, G. (1987). The genome of Shope fibroma virus, a tumorigenic poxvirus, contains a growth factor gene with sequence similarity to those encoding epidermal growth factor and transforming growth factor alpha. *Mol. Cell. Biol.* 7, 535–540.

Chen, P., Xie, H., and Wells, A. (1996). Mitogenic signaling from the egf receptor is attenuated by a phospholipase C-gamma/protein kinase C feedback mechanism. *Mol. Biol. Cell.* 7, 871–881.

Chen, S. L., Lin, S. T., Tsai, T. C., Hsiao, W. C., and Tsao, Y. P. (2007). ErbB4 (JM-b/CYT-1)-induced expression and phosphorylation of c-Jun is abrogated by human papillomavirus type 16 E5 protein. *Oncogene* 26, 42–53.

Cho, H. S., Mason, K., Ramyar, K. X., Stanley, A. M., Gabelli, S. B., Denney, D. W., et al. (2003). Structure of the extracellular region of HER2 alone and in complex with the Herceptin Fab. *Nature* 421, 756–760.

Citri, A., and Yarden, Y. (2006). EGF-ERBB signalling: towards the systems level. *Nat. Rev. Mol. Cell Biol.* 7, 505–516.

Cohen, B. D., Goldstein, D. J., Rutledge, L., Vass, W. C., Lowy, D. R., Schlegel, R., et al. (1993). Transformation-specific interaction of the bovine papillomavirus E5 oncoprotein with the platelet-derived growth factor receptor transmembrane domain and the epidermal growth factor receptor cytoplasmic domain. *J. Virol.* 67, 5303–5311.

Conrad, M., Goldstein, D., Andresson, T., and Schlegel, R. (1994). The E5 protein of HPV-6, but not HPV-16, associates efficiently with cellular growth factor receptors. *Virology* 200, 796–800.

Cooper, O., Vlotides, G., Fukuoka, H., Greene, M. I., and Melmed, S. (2011). Expression and function of ErbB receptors and ligands in the pituitary. *Endocr. Relat. Cancer* 18, 197–211.

da Fonseca, F. G., Silva, R. L., Marques, J. T., Ferreira, P. C., and Kroon, E. G. (1999). The genome of cowpox virus contains a gene related to those encoding the epidermal growth factor, transforming growth factor alpha and vaccinia growth factor. *Virus Genes* 18, 151–160.

Dahlhoff, M., Blutke, A., Wanke, R., Wolf, E., and Schneider, M. R. (2011). *In vivo* evidence for epidermal growth factor receptor (EGFR)-mediated release of prolactin from the pituitary gland. *J. Biol. Chem.* 286, 39297–39306.

Dammann, C. E., Nassimi, N., Liu, W., and Nielsen, H. C. (2006). ErbB receptor regulation by dexamethasone in mouse type II epithelial cells. *Eur. Respir. J.* 28, 1117–1123.

Dan, L., Shi, M., Duan, H., Han, C., and Guo, N. (2010). Erbin, a negative regulator in diverse signal pathways. *Curr. Protein Pept. Sci.* 11, 759–764.

Das, P. M., Thor, A. D., Edgerton, S. M., Barry, S. K., Chen, D. F., and Jones, F. E. (2010). Reactivation of epigenetically silenced HER4/ERBB4 results in apoptosis of breast tumor cells. *Oncogene* 29, 5214–5219.

Davies, D. E., Polosa, R., Puddicombe, S. M., Richter, A., and Holgate, S. T. (1999). The epidermal growth factor receptor and its ligand family: their potential role. *Allergy* 54, 771–783.

Davis, K. L., Stewart, D. G., Friedman, J. I., Buchsbaum, M., Harvey, P. D., Hof, P. R., et al. (2003). White matter changes in schizophrenia: evidence for myelin-related dysfunction. *Arch. Gen. Psychiatry* 60, 443–456.

Depboylu, C., Hollerhage, M., Schnurrbusch, S., Brundin, P., Oertel, W. H., Schrattenholz, A., et al. (2012). Neuregulin-1 receptor tyrosine kinase ErbB4 is upregulated in midbrain dopaminergic neurons in Parkinson disease. *Neurosci. Lett.* 531, 209–214.

Derynck, R., Roberts, A. B., Winkler, M. E., Chen, E. Y., and Goeddel, D. V. (1984). Human transforming growth factor-alpha: precursor structure and expression in *E. coli*. *Cell* 38, 287–297.

Downward, J., Yarden, Y., Mayes, E., Scrace, G., Totty, N., Stockwell, P., et al. (1984). Close similarity of epidermal growth factor receptor and v-erb-B oncogene protein sequences. *Nature* 307, 521–527.

Elenius, K., Corfas, G., Paul, S., Choi, C. J., Rio, C., Plowman, G. D., et al. (1997). A novel juxtamembrane domain isoform of HER4/ErbB4. Isoform-specific tissue distribution and differential processing in response to phorbol ester. *J. Biol. Chem.* 272, 26761–26768.

Eppstein, D. A., Marsh, Y. V., Schreiber, A. B., Newman, S. R., Todaro, G. J., and Nestor, J. J. Jr. (1985). Epidermal growth factor receptor occupancy inhibits vaccinia virus infection. *Nature* 318, 663–665.

Fallon, L., Belanger, C. M., Corera, A. T., Kontogiannea, M., Regan-Klapisz, E., Moreau, F., et al. (2006). A regulated interaction with the UIM protein Eps15 implicates parkin in EGF receptor trafficking and PI(3)K-Akt signalling. *Nat. Cell Biol.* 8, 834–842.

Falls, D. L. (2003). Neuregulins: functions, forms, and signaling strategies. *Exp. Cell Res.* 284, 14–30.

Falls, D. L., Rosen, K. M., Corfas, G., Lane, W. S., and Fischbach, G. D. (1993). ARIA, a protein that stimulates acetylcholine receptor synthesis, is a member of the neu ligand family. *Cell* 72, 801–815.

Fazzari, P., Paternain, A. V., Valiente, M., Pla, R., Lujan, R., Lloyd, K., et al. (2010). Control of cortical GABA circuitry development by Nrg1 and ErbB4 signalling. *Nature* 464, 1376–1380.

Finigan, J. H., Faress, J. A., Wilkinson, E., Mishra, R. S., Nethery, D. E., Wyler, D., et al. (2011). Neuregulin-1-human epidermal receptor-2 signaling is a central regulator of pulmonary epithelial permeability and acute lung injury. *J. Biol. Chem.* 286, 10660–10670.

Flickinger, T. W., Maihle, N. J., and Kung, H. J. (1992). An alternatively processed mRNA from the avian c-erbB gene encodes a soluble, truncated form of the receptor that can block ligand-dependent transformation. *Mol. Cell Biol.* 12, 883–893.

Flores, A. I., Mallon, B. S., Matsui, T., Ogawa, W., Rosenzweig, A., Okamoto, T., et al. (2000). Akt-mediated survival of oligodendrocytes induced by neuregulins. *J. Neurosci.* 20, 7622–7630.

Flynn, S. W., Lang, D. J., Mackay, A. L., Goghari, V., Vavasour, I. M., Whittall, K. P., et al. (2003). Abnormalities of myelination in schizophrenia detected *in vivo* with MRI, and post-mortem with analysis of oligodendrocyte proteins. *Mol. Psychiatry* 8, 811–820.

Fujikawa, A., Chow, J. P., Shimizu, H., Fukada, M., Suzuki, R., and Noda, M. (2007). Tyrosine phosphorylation of ErbB4 is enhanced by PSD95 and repressed by protein tyrosine phosphatase receptor type Z. *J. Biochem.* 142, 343–350.

Futamura, T., Kakita, A., Tohmi, M., Sotoyama, H., Takahashi, H., and Nawa, H. (2003). Neonatal perturbation of neurotrophic signaling results in abnormal sensorimotor gating and social interaction in adults: implication for epidermal growth factor in cognitive development. *Mol. Psychiatry* 8, 19–29.

Futamura, T., Toyooka, K., Iritani, S., Niizato, K., Nakamura, R., Tsuchiya, K., et al. (2002). Abnormal

expression of epidermal growth factor and its receptor in the forebrain and serum of schizophrenic patients. *Mol. Psychiatry* 7, 673–682.

Gajendran, N., Kapfhammer, J. P., Lain, E., Canepari, M., Vogt, K., Wisden, W., et al. (2009). Neuregulin signaling is dispensable for NMDA- and GABA(A)-receptor expression in the cerebellum *in vivo*. *J. Neurosci.* 29, 2404–2413.

Gan, H. K., Kaye, A. H., and Luwor, R. B. (2009). The EGFRvIII variant in glioblastoma multiforme. *J. Clin. Neurosci.* 16, 748–754.

Ganguly, N. (2012). Human papillomavirus-16 E5 protein: oncogenic role and therapeutic value. *Cell Oncol. (Dordr).* 35, 67–76.

Garcia, R. A., Vasudevan, K., and Buonanno, A. (2000). The neuregulin receptor ErbB-4 interacts with PDZ-containing proteins at neuronal synapses. *Proc. Natl. Acad. Sci. U.S.A.* 97, 3596–3601.

Garrett, T. P., McKern, N. M., Lou, M., Elleman, T. C., Adams, T. E., Lovrecz, G. O., et al. (2003). The crystal structure of a truncated ErbB2 ectodomain reveals an active conformation, poised to interact with other ErbB receptors. *Mol. Cell* 11, 495–505.

Gerecke, K. M., Wyss, J. M., and Carroll, S. L. (2004). Neuregulin-1beta induces neurite extension and arborization in cultured hippocampal neurons. *Mol. Cell. Neurosci.* 27, 379–393.

Hahn, C. G., Wang, H. Y., Cho, D. S., Talbot, K., Gur, R. E., Berrettini, W. H., et al. (2006). Altered neuregulin 1-erbB4 signaling contributes to NMDA receptor hypofunction in schizophrenia. *Nat. Med.* 12, 824–828.

Higashiyama, S., Abraham, J. A., Miller, J., Fiddes, J. C., and Klagsbrun, M. (1991). A heparin-binding growth factor secreted by macrophage-like cells that is related to EGF. *Science* 251, 936–939.

Higashiyama, S., Horikawa, M., Yamada, K., Ichino, N., Nakano, N., Nakagawa, T., et al. (1997). A novel brain-derived member of the epidermal growth factor family that interacts with ErbB3 and ErbB4. *J. Biochem.* 122, 675–680.

Higashiyama, S., Iwabuki, H., Morimoto, C., Hieda, M., Inoue, H., and Matsushita, N. (2008). Membrane-anchored growth factors, the epidermal growth factor family: beyond receptor ligands. *Cancer Sci.* 99, 214–220.

Ho, W. H., Armanini, M. P., Nuijens, A., Phillips, H. S., and Osheroff, P. L. (1995), Sensory and motor neuron-derived factor. A novel heregulin variant highly expressed in sensory and motor neurons. *J. Biol. Chem.* 270, 14523–14532.

Hobbs, S. S., Coffing, S. L., Le, A. T., Cameron, E. M., Williams, E. E., Andrew, M., et al. (2002). Neuregulin isoforms exhibit distinct patterns of ErbB family receptor activation. *Oncogene* 21, 8442–8452.

Holmes, W. E., Sliwkowski, M. X., Akita, R. W., Henzel, W. J., Lee, J., Park, J. W., et al. (1992). Identification of heregulin, a specific activator of p185erbB2. *Science* 256, 1205–1210.

Huang, Y. Z., Wang, Q., Xiong, W. C., and Mei, L. (2001). Erbin is a protein concentrated at postsynaptic membranes that interacts with PSD-95. *J. Biol. Chem.* 276, 19318–19326.

Huang, Y. Z., Won, S., Ali, D. W., Wang, Q., Tanowitz, M., Du, Q. S., et al. (2000). Regulation of neuregulin signaling by PSD-95 interacting with ErbB4 at CNS. *Neuron* 26, 443–455.

Inoue, H., Lin, L., Lee, X., Shao, Z., Mendes, S., Snodgrass-Belt, P., et al. (2007). Inhibition of the leucine-rich repeat protein LINGO-1 enhances survival, structure, and function of dopaminergic neurons in Parkinson's disease models. *Proc. Natl. Acad. Sci. U.S.A.* 104, 14430–14435.

Iwakura, Y., Piao, Y. S., Mizuno, M., Takei, N., Kakita, A., Takahashi, H., et al. (2005). Influences of dopaminergic lesion on epidermal growth factor-ErbB signals in Parkinson's disease and its model: neurotrophic implication in nigrostriatal neurons. *J. Neurochem.* 93, 974–983.

Iwakura, Y., Zheng, Y., Sibilia, M., Abe, Y., Piao, Y. S., Yokomaku, D., et al. (2011). Qualitative and quantitative re-evaluation of epidermal growth factor-ErbB1 action on developing midbrain dopaminergic neurons *in vivo* and *in vitro*: target-derived neurotrophic signaling (Part 1). *J. Neurochem.* 118, 45–56.

Iwamoto, R., and Mekada, E. (2000). Heparin-binding EGF-like growth factor: a juxtacrine growth factor. *Cytokine Growth Factor Rev.* 11, 335–344.

Kainulainen, V., Sundvall, M., Maatta, J. A., Santiestevan, E., Klagsbrun, M., and Elenius, K. (2000). A natural ErbB4 isoform that does not activate phosphoinositide 3-kinase mediates proliferation but not survival or chemotaxis. *J. Biol. Chem.* 275, 8641–8649.

Kanazawa, T., Glatt, S. J., Tsutsumi, A., Kikuyama, H., Koh, J., Yoneda, H., et al. (2007). Schizophrenia is not associated with the functional candidate gene ERBB3: results from a case-control study. *Am. J. Med. Genet. B Neuropsychiatr. Genet.* 144B, 113–116.

Kato, T., Abe, Y., Sotoyama, H., Kakita, A., Kominami, R., Hirokawa, S., et al. (2011). Transient exposure of neonatal mice to neuregulin-1 results in hyperdopaminergic states in adulthood: implication in neurodevelopmental hypothesis for schizophrenia. *Mol. Psychiatry.* 16, 307–320.

Kim, M., Yang, H., Kim, S. K., Reche, P. A., Tirabassi, R. S., Hussey, R. E., et al. (2004). Biochemical and functional analysis of smallpox growth factor (SPGF) and anti-SPGF monoclonal antibodies. *J. Biol. Chem.* 279, 25838–25848.

Kinugasa, Y., Ishiguro, H., Tokita, Y., Oohira, A., Ohmoto, H., and Higashiyama, S. (2004). Neuroglycan, C, a novel member of the neuregulin family. *Biochem. Biophys. Res. Commun.* 321, 1045–1049.

Kitada, T., Asakawa, S., Hattori, N., Matsumine, H., Yamamura, Y., Minoshima, S., et al. (1998). Mutations in the parkin gene cause autosomal recessive juvenile parkinsonism. *Nature* 392, 605–608.

Klouche, M., Carruba, G., Castagnetta, L., and Rose-John, S. (2004). Virokines in the pathogenesis of cancer: focus on human herpesvirus 8. *Ann. N.Y. Acad. Sci.* 1028, 329–339.

Kramer, A., Yang, F. C., Snodgrass, P., Li, X., Scammell, T. E., Davis, F. C., et al. (2001). Regulation of daily locomotor activity and sleep by hypothalamic EGF receptor signaling. *Science* 294, 2511–2515.

Kraus, M. H., Issing, W., Miki, T., Popescu, N. C., and Aaronson, S. A. (1989). Isolation and characterization of ERBB3, a third member of the ERBB/epidermal growth factor receptor family: evidence for overexpression in a subset of human mammary tumors. *Proc. Natl. Acad. Sci. U.S.A.* 86, 9193–9197.

Law, A. J., Kleinman, J. E., Weinberger, D. R., and Weickert, C. S. (2007). Disease-associated intronic variants in the ErbB4 gene are related to altered ErbB4 splice-variant expression in the brain in schizophrenia. *Hum. Mol. Genet.* 16, 129–141.

Lee, H., Akita, R. W., Sliwkowski, M. X., and Maihle, N. J. (2001). A naturally occurring secreted human ErbB3 receptor isoform inhibits heregulin-stimulated activation of ErbB2, ErbB3, and ErbB4. *Cancer Res.* 61, 4467–4473.

Lee, H., and Maihle, N. J. (1998). Isolation and characterization of four alternate c-erbB3 transcripts expressed in ovarian carcinoma-derived cell lines and normal human tissues. *Oncogene* 16, 3243–3252.

Li, H., Chou, S. J., Hamasaki, T., Perez-Garcia, C. G., and O'Leary, D. D. (2012). Neuregulin repellent signaling via ErbB4 restricts GABAergic interneurons to migratory paths from ganglionic eminence to cortical destinations. *Neural. Dev.* 7:10. doi: 10.1186/1749-8104-7-10

Liang, C., Tao, Y., Shen, C., Tan, Z., Xiong, W. C., and Mei, L. (2012). Erbin is required for myelination in regenerated axons after injury. *J. Neurosci.* 32, 15169–15180.

Lin, S. H., Cheng, C. J., Lee, Y. C., Ye, X., Tsai, W. W., Kim, J., et al. (2008). A 45-kDa ErbB3 secreted by prostate cancer cells promotes bone formation. *Oncogene* 27, 5195–5203.

Liu, B., Chen, H., Johns, T. G., and Neufeld, A. H. (2006). Epidermal growth factor receptor activation: an upstream signal for transition of quiescent astrocytes into reactive astrocytes after neural injury. *J. Neurosci.* 26, 7532–7540.

Liu, P., Cleveland, T. E. 4th., Bouyain, S., Byrne, P. O., Longo, P. A., and Leahy, D. J. (2012). A single ligand is sufficient to activate EGFR dimers. *Proc. Natl. Acad. Sci. U.S.A.* 109, 10861–10866.

Mahar, I., Tan, S., Davoli, M. A., Dominguez-Lopez, S., Qiang, C., Rachalski, A., et al. (2011). Subchronic peripheral neuregulin-1 increases ventral hippocampal neurogenesis and induces antidepressant-like effects. *PLoS ONE* 6:e26610. doi: 10.1371/journal.pone.0026610

Makinodan, M., Rosen, K. M., Ito, S., and Corfas, G. (2012). A critical period for social experience-dependent oligodendrocyte maturation and myelination. *Science* 337, 1357–1360.

Martin, P., Vass, W. C., Schiller, J. T., Lowy, D. R., and Velu, T. J. (1989). The bovine papillomavirus E5 transforming protein can stimulate the transforming activity of EGF and CSF-1 receptors. *Cell* 59, 21–32.

Mei, L., and Xiong, W. C. (2008). Neuregulin 1 in neural development, synaptic plasticity and schizophrenia. *Nat. Rev. Neurosci.* 9, 437–452.

Mizuno, M., Kawamura, H., Takei, N., and Nawa, H. (2008). The

anthraquinone derivative Emodin ameliorates neurobehavioral deficits of a rodent model for schizophrenia. *J. Neural. Transm.* 115, 521–530.

Moghal, N., and Sternberg, P. W. (1999). Multiple positive and negative regulators of signaling by the EGF-receptor. *Curr. Opin. Cell Biol.* 11, 190–196.

Muraoka-Cook, R. S., Feng, S. M., Strunk, K. E., and Earp, H. S. 3rd. (2008). ErbB4/HER4: role in mammary gland development, differentiation and growth inhibition. *J. Mammary Gland Biol. Neoplasia.* 13, 235–246.

Nagano, T., Namba, H., Abe, Y., Aoki, H., Takei, N., and Nawa, H. (2007). *In vivo* administration of epidermal growth factor and its homologue attenuates developmental maturation of functional excitatory synapses in cortical GABAergic neurons. *Eur. J. Neurosci.* 25, 380–390.

Nakanishi, K., Aono, S., Hirano, K., Kuroda, Y., Ida, M., Tokita, Y., et al. (2006). Identification of neurite outgrowth-promoting domains of neuroglycan C, a brain-specific chondroitin sulfate proteoglycan, and involvement of phosphatidylinositol 3-kinase and protein kinase C signaling pathways in neuritogenesis. *J. Biol. Chem.* 281, 24970–24978.

Namba, H., Nagano, T., Iwakura, Y., Xiong, H., Jourdi, H., Takei, N., et al. (2006). Transforming growth factor alpha attenuates the functional expression of AMPA receptors in cortical GABAergic neurons. *Mol. Cell. Neurosci.* 31, 628–641.

Namba, H., Zheng, Y., Abe, Y., and Nawa, H. (2009). Epidermal growth factor administered in the periphery influences excitatory synaptic inputs onto midbrain dopaminergic neurons in postnatal mice. *Neuroscience* 158, 1731–1741.

Norton, N., Moskvina, V., Morris, D. W., Bray, N. J., Zammit, S., Williams, N. M., et al. (2006). Evidence that interaction between neuregulin 1 and its receptor erbB4 increases susceptibility to schizophrenia. *Am. J. Med. Genet. B. Neuropsychiatr. Genet*. 141B, 96–101.

Olayioye, M. A., Graus-Porta, D., Beerli, R. R., Rohrer, J., Gay, B., and Hynes, N. E. (1998). ErbB-1 and ErbB-2 acquire distinct signaling properties dependent upon their dimerization partner. *Mol. Cell Biol.* 18, 5042–5051.

Olayioye, M. A., Neve, R. M., Lane, H. A., and Hynes, N. E. (2000). The ErbB signaling network: receptor heterodimerization in development and cancer. *EMBO J.* 19, 3159–3167.

Ono, M., Raab, G., Lau, K., Abraham, J. A., and Klagsbrun, M. (1994). Purification and characterization of transmembrane forms of heparin-binding EGF-like growth factor. *J. Biol. Chem.* 269, 31315–31321.

Opgenorth, A., Nation, N., Graham, K., and McFadden, G. (1993). Transforming growth factor alpha, Shope fibroma growth factor, and vaccinia growth factor can replace myxoma growth factor in the induction of myxomatosis in rabbits. *Virology* 192, 701–709.

Ortega, M. C., Bribian, A., Peregrin, S., Gil, M. T., Marin, O., and de Castro, F. (2012). Neuregulin-1/ErbB4 signaling controls the migration of oligodendrocyte precursor cells during development. *Exp. Neurol.* 235, 610–620.

Oyagi, A., Moriguchi, S., Nitta, A., Murata, K., Oida, Y., Tsuruma, K., et al. (2011). Heparin-binding EGF-like growth factor is required for synaptic plasticity and mamory formation. *Brain Res.* 1419, 97–104.

Oyagi, A., Oida, Y., Kakefuda, K., Shimazawa, M., Shioda, N., Moriguchi, S., et al. (2009). Generation and characterization of conditional heparin-binding EGF-like growth factor knockout mice. *PLoS ONE* 4:e7461. doi: 10.1371/journal.pone.0007461

Peles, E., Bacus, S. S., Koski, R. A., Lu, H. S., Wen, D., Ogden, S. G., et al. (1992). Isolation of the neu/HER-2 stimulatory ligand: a 44 kd glycoprotein that induces differentiation of mammary tumor cells. *Cell* 69, 205–216.

Perez-Torres, M., Valle, B. L., Maihle, N. J., Negron-Vega, L., Nieves-Alicea, R., and Cora, E. M. (2008). Shedding of epidermal growth factor receptor is a regulated process that occurs with overexpression in malignant cells. *Exp. Cell Res.* 314, 2907–2918.

Pezzoli, G., Zecchinelli, A., Ricciardi, S., Burke, R. E., Fahn, S., Scarlato, G., et al. (1991). Intraventricular infusion of epidermal growth factor restores dopaminergic pathway in hemiparkinsonian rats. *Mov. Disord.* 6, 281–287.

Pitcher, G. M., Kalia, L. V., Ng, D., Goodfellow, N. M., Yee, K. T., Lambe, E. K., et al. (2011). Schizophrenia susceptibility pathway neuregulin 1-ErbB4 suppresses Src upregulation of NMDA receptors. *Nat. Med.* 17, 470–478.

Pitfield, S. E., Bryant, I., Penington, D. J., Park, G., and Riese, D. J. 2nd. (2006). Phosphorylation of ErbB4 on tyrosine 1056 is critical for ErbB4 coupling to inhibition of colony formation by human mammary cell lines. *Oncol. Res.* 16, 179–193.

Plowman, G. D., Culouscou, J. M., Whitney, G. S., Green, J. M., Carlton, G. W., Foy, L., et al. (1993). Ligand-specific activation of HER4/p180erbB4, a fourth member of the epidermal growth factor receptor family. *Proc. Natl. Acad. Sci. U.S.A.* 90, 1746–1750.

Rose-John, S., and Heinrich, P. C. (1994). Soluble receptors for cytokines and growth factors: generation and biological function. *Biochem. J.* 300(Pt 2), 281–290.

Sasada, R., Ono, Y., Taniyama, Y., Shing, Y., Folkman, J., and Igarashi, K. (1993). Cloning and expression of cDNA encoding human betacellulin, a new member of the EGF family. *Biochem. Biophys. Res. Commun.* 190, 1173–1179.

Satoh, K., Kikuchi, S., Sekimata, M., Kabuyama, Y., Homma, M. K., and Homma, Y. (2001). Involvement of ErbB-2 in rheumatoid synovial cell growth. *Arthritis Rheum.* 44, 260–265.

Schechter, A. L., Stern, D. F., Vaidyanathan, L., Decker, S. J., Drebin, J. A., Greene, M. I., et al. (1984). The neu oncogene: an erb-B-related gene encoding a 185, 000-Mr tumour antigen. *Nature* 312, 513–516.

Schejter, E. D., and Shilo, B. Z. (1989). The Drosophila EGF receptor homolog (DER) gene is allelic to faint little ball, a locus essential for embryonic development. *Cell* 56, 1093–1104.

Semba, K., Kamata, N., Toyoshima, K., and Yamamoto, T. (1985). A v-erbB-related protooncogene, c-erbB-2, is distinct from the c-erbB-1/epidermal growth factor-receptor gene and is amplified in a human salivary gland adenocarcinoma. *Proc. Natl. Acad. Sci. U.S.A.* 82, 6497–6501.

Seroogy, K. B., Gall, C. M., Lee, D. C., and Kornblum, H. I. (1995). Proliferative zones of postnatal rat brain express epidermal growth factor receptor mRNA. *Brain Res.* 670, 157–164.

Seshadri, S., Kamiya, A., Yokota, Y., Prikulis, I., Kano, S., Hayashi-Takagi, A., et al. (2010). Disrupted-in-Schizophrenia-1 expression is regulated by beta-site amyloid precursor protein cleaving enzyme-1-neuregulin cascade. *Proc. Natl. Acad. Sci. U.S.A.* 107, 5622–5627.

Sharif, A., Duhem-Tonnelle, V., Allet, C., Baroncini, M., Loyens, A., Kerr-Conte, J., et al. (2009). Differential erbB signaling in astrocytes from the cerebral cortex and the hypothalamus of the human brain. *Glia* 57, 362–379.

Sharif, A., and Prevot, V. (2010). ErbB receptor signaling in astrocytes: a mediator of neuron-glia communication in the mature central nervous system. *Neurochem. Int.* 57, 344–358.

Shing, Y., Christofori, G., Hanahan, D., Ono, Y., Sasada, R., Igarashi, K., et al. (1993). Betacellulin: a mitogen from pancreatic beta cell tumors. *Science* 259, 1604–1607.

Shoyab, M., Plowman, G. D., McDonald, V. L., Bradley, J. G., and Todaro, G. J. (1989). Structure and function of human amphiregulin: a member of the epidermal growth factor family. *Science* 243, 1074–1076.

Sibilia, M., Steinbach, J. P., Stingl, L., Aguzzi, A., and Wagner, E. F. (1998). A strain-independent postnatal neurodegeneration in mice lacking the EGF receptor. *EMBO J.* 17, 719–731.

Sibilia, M., and Wagner, E. F. (1995). Strain-dependent epithelial defects in mice lacking the EGF receptor. *Science* 269, 234–238.

Sierke, S. L., Cheng, K., Kim, H. H., and Koland, J. G. (1997). Biochemical characterization of the protein tyrosine kinase homology domain of the ErbB3 (HER3) receptor protein. *Biochem. J.* 322, 757–763.

Silberberg, G., Darvasi, A., Pinkas-Kramarski, R., and Navon, R. (2006). The involvement of ErbB4 with schizophrenia: association and expression studies. *Am. J. Med. Genet. B Neuropsychiatr. Genet*. 141B, 142–148.

Singh, A. B., and Harris, R. C. (2005). Autocrine, paracrine and juxtacrine signaling by EGFR ligands. *Cell Signal.* 17, 1183–1193.

Snodgrass-Belt, P., Gilbert, J. L., and Davis, F. C. (2005). Central administration of transforming growth factor-alpha and neuregulin-1 suppress active behaviors and cause weight loss in hamsters. *Brain Res.* 1038, 171–182.

Sotoyama, H., Zheng, Y., Iwakura, Y., Mizuno, M., Aizawa, M., Shcherbakova, K., et al. (2011). Pallidal hyperdopaminergic innervation underlying D2 receptor-dependent behavioral deficits in the schizophrenia animal model established by EGF.

PLoS ONE 6:e25831. doi: 10.1371/journal.pone.0025831

Stefansson, H., Sigurdsson, E., Steinthorsdottir, V., Bjornsdottir, S., Sigmundsson, T., Ghosh, S., et al. (2002). Neuregulin 1 and susceptibility to schizophrenia. *Am. J. Hum. Genet.* 71, 877–892.

Stewart, D. G., and Davis, K. L. (2004). Possible contributions of myelin and oligodendrocyte dysfunction to schizophrenia. *Int. Rev. Neurobiol.* 59, 381–424.

Stoll, S. W., and Elder, J. T. (1998). Retinoid regulation of heparin-binding EGF-like growth factor gene expression in human keratinocytes and skin. *Exp. Dermatol.* 7, 391–397.

Strachan, L., Murison, J. G., Prestidge, R. L., Sleeman, M. A., Watson, J. D., and Kumble, K. D. (2001). Cloning and biological activity of epigen, a novel member of the epidermal growth factor superfamily. *J. Biol. Chem.* 276, 18265–18271.

Sundvall, M., Peri, L., Maatta, J. A., Tvorogov, D., Paatero, I., Savisalo, M., et al. (2007). Differential nuclear localization and kinase activity of alternative ErbB4 intracellular domains. *Oncogene* 26, 6905–6914.

Sundvall, M., Veikkolainen, V., Kurppa, K., Salah, Z., Tvorogov, D., van Zoelen, E. J., et al. (2010). Cell death or survival promoted by alternative isoforms of ErbB4. *Mol. Biol. Cell.* 21, 4275–4286.

Tan, W., Dean, M., and Law, A. J. (2010). Molecular cloning and characterization of the human ErbB4 gene: identification of novel splice isoforms in the developing and adult brain. *PLoS ONE* 5:e12924. doi: 10.1371/journal.pone.0012924

Tao, Y., Dai, P., Liu, Y., Marchetto, S., Xiong, W. C., Borg, J. P., et al. (2009). Erbin regulates NRG1 signaling and myelination. *Proc. Natl. Acad. Sci. U.S.A.* 106, 9477–9482.

Thuret, S., Alavian, K. N., Gassmann, M., Lloyd, C. K., Smits, S. M., Smidt, M. P., et al. (2004). The neuregulin receptor, ErbB4, is not required for normal development and adult maintenance of the substantia nigra pars compacta. *J. Neurochem.* 91, 1302–1311.

Ting, A. K., Chen, Y., Wen, L., Yin, D. M., Shen, C., Tao, Y., et al. (2011). Neuregulin 1 promotes excitatory synapse development and function in GABAergic interneurons. *J. Neurosci.* 31, 15–25.

Tohmi, M., Tsuda, N., Mizuno, M., Takei, N., Frankland, P. W., and Nawa, H. (2005). Distinct influences of neonatal epidermal growth factor challenge on adult neurobehavioral traits in four mouse strains. *Behav. Genet.* 35, 615–629.

Toyoda, H., Komurasaki, T., Uchida, D., Takayama, Y., Isobe, T., Okuyama, T., et al. (1995). Epiregulin. A novel epidermal growth factor with mitogenic activity for rat primary hepatocytes. *J. Biol. Chem.* 270, 7495–7500.

Tse, C., Gauchez, A. S., Jacot, W., and Lamy, P. J. (2012). HER2 shedding and serum HER2 extracellular domain: biology and clinical utility in breast cancer. *Cancer Treat Rev.* 38, 133–142.

Twardzik, D. R., Brown, J. P., Ranchalis, J. E., Todaro, G. J., and Moss, B. (1985). Vaccinia virus-infected cells release a novel polypeptide functionally related to transforming and epidermal growth factors. *Proc. Natl. Acad. Sci. U.S.A.* 82, 5300–5304.

Uchida, T., Wada, K., Akamatsu, T., Yonezawa, M., Noguchi, H., Mizoguchi, A., et al. (1999). A novel epidermal growth factor-like molecule containing two follistatin modules stimulates tyrosine phosphorylation of erbB-4 in MKN28 gastric cancer cells. *Biochem. Biophys. Res. Commun.* 266, 593–602.

Veikkolainen, V., Vaparanta, K., Halkilahti, K., Iljin, K., Sundvall, M., and Elenius, K. (2011). Function of ERBB4 is determined by alternative splicing. *Cell Cycle* 10, 2647–2657.

Ventrella, L. L. (1993). Effect of intracerebroventricular infusion of epidermal growth factor in rats. *J. Neurosurg. Sci.* 37, 1–8.

Venuti, A., Paolini, F., Nasir, L., Corteggio, A., Roperto, S., Campo, M. S., et al. (2011). Papillomavirus E5: the smallest oncoprotein with many functions. *Mol. Cancer* 10, 140.

Vidal, G. A., Naresh, A., Marrero, L., and Jones, F. E. (2005). Presenilin-dependent gamma-secretase processing regulates multiple ERBB4/HER4 activities. *J. Biol. Chem.* 280, 19777–19783.

Vullhorst, D., Neddens, J., Karavanova, I., Tricoire, L., Petralia, R. S., McBain, C. J., et al. (2009). Selective expression of ErbB4 in interneurons, but not pyramidal cells, of the rodent hippocampus. *J. Neurosci.* 29, 12255–12264.

Waddington, L. J., and Buckley, P. (1996). *The Neurodevelopmental Basis of Schizophrenia.* London: Chapman and Hall.

Ward, T. M., Iorns, E., Liu, X., Hoe, N., Kim, P., Singh, S., et al. (2012). Truncated p110 ERBB2 induces mammary epithelial cell migration, invasion and orthotopic xenograft formation, and is associated with loss of phosphorylated STAT5. *Oncogene* 2, 256.

Watanabe, Y., Fukui, N., Nunokawa, A., Muratake, T., Kaneko, N., Kitamura, H., et al. (2007). No association between the ERBB3 gene and schizophrenia in a Japanese population. *Neurosci. Res.* 57, 574–578.

Werner, M. H., Nanney, L. B., Stoscheck, C. M., and King, L. E. (1988). Localization of immunoreactive epidermal growth factor receptors in human nervous system. *J. Histochem. Cytochem.* 36, 81–86.

Wong, R. W., and Guillaud, L. (2004). The role of epidermal growth factor and its receptors in mammalian CNS. *Cytokine Growth Factor Rev.* 15, 147–156.

Woo, R. S., Li, X. M., Tao, Y., Carpenter-Hyland, E., Huang, Y. Z., Weber, J., et al. (2007). Neuregulin-1 enhances depolarization-induced GABA release. *Neuron* 54, 599–610.

Xia, W., Liu, Z., Zong, R., Liu, L., Zhao, S., Bacus, S. S., et al. (2011). Truncated ErbB2 expressed in tumor cell nuclei contributes to acquired therapeutic resistance to ErbB2 kinase inhibitors. *Mol. Cancer Ther.* 10, 1367–1374.

Yamazaki, H., Fukui, Y., Ueyama, Y., Tamaoki, N., Kawamoto, T., Taniguchi, S., et al. (1988). Amplification of the structurally and functionally altered epidermal growth factor receptor gene (c-erbB) in human brain tumors. *Mol. Cell Biol.* 8, 1816–1820.

Yarden, Y., and Pines, G. (2012). The ERBB network: at last, cancer therapy meets systems biology. *Nat. Rev. Cancer* 12, 553–563.

Ye, Y. K., Lin, Y. Z., and Tam, J. P. (1988). Shope fibroma virus growth factor exhibits epidermal growth factor activities in newborn mice. *Biochem. Biophys. Res. Commun.* 154, 497–501.

Zeng, N., Liu, L., McCabe, M. G., Jones, D. T., Ichimura, K., and Collins, V. P. (2009). Real-time quantitative polymerase chain reaction (qPCR) analysis with fluorescence resonance energy transfer (FRET) probes reveals differential expression of the four ERBB4 juxtamembrane region variants between medulloblastoma and pilocytic astrocytoma. *Neuropathol. Appl. Neurobiol.* 35, 353–366.

Zhang, L., Fletcher-Turner, A., Marchionni, M. A., Apparsundaram, S., Lundgren, K. H., Yurek, D. M., et al. (2004). Neurotrophic and neuroprotective effects of the neuregulin glial growth factor-2 on dopaminergic neurons in rat primary midbrain cultures. *J. Neurochem.* 91, 1358–1368.

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Novel molecular changes induced by *Nrg1* hypomorphism and *Nrg1*-cannabinoid interaction in adolescence: a hippocampal proteomic study in mice

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Neuregulin 1 (*NRG1*) is linked to an increased risk of developing schizophrenia and cannabis dependence. Mice that are hypomorphic for *Nrg1* (*Nrg1* HET mice) display schizophrenia-relevant behavioral phenotypes and aberrant expression of serotonin and glutamate receptors. *Nrg1* HET mice also display idiosyncratic responses to the main psychoactive constituent of cannabis, Δ^9-tetrahydrocannabinol (THC). To gain traction on the molecular pathways disrupted by *Nrg1* hypomorphism and *Nrg1*-cannabinoid interactions we conducted a proteomic study. Adolescent wildtype (WT) and *Nrg1* HET mice were exposed to repeated injections of vehicle or THC and their hippocampi were submitted to 2D gel proteomics. Comparison of WT and *Nrg1* HET mice identified proteins linked to molecular changes in schizophrenia that have not been previously associated with *Nrg1*. These proteins are involved in vesicular release of neurotransmitters such as SNARE proteins; enzymes impacting serotonergic neurotransmission, and proteins affecting growth factor expression. *Nrg1* HET mice treated with THC expressed a distinct protein expression signature compared to WT mice. Replicating prior findings, THC caused proteomic changes in WT mice suggestive of greater oxidative stress and neurodegeneration. We have previously observed that THC selectively increased hippocampal NMDA receptor binding of adolescent *Nrg1* HET mice. Here we observed outcomes consistent with heightened NMDA-mediated glutamatergic neurotransmission. This included differential expression of proteins involved in NMDA receptor trafficking to the synaptic membrane; lipid raft stabilization of synaptic NMDA receptors; and homeostatic responses to dampen excitotoxicity. These findings uncover novel proteins altered in response to *Nrg1* hypomorphism and *Nrg1*-cannabinoid interactions that improves our molecular understanding of *Nrg1* signaling and *Nrg1*-mediated genetic vulnerability to the neurobehavioral effects of cannabinoids.

Keywords: *Nrg1*, THC, mouse, hippocampus, schizophrenia, proteomics

INTRODUCTION

Neuregulin 1 (*Nrg1*) is a neurotrophic factor that mediates its effects by binding ErbB receptor tyrosine kinases. *Nrg1* regulates axonal guidance, myelination, and GABAergic and glutamatergic neurotransmission (Mei and Xiong, 2008). The human *NRG1* gene has been linked to schizophrenia by genetic studies (Stefansson et al., 2002; Ayalew et al., 2012) and altered expression of *NRG1* isoforms has been measured in schizophrenia patients (Hashimoto et al., 2004; Chong et al., 2008; Marballi et al., 2012; Weickert et al., 2012). *NRG1* variants have been associated with dysfunction in a number of schizophrenia-relevant "endophenotypes" including sensorimotor gating as measured by prepulse inhibition of startle (PPI) (Hong et al., 2008; Roussos et al., 2011; Greenwood et al., 2012) and working memory (Chong et al., 2008).

Use of transgenic murine models can be useful in exploring the role of *Nrg1* in molecular neurobiology and behavior. The most extensively studied mouse model of *Nrg1* dysfunction is the *Nrg1* transmembrane heterozygous (*Nrg1* HET) mouse which exhibits locomotor hyperactivity and protocol-dependent PPI deficits (Stefansson et al., 2002; Karl et al., 2007; Spencer et al., 2012). These mice display altered anxiety profiles, inhibited preference for social novelty and increased levels of aggressive social interaction as well as impaired performance in novel object

recognition and fear conditioning paradigms (Karl et al., 2007; O'Tuathaigh et al., 2007, 2008; Duffy et al., 2010; Desbonnet et al., 2012). Hypo-phosphorylation of the NR2B subunit of the NMDA receptor is observed in *Nrg1* HET mice (Bjarnadottir et al., 2007). Together, these findings provide some clues of the molecular and neurobiological alterations that mediate the aberrant behavioral phenotypes exhibited by *Nrg1* HET mice.

Adolescence is particularly relevant to schizophrenia given the onset of the disorder typically occurs in late adolescence. During adolescence there exists significant synaptic pruning and a shift between utilization of mesolimbic and mesocortical areas of the brain which indicates a high level of neural development during this period (Giedd et al., 1999; Spear, 2000; Casey et al., 2008). *Nrg1* HET mice display developmentally-specific neurobiological and behavioral phenotypes, for example, adolescent *Nrg1* HET mice have reduced 5-HT_{2A} receptor expression in the insular and cingulate cortices (Long et al., 2013) in contrast to the global increase in $5HT_{2A}$ receptor expression observed in adult *Nrg1* HET mice relative to controls (Dean et al., 2008). *Nrg1* HET mice display an enhanced stress-induced release of corticosterone relative to wildtype (WT) controls at 3–4 months of age, an effect that disappears by 5–6 months (Chesworth et al., 2012). Together these findings point toward the *Nrg1* HET mouse being a particularly suitable model for demonstrating a role for *Nrg1* in developmental stage-specific neurobehavioral alterations.

Drug dependence and schizophrenia are comorbid disorders that may have common genetic and neurobiological substrates. Genetic vulnerability is thought to explain why only a subset of cannabis users become dependent on cannabis or develop psychosis. A recent study demonstrated that *NRG1* increased the risk of cannabis dependence in African-Americans (Han et al., 2012). We have shown *Nrg1* HET mice display distinct schizophrenia-relevant neurobehavioral responses to cannabinoids, including the main psychoactive constituent of cannabis, Δ^9-tetrahydrocannabinol (THC). Acute cannabinoid exposure promoted PPI facilitation in *Nrg1* HET mice but PPI deficits in WT mice (Boucher et al., 2007a, 2011). *Nrg1* genotype also modulated tolerance to the effects of cannabinoids, with *Nrg1* HET mice developing tolerance more rapidly to locomotor suppression and hypothermia than WT mice, but conversely showing a lack of tolerance to cannabinoid-induced anxiety unlike WT mice (Boucher et al., 2011). The acute and repeated effects of cannabinoids correlate with selective changes in Fos transcription factor expression in the lateral septum of *Nrg1* HET mice that were not observed in WT mice (Boucher et al., 2007b, 2011). In adolescence *Nrg1* modulated the effects of repeated THC exposure on the expression of neurotransmitter receptors relevant to the pathophysiology of schizophrenia (i.e., CB_1, NMDA, and 5-HT_{2A} receptors) (Long et al., 2013).

The hippocampus may be an important region for *Nrg1*-cannabinoid interactions as both endocannabinoid and *Nrg1*-ErbB systems are highly expressed in this brain region (Herkenham et al., 1990; Tsou et al., 1998; Vullhorst et al., 2009). We have observed increased brain transcriptional activity in the lateral septum at baseline and following cannabinoid exposure in *Nrg1* HET mice, both of which might reflect downstream effects of aberrant activity in the hippocampus as part of the septohippocampal system. Therefore, molecular changes in the hippocampus may subserve the distinct neurobehavioral phenotypes displayed by *Nrg1* HET mice as well as their altered response to THC. Of particular interest is our observation that adolescent THC–treated *Nrg1* HET mice display increased NMDA receptor expression in the hippocampus, something not observed in THC-treated WT mice (Long et al., 2013). Here we aim to gain some traction on the molecular mechanisms involved in the aberrant phenotypes exhibited by *Nrg1* HET mice at baseline and when exposed to THC using a proteomic approach which allows us to detect changes in hundreds of different proteins in the hippocampus.

MATERIALS AND METHODS

ANIMALS AND DRUG TREATMENT

At the commencement of the study adolescent male *Nrg1* HET mice and WT littermates (C57/BL6 background strain) were at an age of post-natal day (PND) 31 ± 2. The study was restricted to male mice as male *Nrg1* HET mice appear more vulnerable to the effects of cannabinoids (Long et al., 2010). Mice were pair-housed at Neuroscience Research Australia with limited environmental enrichment [certified polycarbonate mouse igloo (Bioserv, USA) and a metal ring in the cage lid] under a 12 h light/dark schedule (lights on 08:30 h) and genotyped as previously detailed (Karl et al., 2007). Food and water were available *ad libitum*. THC (THC Pharm GmbH, Germany) was suspended in a 1:1:18 mixture of ethanol:Tween 80:0.9% saline and injected intraperitoneally at a volume of 10 ml/kg. Mice were injected daily with either 10 mg/kg of THC or vehicle for 21 days. During this time mice were repeatedly behaviorally tested, the results of which are published elsewhere (Long et al., 2013). Two days following the completion of treatment, the mice ($n = 8$) were euthanized by cervical dislocation, with both hippocampi dissected out and snap frozen on dry ice for proteomic analysis. Research and animal care procedures were approved by the University of New South Wales Animal Care and Ethics Committee and were in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes.

PROTEIN EXTRACTION

Protein extraction was performed using a protocol optimized for cytosolic proteins (Quinn et al., 2008). Hippocampal tissue was homogenized in buffer consisting of 7 M urea, 2 M thiourea, 1% C7bZO and 40 mM Tris, sonicated and pelleted. The supernatant was reduced and alkylated in 5 mM tributylphosphine (TBP) and 10 mM acrylamide monomer and quenched using 10 mM dithiothreitol (DTT). The mixture was acidified to pH 6.0 using concentrated citric acid and precipitated with acetone. The precipitate was pelleted, air-dried and resuspended in 7 M urea, 2 M thiourea and 1% C7bZO.

2D GEL ELECTROPHORESIS

Sample protein concentration was determined using the Bradford Protein Assay (Thermoscientific, USA). Immobilized pH gradient strips (IPG strips; 11 cm, pH 4–7) were rehydrated with samples containing 600 μg protein, and samples were separated by isoelectric point (pI). Strips were equilibrated in

SDS equilibration buffer and loaded onto SDS-PAGE gradient gels (8–16%, 10 × 15 cm) and separated by molecular weight using an ElectrophoretIQ3 system (30 mA/gel, 25°C for 110 min; Proteome Systems, Australia). Gels were fixed with methanol [25% (v/v)] and acetic acid [10% (v/v)] and visualized using Flamingo Fluorescent gel stain (BioRad, USA).

IMAGE ANALYSIS

Gels were analyzed using Phoretix 2D Expression software (Nonlinear Dynamics Ltd, UK). Averaged gels were created for each experimental group and averaging parameters were set at 70%. Single factor ANOVAs ($p < 0.05$) of spot volume were performed to determine the effect of genotype in vehicle-treated animals, the effect of THC administration in WT mice and the effect of THC administration in *Nrg1* HET mice.

MASS SPECTROMETRY AND PROTEIN IDENTIFICATION

Protein spots that were identified as significantly altered were digested in 12.5 ng/mL trypsin (Roche, USA) and 25 nM NH_4HCO_3/0.1% trifluroacetic acid and purified using C_{18} purification tips (Eppendorf, Germany) before being eluted in 3 μL of matrix solution. Spots were analyzed using an Applied Biosystems QSTAR MALDI-TOF mass spectrometer (Australian Proteome Analysis Facility, University of Sydney). MALDI spectra were matched against the Swiss-Prot database using the MASCOT search engine with matches determined by molecular weight search score (MWS) and sequence coverage in conjunction with pI and molecular weight as estimated from gels.

IMMUNOBLOTTING

Ten ug of protein per lane was separated by electrophoresis using 10% precast NuPage gels (Invitrogen, USA) and run at 110 V for 2 h. The samples were transferred to PVDF membranes. Membranes were sequentially incubated with Syntaxin-1A antibody (Sigma Aldrich, USA 1:1500) then swine anti-rabbit secondary antibody (DAKO, Australia, 1:200) and rabbit Peroxidase-Anti-Peroxidase (Sigma-Aldrich, USA 1:200) and DAB (DAKO, Australia). Membranes were stripped using Re-blot plus strong antibody stripping solution (Millipore, Australia) and incubated sequentially with Abcam rat monoclonal YL1/2 α-tubulin (TUBA) antibody (Sapphire Biosciences Pty Ltd, Australia, 1:) then anti-rat IgG (HþL) horseradish peroxidase conjugate (Santa Cruz Biotechnology Inc, USA) and visualized using a Syngene G:Box.

RESULTS

Here we present the results of a hippocampal proteomic study conducted on adolescent *Nrg1* HET mice and WT control mice treated with or without THC. The averaged gels for WT vehicle, WT THC, *Nrg1* HET vehicle and *Nrg1* HET THC contained 870, 821, 761, and 742 spots respectively. 26 spots were significantly different between WT vehicle and *Nrg1* HET vehicle mice. Of these spots, 17 proteins were identified using MALDI-TOF MS and the fold changes from control WT mice are listed in **Table 1**. **Figure 1A** shows a representative 2D gel image of protein expression in the hippocampus of a WT mouse administered vehicle. Normalized spot volumes are depicted for three representative proteins are shown in **Figure 1B**, i.e., syntaxin 1A (STX1A), beta-soluble N-ethylmaleimide-sensitive factor attachment protein (β-SNAP) and glypican 6 (GPC6). Western blotting results confirmed *Nrg1* HET mice displayed increased expression of STX1A compared to WT mice (see **Figure 1B**).

THC induced changes in 28 spots and 23 spots in WT and *Nrg1* HET mice respectively relative to vehicle-treated mice within the same genotype. From these comparisons, 4 and 10 proteins were identified as being significantly altered by THC exposure in adolescent WT and *Nrg1* HET mice and fold changes are listed in **Table 2** (relative to WT mice treated with vehicle) and 3 (relative to *Nrg1* HET mice treated with vehicle) respectively. **Figure 2A** shows a representative 2D gel image of protein expression in the hippocampus of an adolescent WT mouse administered repeated THC injections. Normalized spot volumes are depicted for 3 representative proteins in **Figure 2B**, i.e., glutathione S-transferase Mu 2 (GSTM2), calretinin (CALB2) and ADP-ribosylation factor-like protein 1 (ARL1). *Nrg1* HET mice treated with THC displayed a distinct protein expression profile to WT mice exposed to the drug. **Figure 3A** shows a representative 2D gel image of protein expression in the hippocampus of an adolescent *Nrg1* HET mouse administered repeated THC. Normalized spot volumes of three representative proteins are depicted in **Figure 3B**, i.e., G-protein-signaling modulator 2 (GPSM2), apolipoprotein A1 (APOA1) and N-acyl-phosphatidylethanolamine-hydrolyzing phospholipase D (NAPEPLD).

DISCUSSION

Nrg1 HET mice, displayed altered expression of a number of proteins involved in the vesicular release of neurotransmitters including STX1A, syntaxin 7 (STX7) and β-SNAP; serotonergic neurotransmission including tryptophan 5-hydroxylase 1 (TPH1) and serotonin N-acetyltransferase (AA-NAT); growth factor expression and regulation including secreted protein acidic and rich in cysteine (SPARC) and GPC6, and; cell survival and regulators of inflammatory cytokines including cell death regulator Aven (AVEN), TNFAIP3-interacting protein 2 (ABIN2) and regulator of G-protein signaling 10 (RGS10). We replicated prior findings in rodents without genetic modification showing THC reduced the hippocampal expression of GSTM2 and affected the expression of heat shock proteins (here HSPA4). We also identified novel proteins changed in response to repeated THC exposure, that is, CALB2 and ARL1. Unlike WT mice, *Nrg1* HET mice administered THC displayed altered expression of proteins involved in NMDA receptor trafficking to the synaptic membrane including GPSM2; lipid raft stabilization of receptors at the synaptic membrane including flotillin-1 (FLOT1); homeostatic responses to dampen excessive glutamatergic transmission, including NAPEPLD, and excitotoxicity and apoptosis including programmed cell death protein 2 (PCD2). **Figure 4** is a schematic proposing an overview of the proteins found to have altered expression in the current study and their potential functional significance. Proteomics may produce false positives and fold changes < 1.5 should be interpreted cautiously. Nevertheless, these results, while suggestive rather than conclusive, provide

Table 1 | Adolescent hippocampal proteomics comparing adolescent vehicle-treated WT and *Nrg1* HET mice.

Spot number	Protein name	Abbreviation	UniProt accession number	PI	Mass (Da)	MWS	No. of peptides matched	% seq cover	Fold change	*T*-test (p)
VESICLE FUNCTION PROTEINS										
11755	Syntaxin-1A	STX1A	O35526	5.14	33,054	63	5	20	2.996	0.00123
11358	Beta-soluble NSF attachment protein	β-SNAP	P28663	5.32	33,557	80	5	33	−8.897	0.02521
11673	Syntaxin-7	STX7	O70439	5.6	29,821	55	4	22	2.118	0.00333
11737	Dynactin subunit 2	DCTN2	Q99KJ8	5.14	44,117	94	8	21	2.96	0.03845
11800	ADP-ribosyl cyclase 2	BST-1	Q64277	5.49	34,616	85	5	37	−1.516	0.04099
SEROTONERGIC NEUROTRANSMISSION										
11967	Tryptophan 5-hydroxylase 1	TPH1	P17532	6.06	51,343	82	7	17	−3.353	0.04087
11670	Serotonin N-acetyltransferase	AA-NAT	O88816	7.01	23,069	56	3	26	1.549	0.00076
GROWTH FACTORS										
11646	Secreted protein acidic and rich in cysteine	SPARC	P07214	4.77	34,450	71	5	16	1.388	0.04557
11294	Glypican 6	GPC6	Q9R087	5.32	63,057	86	7	20	2.515	0.01623
11419	Fibroblast growth factor 14	FGF14	P70379	10.11	27,764	74	5	29	−1.61	0.02358
CELL SURVIVAL PROTEINS										
11005	TNFAIP3-interacting protein 2	ABIN2	Q99JG7	6.03	49,094	78	8	23	−1.792	0.00449
11105	Cell death regulator Aven	AVEN	Q9D9K3	4.92	37,195	59	5	21	−1.887	0.03471
12856	Regulator of G-protein signaling 10	RGS10	Q9CQE5	6.36	21,151	66	4	44	1.805	8.22E-05
OTHER										
12115	Phosphoserine phosphatase	PSPH	Q99LS3	5.81	25,096	56	4	36	1.434	0.01847
11051	Calcium/calmodulin-dependent 3′,5′-cyclic nucleotide phosphodiesterase 1A	PDE1A	Q61481	5.67	64,529	82	7	15	−1.514	0.00902
11988	Galanin-like peptide	GALP	Q810H5	6.41	12, 773	61	3	35	−5.496	0.03014
12051	Glyoxalase domain-containing protein 5	GLOD5	Q9D8I3	5.12	16, 595	59	4	40	−1.827	0.03871

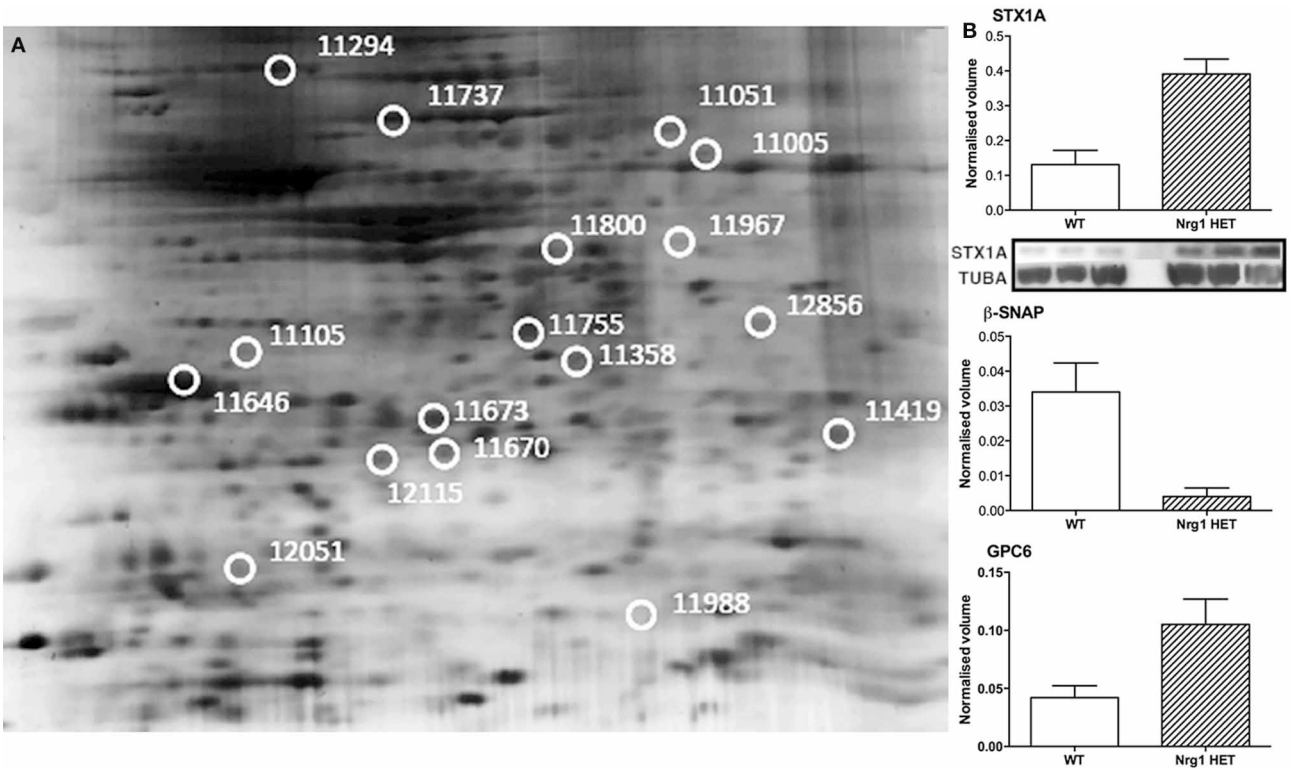

FIGURE 1 | Adolescent hippocampal proteomics comparing adolescent vehicle-treated WT and *Nrg1* HET mice. (A) Representative 2DE gel images showing positions of significantly altered protein spots [the horizontal axis split by isoelectric point, vertical axis split by molecular weight; STX1A (spot 11755); TPH1 (spot 11967); β-SNAP (spot 11358); SPARC (spot 11646); GPC6 (spot 11294)]; and **(B)** Exemplar normalized volumes of altered proteins i.e., STX1A, β-SNAP and GPC6. The inset for STX1A shows immunoblots against STX1A and loading control alpha-tubulin (TUBA), confirming greater expression of STX1A in *Nrg1* HET versus WT mice.

a platform for future work to confirm and more fully characterize the role of various novel proteins in the effects of *Nrg1* hypomorphism, THC and *Nrg1*-THC interactions.

DISTINCT PROTEIN EXPRESSION IN THE HIPPOCAMPUS OF ADOLESCENT *Nrg1* HET AND WT MICE

Disordered neurotransmission is involved in the pathophysiology of schizophrenia and *NRG1*, a schizophrenia susceptibility gene, regulates neurotransmitter receptor expression and synaptic plasticity (Mei and Xiong, 2008). Here we provide evidence that heterozygous deletion of *Nrg1* alters numerous proteins involved in the transport, fusion and recycling of synaptic vesicles, all processes critical to neurotransmitter release and synaptic function. The soluble N-ethylmaleimide-sensitive fusion attachment protein receptor (SNARE) complex regulates exocytotic release of neurotransmitters from presynaptic terminals and alterations in SNARE mRNA and protein is observed in post-mortem schizophrenia brain (Ramakrishnan et al., 2012). Here we show for the first time that *Nrg1* hypomorphism alters the expression of various SNARE proteins including STX1A, STX7, and β-SNAP.

STX1A was increased almost three-fold in *Nrg1* HET mice relative to WT controls and this change was confirmed by Western blot analysis. Located within the pre-synaptic membrane, STX1A combines with 25 kDa synaptosome-associated protein SNAP25 and vesicle-associated membrane protein 2 to form a complex that drives vesicle and presynaptic membrane fusion necessary for neurotransmitter exocytosis. Concordant with our findings in *Nrg1* HET mice, STX1A is upregulated in the hippocampus and cingulate cortex of schizophrenic patients (Gabriel et al., 1997; Honer et al., 1997; Sokolov et al., 2000; Clark et al., 2007). We also demonstrate here that STX7, a member of an endocytic SNARE complex, was upregulated in *Nrg1* HET mice compared to WT mice. STX7 mediates endocytic trafficking from early endosomes to late endosomes, and is necessary for fusion of late endosomes to lysosomes (Mullock et al., 2000; Nakamura et al., 2000). β-SNAP displayed an almost 9-fold reduction in expression in *Nrg1* HET mice compared to WT mice. β-SNAP belongs to a class of proteins known as SNAPs, which form complexes with SNARE proteins to assist with membrane fusion before being dissociated by the ATPase N-ethylmaleimide-sensitive factor. In contrast to other SNAPs, β-SNAP is localized to neural tissue, including hippocampal cells (Schiavo et al., 1995).

Nrg1 HET mice also displayed altered expression of several proteins involved in protein transport between the endoplasmic reticulum (ER) and Golgi apparatus. These included β-SNAP, dynactin subunit 2 (DCTN2) and ADP-ribosyl cyclase 2 (BST-1). Transport of protein from the ER to the Golgi is important to protein sorting and the dispatch of protein to cellular

Table 2 | Adolescent hippocampal proteomics comparing adolescent WT mice treated with vehicle and THC.

Spot number	Protein name	Abbreviation	UniProt accession number	PI	Mass (Da)	MWS	No. of peptides matched	% seq cover	Fold change	*T*-test (p)
OXIDATION REGULATION PROTEINS										
11418	Glutathione S-transferase Mu 2	GSTM2	P15626	6.9	25,717	73	5	20	−1.759	0.02679
11769	Heat shock 70 kDa protein 4	HSPA4	Q61316	5.15	94,133	95	8	14	2.137	0.04305
OTHER										
11103	Calretinin	CALB2	Q08331	4.94	31,373	66	4	21	−1.748	0.03045
11677	ADP-ribosylation factor-like protein 1	ARL1	P61211	5.63	20,412	57	3	20	−2.869	0.04051

locations. An increased expression of DCTN2 was also observed in *Nrg1* HET mice. DCTN2 is a functional subunit of dynactin, a component of the dynein-dynactin system. DCTN2 overexpression inhibits dynactin, and therefore dynein functions such as dynein-dependent maintenance of membrane organelle distribution (Burkhardt et al., 1997). DCTN2 is associated with syntaxin 18, an ER-localized SNARE involved in membrane trafficking between the ER and Golgi (Arasaki et al., 2006). BST-1, which was downregulated in *Nrg1* HET mice, is also implicated in ER to Golgi transport as it suppressed such trafficking in yeast cells (Sompol et al., 2011).

Previous studies suggest that *Nrg1* hypomorphism affects serotonergic neurotransmission, by altering the expression of 5-HT_{2A} receptors and the serotonin transporter in various brain regions of both adolescent and adult mice (Dean et al., 2008; Long et al., 2013). The observation that *Nrg1* HET mice had reduced expression of TPH1 and increased level of AA-NAT is consistent with this notion. TPH1 is one of two isoforms of the enzyme involved in the rate-limiting synthesis of serotonin. Polymorphism in *TPH1* is associated with increased risk for various psychiatric disorders including schizophrenia and bipolar disorder (Saetre et al., 2010; Seifuddin et al., 2012) and varies with neurodevelopment with peaks at PND 21 before decreasing in adulthood (Nakamura et al., 2006). Given the autocrine role of serotonin in guiding the development of serotonergic neurons (Gaspar et al., 2003), reduced TPH1 expression in adolescent *Nrg1* HET mice may reflect aberrant development of serotonergic brain circuitry in these mice. AA-NAT, which converts serotonin to N-acetylserotonin, was also upregulated in the hippocampus of *Nrg1* HET mice. This role of this enzyme is well characterized in the pineal gland due to its involvement in melatonin synthesis and sleep-wake cycles (Zheng and Cole, 2002). However, the function of AA-NAT in other brain regions including the hippocampus is poorly understood. AA-NAT is expressed in a non-diurnal dependent manner in the hippocampus (Uz et al., 2002) and promotes hippocampal neuroprogenitor cell proliferation in mice (Sompol et al., 2011).

Given that *Nrg1* is a neurotrophic factor it is not entirely surprising that *Nrg1* HET mice display altered expression of the growth factor fibroblast growth factor 14 (FGF14) and regulators of growth factor protein expression, including SPARC and GPC6. FGF14 knockout mice, like *Nrg1* HET mice, display locomotor hyperactivity, spatial learning deficits and impaired hippocampal long-term potentiation associated with lowered presynaptic vesicle docking (Wozniak et al., 2007; Xiao et al., 2007). The latter effect is relevant given the altered proteins involved in vesicle docking we observed here in *Nrg1* HET mice. The reduced level of FGF14 might be related to the increased expression of GPC6 and SPARC. Glypicans are heparan sulphate proteoglycans that act as co-receptors for growth factors and modulate fibroblast growth factor signaling (Paine-Saunders et al., 1999; Galli et al., 2003). GPC6 has recently been identified as a gene that confers susceptibility to formal thought disorder in schizophrenia and as a factor released from astrocytes that supports the formation of glutamatergic synapses via GluA1 AMPA receptors (Allen et al., 2012; Wang et al., 2012). Similar to GPC6, SPARC is released from astrocytes and modulates the formation

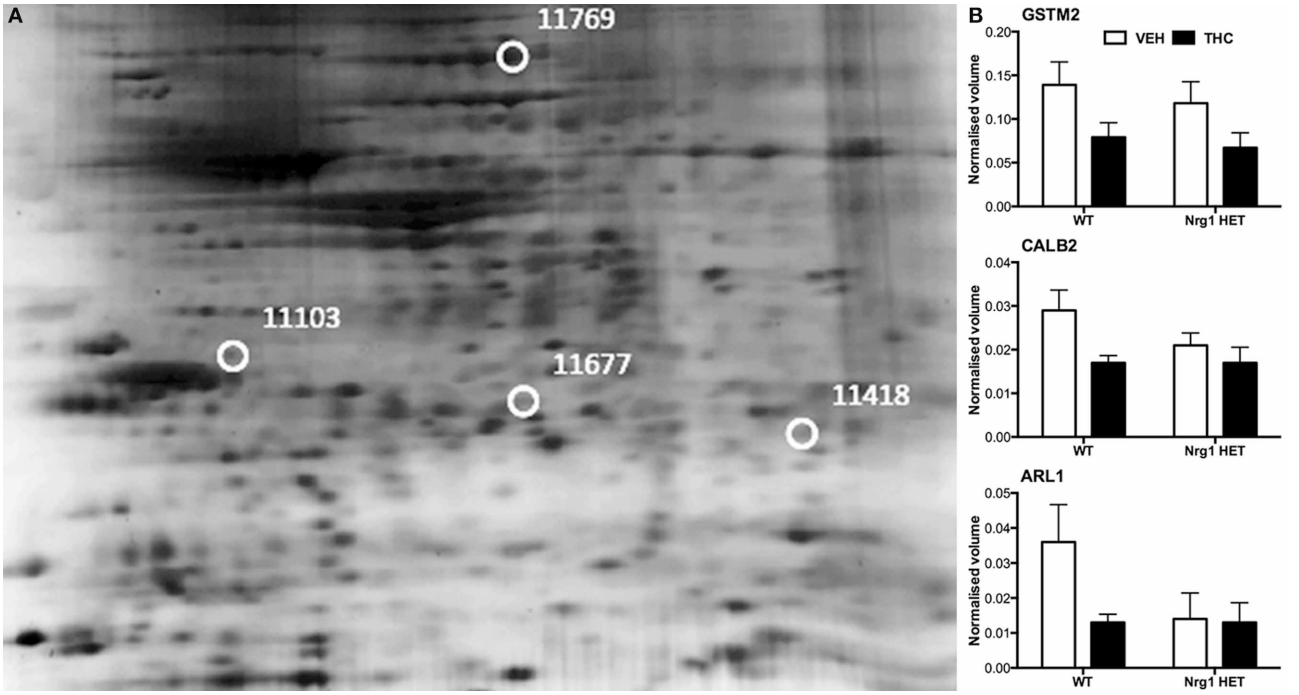

FIGURE 2 | Adolescent hippocampal proteomics comparing adolescent WT mice treated with vehicle and THC. **(A)** Representative 2DE gel images showing positions of significantly altered protein spots, the horizontal axis split by isoelectric point, vertical axis split by molecular weight; [CALB2 (spot 11103); ARL1 (spot 11677); GSTM2 (spot 11418); HSPA4 (spot 11769)]; and **(B)** Exemplar normalized volumes of altered proteins i.e., CALB2, ARL1, and GSTM2.

of excitatory synapses and FGF expression (Kucukdereli et al., 2011; Bradshaw, 2012). Taken together these findings suggest a novel link between *Nrg1* and other interrelated growth factor-related proteins FG14, GPC6, and SPARC, worthy of further examination in future studies.

The hippocampus of adolescent *Nrg1* HET mice displayed altered expression of various proteins that influence cell survival and neuroinflammation including AVEN, ABIN2, and RGS10. *Nrg1* exerts neuroprotective effects via inhibiting apoptosis triggered by various challenges (Chen et al., 2011; Li et al., 2012; Woo et al., 2012). AVEN inhibits apoptosis (Chau et al., 2000; Figueroa et al., 2004; Kutuk et al., 2010), therefore the reduction of AVEN in the hippocampus of *Nrg1* HET mice might confer greater vulnerability to apoptosis in these mice. Adolescent *Nrg1* HET mice displayed a trend toward increased expression of the pro-apoptotic and inflammatory cytokine TNF-α in the hippocampus and schizophrenia patients with a missense mutation in the transmembrane domain of *NRG1* show heightened expression of TNF-α from B cells (Marballi et al., 2010; Desbonnet et al., 2012). This is interesting as ABIN-2 and RGS10, two proteins altered in *Nrg1* HET mice, modulate the action of the TNF-α. ABIN-2 prevents TNF-α mediated pro-apoptotic effects and its decreased expression in *Nrg1* HET mice might reflect again a greater propensity to hippocampal apoptosis (Verstrepen et al., 2009). Perhaps as a compensatory mechanism *Nrg1* HET mice showed increased expression of RGS10, a protein which renders cells resistant to TNF-α induced apoptosis (Lee et al., 2012). Future studies are required to confirm whether altered apoptosis and neuroinflammation exists in *Nrg1* HET mice.

DIFFERENTIAL EFFECTS OF THC ON THE PROTEOME OF *Nrg1* HET MICE vs. WT MICE

Our prior research shows that *Nrg1* heterozygotes display an altered neurobehavioral response to cannabinoids (Boucher et al., 2007a,b, 2011; Arnold et al., 2012; Long et al., 2013). *Nrg1* mutant mice were more sensitive to the behavioral actions of acute THC compared to WT littermates in a sex-specific manner, with males being selectively affected but not females (Boucher et al., 2007a; Long et al., 2010). In a repeated dosing study, tolerance to cannabinoid-induced hypothermia and locomotor suppression developed more rapidly in *Nrg1* HET than WT mice (Boucher et al., 2011). Conversely, only WT mice developed tolerance to cannabinoid-induced anxiety and *Nrg1* HET mice maintained a persistent anxiogenic response to repeated cannabinoid exposure. Acute and repeated cannabinoid exposure selectively activated expression of Fos transcription factors in the lateral septum of *Nrg1* HET mice but not WT mice (Boucher et al., 2007b, 2011). We also examined whether *Nrg1* hypomorphism confers vulnerability to the neurobehavioral actions of acute or repeated THC exposure in adolescence (Long et al., 2013). THC exposure exacerbated the hyperlocomotor phenotype of *Nrg1* HET mice expressed after withdrawal of the drug. Further, repeated THC administration also promoted differential effects on CB_1 receptor, $5\text{-}HT_{2A}$ and NMDA receptor binding. Notably adolescent THC

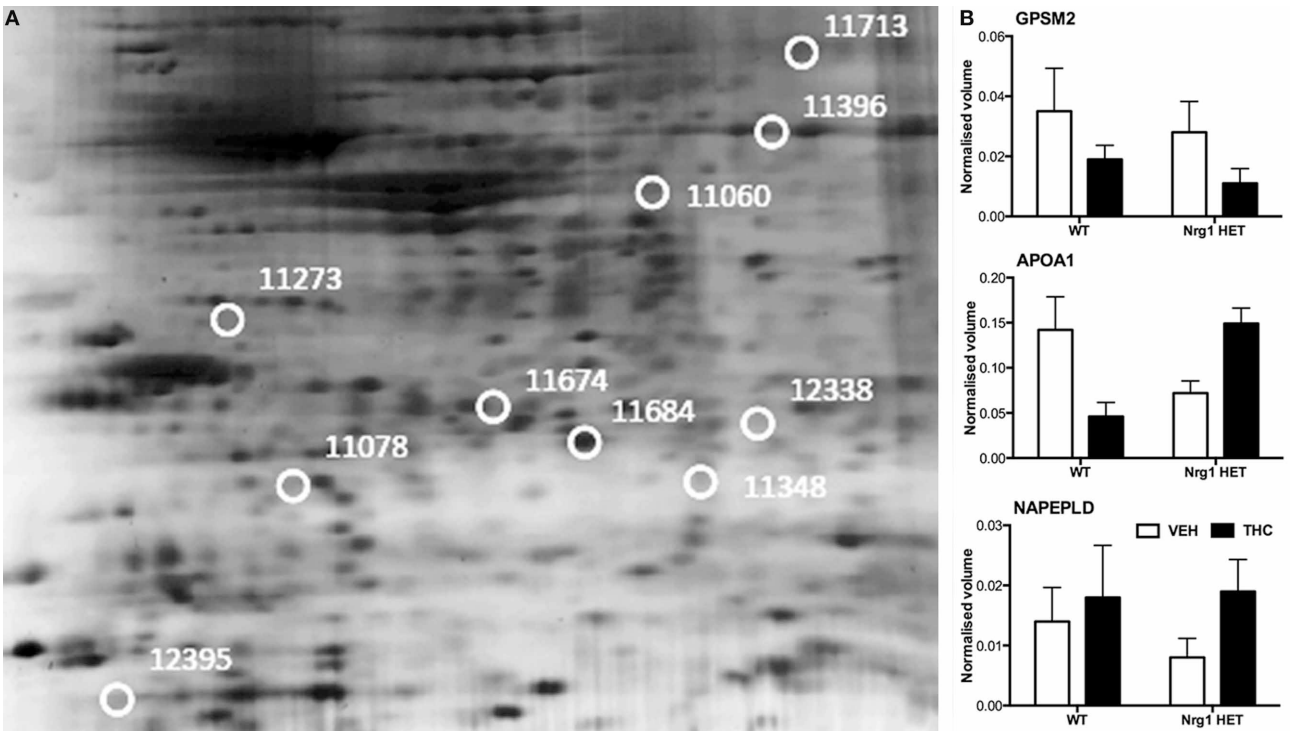

FIGURE 3 | Adolescent hippocampal proteomics comparing adolescent *Nrg1* HET mice treated with vehicle and THC. **(A)** Representative 2D E gel images showing positions of significantly altered protein spots (the horizontal axis split by isoelectric point, vertical axis split by molecular weight; [GPSM2 (spot 11713); APOA1 (spot 11674); NAPEPLD (spot 11060); OTOR (spot 12395)]; and **(B)** exemplar normalized volumes of altered proteins i.e., GPSM2, APOA1 and NAPEPLD.

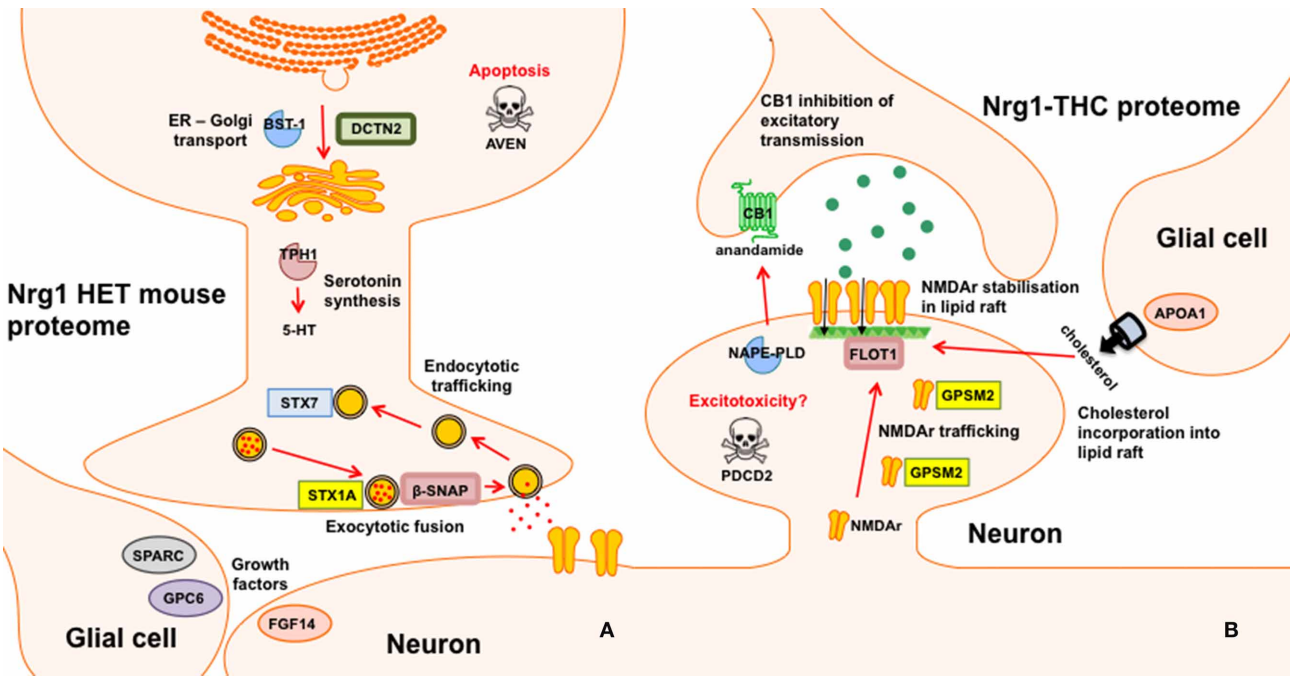

FIGURE 4 | Schematic representation of altered proteins. (A) Proteins with different expression in *Nrg1* HET mice relative to WT controls; and **(B)** Proteins with different expression in THC administered *Nrg1* HET mice relative to vehicle treated controls.

exposure selectively increased NMDA receptor expression in the hippocampus of *Nrg1* HET but not WT mice. Given these findings it is perhaps not surprising that the impact of repeated THC treatment as measured by proteomics was quite distinct in *Nrg1* HET mice vs. WT mice, with no overlap in differentially expressed proteins **Table 3**.

Nevertheless, our findings show some degree of overlap with previous examinations of THC effects on the rodent brain proteome (Quinn et al., 2008; Colombo et al., 2009; Rubino et al., 2009a; Filipeanu et al., 2011; Wang et al., 2011). THC treatment in adolescent rats modulated proteins regulating oxidative stress such as glutathione S-transferase and heat shock proteins (Quinn et al., 2008). Our results replicate the finding that repeated THC exposure decreased the expression of GSTM2 in the hippocampus (Quinn et al., 2008). GSTM2 catalysis the conjugation of reduced glutathione to electrophilic compounds thereby reducing the deleterious effects of reactive oxygen species (ROS) on cellular lipid, protein and DNA. By reducing levels of GSTM2, THC may render the hippocampus more vulnerable to oxidative stress and this may be linked to the long-term memory impairing effects of cannabinoids (Quinn et al., 2008; Boucher et al., 2009). Phencyclidine, another drug of abuse that promotes schizophrenia-relevant behaviors and cognitive dysfunction, also reduced glutathione levels and antioxidant defense enzymes in the rodent brain (Radonjic et al., 2010; Stojković et al., 2012). Interestingly, copy number variants in genes encoding glutathione S-transferase may be involved in susceptibility to schizophrenia (Rodriguez-Santiago et al., 2010). Here we also showed repeated adolescent THC exposure upregulated the expression of heat shock protein 70 kDa in the hippocampus. Previous studies illustrated effects of rodent THC exposure on heat shock protein 70 kDa, heat shock cognate 71 kDa protein and heat shock 60 kDa protein (Bindukumar et al., 2008; Quinn et al., 2008; Colombo et al., 2009; Rubino et al., 2009a; Filipeanu et al., 2011). Heat shock proteins regulate cellular stress responses and provide protection against oxidative stress (Quinn et al., 2008; Stetler et al., 2010) so their increased expression may signify greater oxidative stress in the hippocampus. Heat shock protein 70 kDa may also serve an autophagic function facilitating the clearance of toxic proteins and assisting in neuronal survival (Stetler et al., 2010).

Adolescent THC exposure decreased hippocampal levels of the calcium-binding protein CALB2. CB_1 receptors are expressed on calretinin-positive GABA interneurons in the hippocampus (Marsicano and Lutz, 1999; Morozov et al., 2009). THC exposure in C57/BL6 mice increased expression of this protein in the cerebellum (Colombo et al., 2009). Colombo et al. (2009) analyzed CALB2 expression in the membrane whereas we assessed the cytosolic fraction, therefore it remains possible our finding may reflect translocation of the protein from the cytosol to the membrane. We isolated a novel protein, (ARL1), which was downregulated in response to THC exposure. Alcohol and methamphetamine administration similarly alter expression of this protein (Iwazaki et al., 2008; Kobeissy et al., 2008; Kashem et al., 2009). ARL1 is a Ras GTPase involved in retrograde trafficking of endosomes between the Golgi apparatus and the membrane in mammalian cells (Nishimoto-Morita et al., 2009).

Table 3 | Adolescent hippocampal proteomics comparing adolescent *Nrg1* HET mice treated with vehicle and THC.

Spot number	Protein name	Abbreviation	UniProt accession number	PI	Mass (Da)	MWS	No. of peptides matched	% seq cover	Fold change	*T*-test (p)
NMDA RECEPTOR PHYSIOLOGY										
11396	Flotillin-1	FLOT1	O08917	6.71	47,513	82	6	16	1.54	0.001985
11674	Apolipoprotein A-I	APOA1	Q00623	5.64	30,616	73	5	19	2.069	0.003538
11713	G-protein-signaling modulator 2	GPSM2	Q8VDU0	6.49	75,591	77	7	15	−2.648	0.04831
CELL SURVIVAL/CYTOTOXICITY RELATED PROTEINS										
12338	Programmed cell death protein 2 (fragment)	PDCD2	Q6RI66	5.24	19,190	66	9	19	1.169	0.0391
11060	N-acyl-phosphatidylethanolamine-hydrolyzing phospholipase D	NAPEPLD	Q8BH82	5.63	45,816	78	6	21	2.334	0.02612
11078	Interleukin-2	IL-2	P04351	4.66	19,400	58	3	34	−1.48	0.03401
OTHER										
11273	Translocon-associated protein subunit alpha	SSR1	Q9CY50	4.36	32,065	56	4	20	−3.383	0.02365
11684	Carbonic anhydrase 3	CA3	P16015	6.89	29,366	97		42	−1.293	0.03876
11348	Vacuolar protein-sorting-associated protein 25	VPS25	Q9CQ80	5.97	20,748	62	4	33	−3.211	0.04376
12395	Otoraplin	OTOR	Q9JIE3	4.77	14,328	61	3	21	3.159	0.0296

A THC-induced reduction in ARL1 may then disrupt the distribution of intracellular protein transport in the hippocampus.

Proteins selectively altered in *Nrg1* HET mice treated with THC include those that affect synapse formation and the dynamics of dendritic spines. *Nrg1* is a neurotrophic factor involved in spinogenesis through its modulation of NMDA receptor function (Li et al., 2007; Chen et al., 2008; Barros et al., 2009; Bennett, 2011; Nason et al., 2011). Adolescent THC exposure reduced the density of dendritic spines in the hippocampus via modulation of a number of proteins important to spine dynamics such as PSD-95 and NMDA receptors (Rubino et al., 2009b). *Nrg1* hypomorphism might abnormally increase dendritic spine density in the hippocampus in response to THC as adolescent *Nrg1* HET mice treated with THC displayed increased NMDA receptor binding in the hippocampus (Long et al., 2013). Our proteomic findings indicate altered expression in a number of proteins involved in intracellular trafficking and stabilization of NMDA receptors at the synapse. These include FLOT1, APOA1, and GPSM2.

GPSM2 traffics intracellular NMDA receptors to the synaptic membrane and facilitates spinogenesis by forming a macromolecular complex with NMDA receptors and synapse associated protein 102 (Sans et al., 2005). The reduced level of GPSM2 we observed in THC-treated *Nrg1* HET mice may reflect GPSM2 being incorporated into the macromolecular complex, lowering the observed expression of free, unconjugated GPSM2. Further, *Nrg1* HET mice treated with THC showed a selective increase in FLOT1 expression in the hippocampus, a protein that helps stabilize lipid rafts in the membrane. FLO T1 mediates neurite branching and dendritic spine dynamics in the hippocampus (Swanwick et al., 2010; Raemaekers et al., 2012). It also regulates the formation of glutamatergic synapses and interacts with NMDA receptors, possibly to enhance NMDA receptor clustering or trafficking to the membrane (Allen et al., 2007; Swanwick et al., 2009, 2010). Lipid rafts are constituted by cholesterol and sphingolipids (Mauch et al., 2001; Hering et al., 2003). APOA1, a protein that stimulates cholesterol release from glia, was upregulated in THC exposed *Nrg1* HET mice (Hirsch-Reinshagen et al., 2004; Karten et al., 2005). Therefore, APOA1, by increasing the availability of cholesterol for incorporation into lipid rafts, may have in turn assisted in the molecular events required to stabilize NMDA receptors at the synaptic membrane. Interestingly, APOA1 is altered in schizophrenia brain (Huang et al., 2008).

The increased excitatory transmission mediated by increased NMDA receptors in THC-treated *Nrg1* HET mice might also increase the expression of the apoptotic marker PCD2 and anandamide synthesizing enzyme NAPEPLD (Howlett et al., 2011), proteins reflecting heightened excitotoxicity/apoptosis and a homeostatic attempt to dampen increased NMDA receptor activation respectively. These results are consistent with *Nrg1*-cannabinoid interactions dysregulating the septohippocampal system. Increased excitation in the hippocampus of THC-treated *Nrg1* HET mice might then influence downstream activity of the lateral septum, a region we have repeatedly shown to be selectively activated in *Nrg1* HET mice in response to THC (Boucher et al., 2007b, 2011).

CONCLUSIONS

Using a proteomic approach we have uncovered numerous novel proteins that may be subject to regulation by disturbed *Nrg1* signaling. Our findings also illuminate a potential constellation of molecular changes that may subserve the behavioral abnormalities that are observed in the *Nrg1* transmembrane domain heterozygous mouse as well as their idiosyncratic response to repeated cannabinoid treatment. This may have implications for our overall understanding of genetic vulnerability to schizophrenia and to the exacerbation of psychosis sometimes caused by cannabis.

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REFERENCES

Allen, J. A., Halverson-Tamboli, R. A., and Rasenick, M. M. (2007). Lipid raft microdomains and neurotransmitter signalling. *Nat. Rev. Neurosci.* 8, 128–140.

Allen, N. J., Bennett, M. L., Foo, L. C., Wang, G. X., Chakraborty, C., Smith, S. J., et al. (2012). Astrocyte glypicans 4 and 6 promote formation of excitatory synapses via GluA1 AMPA receptors. *Nature* 486, 410–414.

Arasaki, K., Taniguchi, M., Tani, K., and Tagaya, M. (2006). RINT-1 regulates the localization and entry of ZW10 to the syntaxin 18 complex. *Mol. Biol. Cell* 17, 2780–2788.

Arnold, J. C., Boucher, A. A., and Karl, T. (2012). The yin and yang of cannabis-induced psychosis: the actions of delta 9-tetrahydrocannabinol and cannabidiol in rodent models of Schizophrenia. *Curr. Pharm. Des.* 18, 5113–5130.

Ayalew, M., Le-Niculescu, H., Levey, D. F., Jain, N., Changala, B., Patel, S. D., et al. (2012). Convergent functional genomics of schizophrenia: from comprehensive understanding to genetic risk prediction. *Mol. Psychiatry* 17, 887–905.

Barros, C. S., Calabrese, B., Chamero, P., Roberts, A. J., Korzus, E., Lloyd, K., et al. (2009). Impaired maturation of dendritic spines without disorganization of cortical cell layers in mice lacking *NRG1*/ErbB signaling in the central nervous system. *Proc. Natl. Acad. Sci. U.S.A.* 106, 4507–4512.

Bennett, M. R. (2011). Schizophrenia: susceptibility genes, dendritic-spine pathology and gray matter loss. *Prog. Neurobiol.* 95, 275–300.

Bindukumar, B., Mahajan, S. D., Reynolds, J. L., Hu, Z., Sykes, D. E., Aalinkeel, R., et al. (2008). Genomic and proteomic analysis of the effects of cannabinoids on normal human astrocytes. *Brain Res.* 1191, 1–11.

Bjarnadottir, M., Misner, D. L., Haverfield-Gross, S., Bruun, S., Helgason, V. G., Stefansson, H., et al. (2007). Neuregulin1 (*NRG1*) signaling through Fyn modulates NMDA receptor phosphorylation: differential synaptic function in *NRG1* ± knock-outs compared with wild-type mice. *J. Neurosci.* 27, 4519–4529.

Boucher, A. A., Arnold, J. C., Duffy, L., Schofield, P. R., Micheau, J., and Karl, T. (2007a). Heterozygous neuregulin 1 mice are more sensitive to the behavioural effects

of Delta9-tetrahydrocannabinol. *Psychopharmacology (Berl.)* 192, 325–336.

Boucher, A. A., Hunt, G. E., Karl, T., Micheau, J., McGregor, I. S., and Arnold, J. C. (2007b). Heterozygous neuregulin 1 mice display greater baseline and Delta(9)-tetrahydrocannabinol-induced c-Fos expression. *Neuroscience* 149, 861–870.

Boucher, A. A., Hunt, G. E., Micheau, J., Huang, X., McGregor, I. S., Karl, T., et al. (2011). The schizophrenia susceptibility gene neuregulin 1 modulates tolerance to the effects of cannabinoids. *Int. J. Neuropsychopharmacol.* 14, 631–643.

Boucher, A. A., Vivier, L., Metna-Laurent, M., Brayda-Bruno, L., Mons, N., Arnold, J. C., et al. (2009). Chronic treatment with Delta(9)-tetrahydrocannabinol impairs spatial memory and reduces zif268 expression in the mouse forebrain. *Behav. Pharmacol.* 20, 45–55.

Bradshaw, A. D. (2012). Diverse biological functions of the SPARC family of proteins. *Int. J. Biochem. Cell Biol.* 44, 480–488.

Burkhardt, J. K., Echeverri, C. J., Nilsson, T., and Vallee, R. B. (1997). Overexpression of the dynamitin (p50) subunit of the dynactin complex disrupts dynein-dependent maintenance of membrane organelle distribution. *J. Cell Biol.* 139, 469–484.

Casey, B. J., Jones, R. M., and Hare, T. A. (2008). The adolescent brain. *Ann. N.Y. Acad. Sci.* 1124, 111–126.

Chau, B. N., Cheng, E. H., Kerr, D. A., and Hardwick, J. M. (2000). Aven, a novel inhibitor of caspase activation, binds Bcl-xL and Apaf-1. *Mol. Cell* 6, 31–40.

Chen, Y., Zhang, M., Li, Q., Guo, Y., Ding, W., Wang, L., et al. (2011). Interfering effect and mechanism of neuregulin on experimental dementia model in rats. *Behav. Brain Res.* 222, 321–325.

Chen, Y. J., Johnson, M. A., Lieberman, M. D., Goodchild, R. E., Schobel, S., Lewandowski, N., et al. (2008). Type III neuregulin-1 is required for normal sensorimotor gating, memory-related behaviors, and corticostriatal circuit components. *J. Neurosci.* 28, 6872–6883.

Chesworth, R., Yulyaningsih, E., Cappas, E., Arnold, J., Sainsbury, A., and Karl, T. (2012). The response of neuregulin 1 mutant mice to acute restraint stress. *Neurosci. Lett.* 515, 82–86.

Chong, V. Z., Thompson, M., Beltaifa, S., Webster, M. J., Law, A. J., and Weickert, C. S. (2008). Elevated neuregulin-1 and ErbB4 protein in the prefrontal cortex of schizophrenic patients. *Schizophr. Res.* 100, 270–280.

Clark, D., Dedova, I., Cordwell, S., and Matsumoto, I. (2007). Altered proteins of the anterior cingulate cortex white matter proteome in schizophrenia. *Proteomics Clin. Appl.* 1, 157–166.

Colombo, G., Rusconi, F., Rubino, T., Cattaneo, A., Martegani, E., Parolaro, D., et al. (2009). Transcriptomic and proteomic analyses of mouse cerebellum reveals alterations in RasGRF1 expression following *in vivo* chronic treatment with delta 9-tetrahydrocannabinol. *J. Mol. Neurosci.* 37, 111–122.

Dean, B., Karl, T., Pavey, G., Boer, S., Duffy, L., and Scarr, E. (2008). Increased levels of serotonin 2A receptors and serotonin transporter in the CNS of neuregulin 1 hypomorphic/mutant mice. *Schizophr. Res.* 99, 341–349.

Desbonnet, L., O'Tuathaigh, C., Clarke, G., O'Leary, C., Petit, E., Clarke, N., et al. (2012). Phenotypic effects of repeated psychosocial stress during adolescence in mice mutant for the schizophrenia risk gene neuregulin-1: a putative model of gene x environment interaction. *Brain Behav. Immun.* 26, 660–671.

Duffy, L., Cappas, E., Lai, D., Boucher, A. A., and Karl, T. (2010). Cognition in transmembrane domain neuregulin 1 mutant mice. *Neuroscience* 170, 800–807.

Figueroa, B. Jr., Chen, S., Oyler, G. A., Hardwick, J. M., and Betenbaugh, M. J. (2004). Aven and Bcl-xL enhance protection against apoptosis for mammalian cells exposed to various culture conditions. *Biotechnol. Bioeng.* 85, 589–600.

Filipeanu, C. M., Guidry, J. J., Leonard, S. T., and Winsauer, P. J. (2011). Delta9-THC increases endogenous AHA1 expression in rat cerebellum and may modulate CB1 receptor function during chronic use. *J. Neurochem.* 118, 1101–1112.

Gabriel, S. M., Haroutunian, V., Powchik, P., Honer, W. G., Davidson, M., Davies, P., et al. (1997). Increased concentrations of presynaptic proteins in the cingulate cortex of subjects with schizophrenia. *Arch. Gen. Psychiatry* 54, 559–566.

Galli, A., Roure, A., Zeller, R., and Dono, R. (2003). Glypican 4 modulates FGF signalling and regulates dorsoventral forebrain patterning in Xenopus embryos. *Development* 130, 4919–4929.

Gaspar, P., Cases, O., and Maroteaux, L. (2003). The developmental role of serotonin: news from mouse molecular genetics. *Nat. Rev. Neurosci.* 4, 1002–1012.

Giedd, J. N., Blumenthal, J., Jeffries, N. O., Castellanos, F. X., Liu, H., Zijdenbos, A., et al. (1999). Brain development during childhood and adolescence: a longitudinal MRI study. *Nat. Neurosci.* 2, 861–863.

Greenwood, T. A., Light, G. A., Swerdlow, N. R., Radant, A. D., and Braff, D. L. (2012). Association analysis of 94 candidate genes and schizophrenia-related endophenotypes. *PLoS ONE* 7:e29630. doi: 10.1371/journal.pone.0029630

Han, S., Yang, B. Z., Kranzler, H. R., Oslin, D., Anton, R., Farrer, L. A., et al. (2012). Linkage analysis followed by association show *NRG1* associated with cannabis dependence in African Americans. *Mol. Psychiatry* 72, 637–644.

Hashimoto, R., Straub, R. E., Weickert, C. S., Hyde, T. M., Kleinman, J. E., and Weinberger, D. R. (2004). Expression analysis of neuregulin-1 in the dorsolateral prefrontal cortex in schizophrenia. *Mol. Psychiatry* 9, 299–307.

Hering, H., Lin, C. C., and Sheng, M. (2003). Lipid rafts in the maintenance of synapses, dendritic spines, and surface AMPA receptor stability. *J. Neurosci.* 23, 3262–3271.

Herkenham, M., Lynn, A. B., Little, M. D., Johnson, M. R., Melvin, L. S., de Costa, B. R., et al. (1990). Cannabinoid receptor localization in brain. *Proc. Natl. Acad. Sci. U.S.A.* 87, 1932–1936.

Hirsch-Reinshagen, V., Zhou, S., Burgess, B. L., Bernier, L., McIsaac, S. A., Chan, J. Y., et al. (2004). Deficiency of ABCA1 impairs apolipoprotein E metabolism in brain. *J. Biol. Chem.* 279, 41197–41207.

Honer, W. G., Falkai, P., Young, C., Wang, T., Xie, J., Bonner, J., et al. (1997). Cingulate cortex synaptic terminal proteins and neural cell adhesion molecule in schizophrenia. *Neuroscience* 78, 99–110.

Hong, L. E., Wonodi, I., Stine, O. C., Mitchell, B. D., and Thaker, G. K. (2008). Evidence of missense mutations on the neuregulin 1 gene affecting function of prepulse inhibition. *Biol. Psychiatry* 63, 17–23.

Howlett, A. C., Reggio, P. H., Childers, S. R., Hampson, R. E., Ulloa, N. M., and Deutsch, D. G. (2011). Endocannabinoid tone versus constitutive activity of cannabinoid receptors. *Br. J. Pharmacol.* 163, 1329–1343.

Huang, J. T., Wang, L., Prabakaran, S., Wengenroth, M., Lockstone, H. E., Koethe, D., et al. (2008). Independent protein-profiling studies show a decrease in apolipoprotein A1 levels in schizophrenia CSF, brain and peripheral tissues. *Mol. Psychiatry* 13, 1118–1128.

Iwazaki, T., McGregor, I. S., and Matsumoto, I. (2008). Protein expression profile in the amygdala of rats with methamphetamine-induced behavioral sensitization. *Neurosci. Lett.* 435, 113–119.

Karl, T., Duffy, L., Scimone, A., Harvey, R. P., and Schofield, P. R. (2007). Altered motor activity, exploration and anxiety in heterozygous neuregulin 1 mutant mice: implications for understanding schizophrenia. *Genes Brain Behav.* 6, 677–687.

Karten, B., Hayashi, H., Francis, G. A., Campenot, R. B., Vance, D. E., and Vance, J. E. (2005). Generation and function of astroglial lipoproteins from Niemann-Pick type C1-deficient mice. *Biochem. J.* 387, 779–788.

Kashem, M. A., Sarker, R., Des Etages, H., Machaalani, R., King, N., McGregor, I. S., et al. (2009). Comparative proteomics in the corpus callosal sub-regions of post-mortem human brain. *Neurochem. Int.* 55, 483–490.

Kobeissy, F. H., Warren, M. W., Ottens, A. K., Sadasivan, S., Zhang, Z., Gold, M. S., et al. (2008). Psychoproteomic analysis of rat cortex following acute methamphetamine exposure. *J. Proteome Res.* 7, 1971–1983.

Kucukdereli, H., Allen, N. J., Lee, A. T., Feng, A., Ozlu, M. I., Conatser, L. M., et al. (2011). Control of excitatory CNS synaptogenesis by astrocyte-secreted proteins Hevin and SPARC. *Proc. Natl. Acad. Sci. U.S.A.* 108, E440–E449.

Kutuk, O., Temel, S. G., Tolunay, S., and Basaga, H. (2010). Aven blocks DNA damage-induced apoptosis by stabilising Bcl-xL. *Eur. J. Cancer* 46, 2494–2505.

Lee, J. K., Chung, J., Druey, K. M., and Tansey, M. G. (2012). RGS10 exerts a neuroprotective role through the PKA/c-AMP response-element (CREB) pathway in dopaminergic neuron-like cells. *J. Neurochem.* 122, 333–343.

Li, B., Woo, R. S., Mei, L., and Malinow, R. (2007). The neuregulin-1 receptor erbB4 controls glutamatergic

synapse maturation and plasticity. *Neuron* 54, 583–597.

Li, Y., Lein, P. J., Liu, C., Bruun, D. A., Giulivi, C., Ford, G. D., et al. (2012). Neuregulin-1 is neuroprotective in a rat model of organophosphate-induced delayed neuronal injury. *Toxicol. Appl. Pharmacol.* 262, 194–204.

Long, L. E., Chesworth, R., Arnold, J. C., and Karl, T. (2010). A follow-up study: acute behavioural effects of Delta(9)-THC in female heterozygous neuregulin 1 transmembrane domain mutant mice. *Psychopharmacology (Berl.)* 211, 277–289.

Long, L. E., Chesworth, R., Huang, X. F., McGregor, I. S., Arnold, J. C., and Karl, T. (2013). Transmembrane domain *Nrg1* mutant mice show altered susceptibility to the neurobehavioural actions of repeated THC exposure in adolescence. *Int. J. Neuropsychopharmacol.* 16, 163–175.

Marballi, K., Cruz, D., Thompson, P., and Walss-Bass, C. (2012). Differential neuregulin 1 cleavage in the prefrontal cortex and hippocampus in schizophrenia and bipolar disorder: preliminary findings. *PLoS ONE* 7:e36431. doi: 10.1371/journal.pone.0036431

Marballi, K., Quinones, M. P., Jimenez, F., Escamilla, M. A., Raventos, H., Soto-Bernardini, M. C., et al. (2010). *In vivo* and *in vitro* genetic evidence of involvement of neuregulin 1 in immune system dysregulation. *J. Mol. Med. (Berl.)* 88, 1133–1141.

Marsicano, G., and Lutz, B. (1999). Expression of the cannabinoid receptor CB1 in distinct neuronal subpopulations in the adult mouse forebrain. *Eur. J. Neurosci.* 11, 4213–4225.

Mauch, D. H., Nagler, K., Schumacher, S., Goritz, C., Muller, E. C., Otto, A., et al. (2001). CNS synaptogenesis promoted by glia-derived cholesterol. *Science* 294, 1354–1357.

Mei, L., and Xiong, W. C. (2008). Neuregulin 1 in neural development, synaptic plasticity and schizophrenia. *Nat. Rev. Neurosci.* 9, 437–452.

Morozov, Y. M., Torii, M., and Rakic, P. (2009). Origin, early commitment, migratory routes, and destination of cannabinoid type 1 receptor-containing interneurons. *Cereb. Cortex* 19(Suppl. 1), i78–i89.

Mullock, B. M., Smith, C. W., Ihrke, G., Bright, N. A., Lindsay, M., Parkinson, E. J., et al. (2000). Syntaxin 7 is localized to late endosome compartments, associates with Vamp 8, and Is required for late endosome-lysosome fusion. *Mol. Biol. Cell* 11, 3137–3153.

Nakamura, K., Sugawara, Y., Sawabe, K., Ohashi, A., Tsurui, H., Xiu, Y., et al. (2006). Late developmental stage-specific role of tryptophan hydroxylase 1 in brain serotonin levels. *J. Neurosci.* 26, 530–534.

Nakamura, N., Yamamoto, A., Wada, Y., and Futai, M. (2000). Syntaxin 7 mediates endocytic trafficking to late endosomes. *J. Biol. Chem.* 275, 6523–6529.

Nason, M. W. Jr., Adhikari, A., Bozinoski, M., Gordon, J. A., and Role, L. W. (2011). Disrupted activity in the hippocampal-accumbens circuit of type III neuregulin 1 mutant mice. *Neuropsychopharmacology* 36, 488–496.

Nishimoto-Morita, K., Shin, H. W., Mitsuhashi, H., Kitamura, M., Zhang, Q., Johannes, L., et al. (2009). Differential effects of depletion of ARL1 and ARFRP1 on membrane trafficking between the trans-Golgi network and endosomes. *J. Biol. Chem.* 284, 10583–10592.

O'Tuathaigh, C. M., Babovic, D., O'Sullivan, G. J., Clifford, J. J., Tighe, O., Croke, D. T., et al. (2007). Phenotypic characterization of spatial cognition and social behavior in mice with 'knockout' of the schizophrenia risk gene neuregulin 1. *Neuroscience* 147, 18–27.

O'Tuathaigh, C. M., O'Connor, A. M., O'Sullivan, G. J., Lai, D., Harvey, R., Croke, D. T., et al. (2008). Disruption to social dyadic interactions but not emotional/anxiety-related behaviour in mice with heterozygous 'knockout' of the schizophrenia risk gene neuregulin-1. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 32, 462–466.

Paine-Saunders, S., Viviano, B. L., and Saunders, S. (1999). GPC6, a novel member of the glypican gene family, encodes a product structurally related to GPC4 and is colocalized with GPC5 on human chromosome 13. *Genomics* 57, 455–458.

Quinn, H. R., Matsumoto, I., Callaghan, P. D., Long, L. E., Arnold, J. C., Gunasekaran, N., et al. (2008). Adolescent rats find repeated Delta(9)-THC less aversive than adult rats but display greater residual cognitive deficits and changes in hippocampal protein expression following exposure. *Neuropsychopharmacology* 33, 1113–1126.

Radonjic, N. V., Knezevic, I. D., Vilimanovich, U., Kravic-Stevovic, T., Marina, L. V., Nikolic, T., et al. (2010). Decreased glutathione levels and altered antioxidant defense in an animal model of schizophrenia: long-term effects of perinatal phencyclidine administration. *Neuropharmacology* 58, 739–745.

Raemaekers, T., Peric, A., Baatsen, P., Sannerud, R., Declerck, I., Baert, V., et al. (2012). ARF6-mediated endosomal transport of Telencephalin affects dendritic filopodia-to-spine maturation. *EMBO J.* 31, 3252–3269.

Ramakrishnan, N. A., Drescher, M. J., and Drescher, D. G. (2012). The SNARE complex in neuronal and sensory cells. *Mol. Cell. Neurosci.* 50, 58–69.

Rodriguez-Santiago, B., Brunet, A., Sobrino, B., Serra-Juhe, C., Flores, R., Armengol, L., et al. (2010). Association of common copy number variants at the glutathione S-transferase genes and rare novel genomic changes with schizophrenia. *Mol. Psychiatry* 15, 1023–1033.

Roussos, P., Giakoumaki, S. G., Adamaki, E., and Bitsios, P. (2011). The influence of schizophrenia-related neuregulin-1 polymorphisms on sensorimotor gating in healthy males. *Biol. Psychiatry* 69, 479–486.

Rubino, T., Realini, N., Braida, D., Alberio, T., Capurro, V., Vigano, D., et al. (2009a). The depressive phenotype induced in adult female rats by adolescent exposure to THC is associated with cognitive impairment and altered neuroplasticity in the prefrontal cortex. *Neurotox. Res.* 15, 291–302.

Rubino, T., Realini, N., Braida, D., Guidi, S., Capurro, V., Vigano, D., et al. (2009b). Changes in hippocampal morphology and neuroplasticity induced by adolescent THC treatment are associated with cognitive impairment in adulthood. *Hippocampus* 19, 763–772.

Saetre, P., Lundmark, P., Wang, A., Hansen, T., Rasmussen, H. B., Djurovic, S., et al. (2010). The tryptophan hydroxylase 1 (TPH1) gene, schizophrenia susceptibility, and suicidal behavior: a multicentre case-control study and meta-analysis. *Am. J. Med. Genet. B Neuropsychiatr. Genet.* 153B, 387–396.

Sans, N., Wang, P. Y., Du, Q., Petralia, R. S., Wang, Y. X., Nakka, S., et al. (2005). mPins modulates PSD-95 and SAP102 trafficking and influences NMDA receptor surface expression. *Nat. Cell Biol.* 7, 1179–1190.

Schiavo, G., Gmachl, M. J., Stenbeck, G., Sollner, T. H., and Rothman, J. E. (1995). A possible docking and fusion particle for synaptic transmission. *Nature* 378, 733–736.

Seifuddin, F., Mahon, P. B., Judy, J., Pirooznia, M., Jancic, D., Taylor, J., et al. (2012). Meta-analysis of genetic association studies on bipolar disorder. *Am. J. Med. Genet. B Neuropsychiatr. Genet.* 159B, 508–518.

Sokolov, B. P., Tcherepanov, A. A., Haroutunian, V., and Davis, K. L. (2000). Levels of mRNAs encoding synaptic vesicle and synaptic plasma membrane proteins in the temporal cortex of elderly schizophrenic patients. *Biol. Psychiatry* 48, 184–196.

Sompol, P., Liu, X., Baba, K., Paul, K. N., Tosini, G., Iuvone, P. M., et al. (2011). N-acetylserotonin promotes hippocampal neuroprogenitor cell proliferation in sleep-deprived mice. *Proc. Natl. Acad. Sci. U.S.A.* 108, 8844–8849.

Spear, L. P. (2000). The adolescent brain and age-related behavioral manifestations. *Neurosci. Biobehav. Rev.* 24, 417–463.

Spencer, J. R., Darbyshire, K. M., Boucher, A. A., and Arnold, J. C. (2012). Adolescent neuregulin 1 heterozygous mice display enhanced behavioural sensitivity to methamphetamine. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 39, 376–381.

Stefansson, H., Sigurdsson, E., Steinthorsdottir, V., Bjornsdottir, S., Sigmundsson, T., Ghosh, S., et al. (2002). Neuregulin 1 and susceptibility to schizophrenia. *Am. J. Hum. Genet.* 71, 877–892.

Stetler, R. A., Gan, Y., Zhang, W., Liou, A. K., Gao, Y., Cao, G., et al. (2010). Heat shock proteins: cellular and molecular mechanisms in the central nervous system. *Prog. Neurobiol.* 92, 184–211.

Stojković, T., Radonjić, N. V., Velimirović, M., Jevtić, G., Popović, V., Doknić, M., et al. (2012). Risperidone reverses phencyclidine induced decrease in glutathione levels and alterations of antioxidant defense in rat brain. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 39, 192–199.

Swanwick, C. C., Shapiro, M. E., Vicini, S., and Wenthold, R. J. (2010). Flotillin-1 mediates neurite branching induced by synaptic adhesion-like molecule 4 in hippocampal neurons. *Mol. Cell. Neurosci.* 45, 213–225.

Swanwick, C. C., Shapiro, M. E., Yi, Z., Chang, K., and Wenthold, R. J. (2009). NMDA receptors interact with flotillin-1 and -2, lipid raft-associated proteins. *FEBS Lett.* 583, 1226–1230.

Tsou, K., Brown, S., Sanudo-Pena, M. C., Mackie, K., and Walker, J. M. (1998). Immunohistochemical distribution of cannabinoid CB1 receptors in the rat central nervous system. *Neuroscience* 83, 393–411.

Uz, T., Qu, T., Sugaya, K., and Manev, H. (2002). Neuronal expression of arylalkylamine N-acetyltransferase (AANAT) mRNA in the rat brain. *Neurosci. Res.* 42, 309–316.

Verstrepen, L., Carpentier, I., Verhelst, K., and Beyaert, R. (2009). ABINs: A20 binding inhibitors of NF-kappa B and apoptosis signaling. *Biochem. Pharmacol.* 78, 105–114.

Vullhorst, D., Neddens, J., Karavanova, I., Tricoire, L., Petralia, R. S., McBain, C. J., et al. (2009). Selective expression of ErbB4 in interneurons, but not pyramidal cells, of the rodent hippocampus. *J. Neurosci.* 29, 12255–12264.

Wang, J., Yuan, W., and Li, M. D. (2011). Genes and pathways co-associated with the exposure to multiple drugs of abuse, including alcohol, amphetamine/methamphetamine, cocaine, marijuana, morphine, and/or nicotine: a review of proteomics analyses. *Mol. Neurobiol.* 44, 269–286.

Wang, K. S., Zhang, Q., Liu, X., Wu, L., and Zeng, M. (2012). PKNOX2 is associated with formal thought disorder in schizophrenia: a meta-analysis of two genome-wide association studies. *J. Mol. Neurosci.* 48, 265–272.

Weickert, C. S., Tiwari, Y., Schofield, P. R., Mowry, B. J., and Fullerton, J. M. (2012). Schizophrenia-associated HapICE haplotype is associated with increased *NRG1* type III expression and high nucleotide diversity. *Transl. Psychiatry* 2:e104. doi: 10.1038/tp.2012.25

Woo, R. S., Lee, J. H., Kim, H. S., Baek, C. H., Song, D. Y., Suh, Y. H., et al. (2012). Neuregulin-1 protects against neurotoxicities induced by Swedish amyloid precursor protein via the ErbB4 receptor. *Neuroscience* 202, 413–423.

Wozniak, D. F., Xiao, M., Xu, L., Yamada, K. A., and Ornitz, D. M. (2007). Impaired spatial learning and defective theta burst induced LTP in mice lacking fibroblast growth factor 14. *Neurobiol. Dis.* 26, 14–26.

Xiao, M., Xu, L., Laezza, F., Yamada, K., Feng, S., and Ornitz, D. M. (2007). Impaired hippocampal synaptic transmission and plasticity in mice lacking fibroblast growth factor 14. *Mol. Cell. Neurosci.* 34, 366–377.

Zheng, W., and Cole, P. A. (2002). Serotonin N-acetyltransferase: mechanism and inhibition. *Curr. Med. Chem.* 9, 1187–1199.

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What does a mouse tell us about *neuregulin 1*—cannabis interactions?

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The link between cannabis and psychosis has been debated although there is substantial epidemiological evidence showing that cannabis increases the risk of psychosis. It has been hypothesized that schizophrenia patients carrying particular risk genes might be more sensitive to the psychosis-inducing effects of cannabis than other patients and healthy test subjects. Here we review the effects of cannabinoids on a mutant mouse model for the schizophrenia candidate gene *neuregulin 1* (*Nrg1*). The studies suggest a complex interaction between cannabis and *Nrg1*: the neuro-behavioral effects of cannabinoids were different in *Nrg1* mutant and control mice and depended on exposure time, sex, and age of test animals. This research provides the first evidence of complex cannabis-*Nrg1* interactions suggesting *Nrg1* as a prime target for future clinical investigations. Furthermore, it highlights that animal model research can broaden our understanding of the complex multi-factorial etiology of schizophrenia. Finally, the findings are important to preventive psychiatry: if the genes that confer genetic vulnerability to cannabis-induced psychosis were identified patients at-high risk could be forewarned of the potential dangers of cannabis abuse.

Keywords: schizophrenia, cannabis, *neuregulin 1*, gene-environment interactions, mouse model

The two-hit hypothesis of schizophrenia states that a combination of genetic and environmental risk factors will cause the development of schizophrenia (Bayer et al., 1999; Rapoport et al., 2005; Caspi and Moffitt, 2006). Scores of genetic risk factors have been suggested for schizophrenia (Allen et al., 2008) and mouse mutants have been developed for most of those candidates (Desbonnet et al., 2009). *Neuregulin 1* (*NRG1*) is one of the more promising schizophrenia candidate genes as associations with schizophrenia have been found in several studies (Stefansson et al., 2002; Tosato et al., 2005; Munafo et al., 2006). However, similar to many other schizophrenia susceptibility genes, recent genome wide associations studies suggest that it is more important to consider an interplay of different genetic and environmental risk factors for schizophrenia to understand the etiology of the disorder (Sanders et al., 2008). Thus, many environmental factors have been considered as risk factors for schizophrenia (Van Os et al., 2010) and cannabis use has been the focus of an ever larger growing list of studies. Cannabis appears to be a component/cumulative cause for schizophrenia and increases the overall risk of developing the disorder by 2-fold (Henquet et al., 2005). Importantly, an increasing number of researchers believe that this risk might be elevated for cannabis users with a genetic vulnerability to schizophrenia (Caspi and Moffitt, 2006). Indeed, a functional polymorphism in the gene for catechol-*O*-methyl transferase (*COMT*) was implicated in conferring vulnerability to cannabis-induced psychosis (Caspi et al., 2005; Henquet et al., 2006). Subsequently, a genetic mouse model for *COMT* was treated with the main psychoactive component of cannabis (Δ^9-tetrahydrocannabinol: THC) during adolescence and exhibited a greater behavioral sensitivity to the long-term effects of THC (O'Tuathaigh et al., 2010) and a genotype-specific response in dopaminergic and GABAergic pathways as well as in the protein expression of cannabinoid 1 receptors (CB1) (Behan et al., 2012). Based on *NRG1's* established role in schizophrenia and the availability of validated mouse mutants for *Nrg1* (Duffy et al., 2008), we investigated over the last decade if *Nrg1* represents a second candidate for gene-cannabis interactions in schizophrenia. The mini review will outline how our mouse research has been instrumental in discovering *Nrg1*-cannabis interactions relevant to schizophrenia and in deciphering potential mechanisms. Our studies not only considered THC but also the cannabinoid cannabidiol (CBD), which is devoid of psychoactive properties and has been reported to block or reverse effects of THC and have antipsychotic properties (Arnold et al., 2012).

The protein Nrg1 influences key neurodevelopmental processes such as myelination, synaptogenesis, neuronal migration, and is involved in the expression and activation of N-methyl-D-aspartic acid (NMDA) receptors (Harrison and Law, 2006; Mei and Xiong, 2008). Importantly, a number of genetic mouse models have been developed for the different isoforms of Nrg1 (Duffy et al., 2008; Mei and Xiong, 2008; Karl et al., 2011). Among those, the heterozygous transmembrane domain *Nrg1* mutant mouse (*Nrg1* HET) has shown compelling face, construct, and partial predictive validity for schizophrenia research (Stefansson

et al., 2002; Karl et al., 2007, 2011; Van Den Buuse et al., 2009; Duffy et al., 2010; Chesworth et al., 2012a,b). Thus, our team has utilized this model to determine the nature of *Nrg1*-cannabis interactions in great detail (for review see Arnold et al., 2012). The clinical relevance of this research has recently been highlighted by a genome-wide linkage and single nucleotide polymorphism association analysis, which discovered *NRG1* as a major candidate for the development of cannabis dependence in African Americans (Han et al., 2012). The findings of our earlier mouse model research will be outlined in the following.

In an initial study we exposed *Nrg1* HET mice to acute doses of THC before testing them in an array of schizophrenia-relevant behavioral paradigms (Powell and Miyakawa, 2006). *Nrg1* mutant mice exhibited an increased sensitivity to the locomotor-suppressant and anxiogenic effects of THC compared to wild type-like littermates (WT). Surprisingly, the mutants also showed improved sensorimotor gating following THC challenge as measured by prepulse inhibition of the startle response (PPI) (Boucher et al., 2007a). Increased PPI is often detected after treatment with antipsychotic drugs which normalize PPI deficits of schizophrenia patients (Geyer et al., 2001). Recent human data suggest that *NRG1* may also confer increased behavioral sensitivity to THC [although *NRG1* polymorphisms worsened THC-induced information processing dysfunction rather than improving it (Stadelmann et al., 2010)]. It is possible that the effects of heterozygous deletion of *Nrg1* in mice might be opposite to that conferred by *NRG1* polymorphisms in patients. Future studies may examine whether mice overexpressing Nrg1 protein display exaggerated THC-induced PPI deficits. Follow-up experiments revealed that the enhanced behavioral response of *Nrg1* HETs to acute THC was sex-specific as female mutants showed no enhanced susceptibility to acute THC and actually developed resistance to aspects of THC-induced social withdrawal (Long et al., 2010a). It is unclear as to why *Nrg1*-cannabinoid interactions are sex-specific. Gender influences the actions of cannabinoids (McGregor and Arnold, 2007) and interactions between gonadal hormones and neuregulin have been demonstrated [(Lacroix-Fralish et al., 2006); but also see Taylor et al., 2011]. Future studies could examine whether Nrg1 expression regulates the modulatory effects of gonadal hormones on cannabinoid receptor sensitivity.

The increased behavioral susceptibility of male *Nrg1* HET mice to THC was accompanied by elevated neuronal activation as measured using c-Fos immunohistochemistry (Boucher et al., 2007b). THC selectively increased c-Fos expression in the ventral part of the lateral septum (LSV) of *Nrg1* mutants. No corresponding effect was observed in control littermates. Interestingly, drugs, which modulate PPI, whether they are pro-psychotic drugs that impair PPI, or anti-psychotic drugs that facilitate PPI, all increase c-Fos expression in the lateral septum (Sumner et al., 2004). Furthermore, *Nrg1* HET mice exhibited a more pronounced enhancement of c-Fos levels in stress-related brain regions (i.e., paraventricular nucleus of hypothalamus and central nucleus of amygdala). In summary, these animal studies provided the very first evidence for an interaction between the schizophrenia risk gene *Nrg1* and cannabis and implied that stress and gender may also influence these interactions.

Chronic cannabis use is more relevant in cannabis-induced psychosis than acute exposure. Thus, our team continued this line of research and determined the neuro-behavioral response of *Nrg1* mutants to long-term cannabinoid exposure. *Nrg1* mutant and control mice were treated chronically with the synthetic CB1 receptor agonist CP 55,940 (Boucher et al., 2011). *Nrg1* hypomorphic mice developed tolerance to the hypothermic and locomotor-suppressant effects of CP 55,940 more rapidly than WT mice. Interestingly, tolerance development toward the anxiogenic effects of the cannabinoid was only observed in control mice whereas *Nrg1* HETs maintained persistent THC-induced anxiety with repeated CP 55,940 dosing. All mice developed tolerance to the genotype-specific effects of acute CP 55,940 on PPI (i.e., impairment in WT and facilitation in *Nrg1* HET mice). Mutant mice showed a selective increase in CP 55,940-induced FosB/ΔFosB expression in the LSV, which is a marker for long-term neuroadaptive changes. These findings suggest that *Nrg1* is not only involved in the acute neuro-behavioral response to cannabinoids but also modulates neuroadaptive responses to long-term cannabinoid challenge. Furthermore, it confirms the LSV as an important brain region for *Nrg1*–cannabinoid interactions. This could be related to the fact that the lateral septum shares reciprocal projections with the hypothalamus and the amygdala and receives cognitive input from the hippocampus and the prefrontal cortex (Sheehan et al., 2004). These brain areas are important in schizophrenia and are characterized by high expression levels of Nrg1, its main receptor ErbB4 and CB1 (Law et al., 2004; Kofalvi, 2008; Neddens and Buonanno, 2011). Future studies should examine in more detail the role of the LSV in mediating the neuro-behavioral effects of cannabinoids in *Nrg1* HET mice and define in particular the involvement of CB1 and ErbB4 receptors.

Human research suggests that adolescence is a time of increased vulnerability to the detrimental effects of cannabis on the development of psychosis (Caspi et al., 2005). Thus, our team exposed adolescent WT and *Nrg1* HET mice to chronic THC (Long et al., 2013). Surprisingly, *Nrg1* mutants appeared less susceptible to THC-induced suppression of investigative social behaviors than control mice. However, adolescent THC exacerbated the hyperlocomotive phenotype characteristic for adult *Nrg1* mutant mice (Karl et al., 2007; Long et al., 2013). *Nrg1* deficiency also modulated the effects of adolescent THC on neurotransmitter systems involved in the pathophysiology of schizophrenia. Radioligand binding analyses found genotype-specific THC effects on CB1 expression in the substantia nigra: *Nrg1* HET mice exhibited reduced CB1 levels drug-free whereas CB1 binding was decreased in WT and increased in *Nrg1* mice post THC challenge. Lower CB1 expression levels in the substantia nigra might be responsible for the observed decreased susceptibility of adolescent *Nrg1* mutant mice (whereas binding studies in adult *Nrg1* HETs found increased levels of CB1 in the same brain region; manuscript currently being submitted). Interestingly, ErbB4 is localized in dopaminergic neurons in the substantia nigra (Abe et al., 2009). Thus, ErbB4 and CB1 might interact in the substantia nigra and thereby regulate the hyper-locomotor phenotype of *Nrg1* mutant mice.

Nrg1 also conferred opposing effects of THC on 5-HT2A receptor expression in the insular cortex and NMDA receptor binding was selectively increased in the hippocampus and cingulate cortex of *Nrg1* HET mice (Long et al., 2013) (for a better mechanistic understanding of *Nrg1*-THC interaction on NMDA receptor expression in the hippocampus see Spencer et al., 2013).

The *cannabis sativa* plant is a mix of over 60 different cannabinoids, one being THC, another being CBD, which blocks, or reverses the effects of THC and other psychotropic drugs such as methamphetamine (Long et al., 2010b). Varying levels of THC and CBD in different cannabis strains could modify the consequences of long-term cannabis consumption and also shift the nature of gene-cannabis interactions such as the one reported here for *Nrg1*. Thus, our team characterized the neuro-behavioral response of *Nrg1* HET mice to acute and chronic CBD (Long et al., 2012) to investigate its potentially therapeutic-like effects in animal models for schizophrenia. CBD did not alter schizophrenia-relevant behaviors such as hyperlocomotion or PPI deficits in our *Nrg1* HET mouse model (Long et al., 2012). Nevertheless, high dose CBD selectively increased social interaction of *Nrg1* mutant mice, which are normally characterized by diminished investigative social behaviors (i.e., social withdrawal) at baseline. Furthermore, chronic CBD also increased GABAA receptor binding in the granular retrosplenial cortex of mutant mice suggesting that *Nrg1* may not only modulate neuro-behavioral actions of THC but also of CBD in a task- and brain region-specific manner. Further research using a variety of CBD doses thereby considering dose and age-effects will address the issue of varying or even opposing effects of different cannabinoids more comprehensively.

In summary, the transmembrane domain *Nrg1* mouse model has enabled the detailed analysis of acute vs. chronic effects of cannabinoids at different stages of brain development. *Nrg1* modulated the behavioral sensitivity of mice to cannabinoids differentially during adolescence and adulthood providing evidence for a role of *Nrg1*-cannabis interactions in schizophrenia. Furthermore, insights into the molecular and neurobiological mechanisms of *Nrg1*-cannabinoid interactions (involvement of CB1, 5-HT2A and NMDA receptors in particular) would not have been possible without utilizing these mouse mutants. Future research will extend on our initial findings and address sex specificity and the opposite effects of CB1 stimulation in adolescence (*Nrg1* mutant less susceptible) and adulthood (*Nrg1* mutants more susceptible) in greater detail. Finally, models of cannabinoid addiction should be considered given the significant comorbidity of schizophrenia and drug dependence.

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REFERENCES

Abe, Y., Namba, H., Zheng, Y., and Nawa, H. (2009). *In situ* hybridization reveals developmental regulation of ErbB1-4 mRNA expression in mouse midbrain: implication of ErbB receptors for dopaminergic neurons. *Neuroscience* 161, 95–110.

Allen, N. C., Bagade, S., McQueen, M. B., Ioannidis, J. P., Kavvoura, F. K., Khoury, M. J., et al. (2008). Systematic meta-analyses and field synopsis of genetic association studies in schizophrenia: the SzGene database. *Nat. Genet.* 40, 827–834.

Arnold, J. C., Boucher, A. A., and Karl, T. (2012). The yin and yang of cannabis-induced psychosis: the actions of delta 9-tetrahydrocannabinol and cannabidiol in rodent models of schizophrenia. *Curr. Pharm. Des.* 18, 5113–5130.

Bayer, T. A., Falkai, P., and Maier, W. (1999). Genetic and non-genetic vulnerability factors in schizophrenia: the basis of the "two hit hypothesis". *J. Psychiatr. Res.* 33, 543–548.

Behan, A. T., Hryniewiecka, M., O'Tuathaigh, C. M., Kinsella, A., Cannon, M., Karayiorgou, M., et al. (2012). Chronic adolescent exposure to delta-9-tetrahydrocannabinol in COMT mutant mice: impact on indices of dopaminergic, endocannabinoid and GABAergic pathways. *Neuropsychopharmacology* 37, 1773–1783.

Boucher, A. A., Arnold, J. C., Duffy, L., Schofield, P. R., Micheau, J., and Karl, T. (2007a). Heterozygous neuregulin 1 mice are more sensitive to the behavioural effects of Delta9-tetrahydrocannabinol. *Psychopharmacology (Berl.)* 192, 325–336.

Boucher, A. A., Hunt, G. E., Karl, T., Micheau, J., McGregor, I. S., and Arnold, J. C. (2007b). Heterozygous neuregulin 1 mice display greater baseline and Delta (9) - tetrahydrocannabinol - induced c-Fos expression. *Neuroscience* 149, 861–870.

Boucher, A. A., Hunt, G. E., Micheau, J., Huang, X., McGregor, I. S., Karl, T., et al. (2011). The schizophrenia susceptibility gene neuregulin 1 modulates tolerance to the effects of cannabinoids. *Int. J. Neuropsychopharmacol.* 14, 631–643.

Caspi, A., and Moffitt, T. E. (2006). Gene-environment interactions in psychiatry: joining forces with neuroscience. *Nat. Rev. Neurosci.* 7, 583–590.

Caspi, A., Moffitt, T. E., Cannon, M., McClay, J., Murray, R., Harrington, H., et al. (2005). Moderation of the effect of adolescent-onset cannabis use on adult psychosis by a functional polymorphism in the catechol-O-methyltransferase gene: longitudinal evidence of a gene X environment interaction. *Biol. Psychiatry* 57, 1117–1127.

Chesworth, R., Downey, L., Logge, W., Killcross, S., and Karl, T. (2012a). Cognition in female transmembrane domain neuregulin 1 mutant mice. *Behav. Brain Res.* 226, 218–223.

Chesworth, R., Yulyaningsih, E., Cappas, E., Arnold, J., Sainsbury, A., and Karl, T. (2012b). The response of neuregulin 1 mutant mice to acute restraint stress. *Neurosci. Lett.* 515, 82–86.

Desbonnet, L., Waddington, J. L., and O'Tuathaigh, C. M. (2009). Mutant models for genes associated with schizophrenia. *Biochem. Soc. Trans.* 37, 308–312.

Duffy, L., Cappas, E., Lai, D., Boucher, A. A., and Karl, T. (2010). Cognition in transmembrane domain neuregulin 1 mutant mice. *Neuroscience* 170, 800–807.

Duffy, L., Cappas, E., Scimone, A., Schofield, P. R., and Karl, T. (2008). Behavioral profile of a heterozygous mutant mouse model for EGF-like domain neuregulin 1. *Behav. Neurosci.* 122, 748–759.

Geyer, M. A., Krebs-Thomson, K., Braff, D. L., and Swerdlow, N. R. (2001). Pharmacological studies of prepulse inhibition models of sensorimotor gating deficits in schizophrenia: a decade in review. *Psychopharmacology (Berl.)* 156, 117–154.

Han, S., Yang, B. Z., Kranzler, H. R., Oslin, D., Anton, R., Farrer, L. A., et al. (2012). Linkage analysis followed by association show NRG1 associated with cannabis dependence in African Americans. *Biol. Psychiatry* 72, 637–644.

Harrison, P. J., and Law, A. J. (2006). Neuregulin 1 and schizophrenia: genetics, gene expression, and neurobiology. *Biol. Psychiatry* 60, 132–140.

Henquet, C., Murray, R., Linszen, D., and Van Os, J. (2005). The environment and schizophrenia: the role of cannabis use. *Schizophr. Bull.* 31, 608–612.

Henquet, C., Rosa, A., Krabbendam, L., Papiol, S., Fananas, L., Drukker, M., et al. (2006). An experimental study of catechol-o-methyltransferase Val158Met moderation of delta-9-tetrahydrocannabinol-induced effects on psychosis and cognition. *Neuropsychopharmacology* 31, 2748–2757.

Karl, T., Burne, T. H., Van Den Buuse, M., and Chesworth, R. (2011). Do transmembrane domain neuregulin 1 mutant mice exhibit a reliable sensorimotor gating deficit? *Behav. Brain Res.* 223, 336–341.

Karl, T., Duffy, L., Scimone, A., Harvey, R. P., and Schofield, P. R. (2007). Altered motor activity, exploration and anxiety in heterozygous neuregulin 1 mutant mice: implications for understanding schizophrenia. *Genes. Brain Behav.* 6, 677–687.

Kofalvi, A. (2008). *Cannbinoids and the Brain.* New York, NY: Springer.

Lacroix-Fralish, M. L., Tawfik, V. L., Nutile-McMenemy, N., Harris, B. T., and Deleo, J. A. (2006). Differential regulation of neuregulin 1 expression by progesterone in astrocytes and neurons. *Neuron Glia Biol.* 2, 2227–2234.

Law, A. J., Shannon Weickert, C., Hyde, T. M., Kleinman, J. E., and Harrison, P. J. (2004). Neuregulin-1 (NRG-1) mRNA and protein in the adult human brain. *Neuroscience* 127, 125–136.

Long, L. E., Chesworth, R., Arnold, J. C., and Karl, T. (2010a). A follow-up study: acute behavioural effects of Delta(9)-THC in female heterozygous neuregulin 1 transmembrane domain mutant mice. *Psychopharmacology (Berl.)* 211, 277–289.

Long, L. E., Chesworth, R., Huang, X. F., McGregor, I. S., Arnold, J. C., and Karl, T. (2010b). A behavioural comparison of acute and chronic Delta9-tetrahydrocannabinol and cannabidiol in C57BL/6JArc mice. *Int. J. Neuropsychopharmacol.* 13, 861–876.

Long, L. E., Chesworth, R., Huang, X. F., McGregor, I. S., Arnold, J. C., and Karl, T. (2013). Transmembrane domain Nrg1 mutant mice show altered susceptibility to the neurobehavioural actions of repeated THC exposure in adolescence. *Int. J. Neuropsychopharmacol.* 16, 163–175.

Long, L. E., Chesworth, R., Huang, X. F., Wong, A., Spiro, A., McGregor, I. S., et al. (2012). Distinct neurobehavioural effects of cannabidiol in transmembrane domain neuregulin 1 mutant mice. *PLoS ONE* 7:e34129. doi: 10.1371/journal.pone.0034129

McGregor, I. S., and Arnold, J. C. (2007). Cannabis reward: biased towards the fairer sex? *Br. J. Pharmacol.* 152, 562–564.

Mei, L., and Xiong, W. C. (2008). Neuregulin 1 in neural development, synaptic plasticity and schizophrenia. *Nat. Rev. Neurosci.* 9, 437–452.

Munafo, M. R., Thiselton, D. L., Clark, T. G., and Flint, J. (2006). Association of the NRG1 gene and schizophrenia: a meta-analysis. *Mol. Psychiatry* 11, 539–546.

Neddens, J., and Buonanno, A. (2011). Expression of the neuregulin receptor ErbB4 in the brain of the rhesus monkey (*Macaca mulatta*). *PLoS ONE* 6:e27337. doi: 10.1371/journal.pone.0027337

O'Tuathaigh, C. M., Hryniewiecka, M., Behan, A., Tighe, O., Coughlan, C., Desbonnet, L., et al. (2010). Chronic adolescent exposure to Delta-9-tetrahydrocannabinol in COMT mutant mice: impact on psychosis-related and other phenotypes. *Neuropsychopharmacology* 35, 2262–2273.

Powell, C. M., and Miyakawa, T. (2006). Schizophrenia-relevant behavioural testing in rodent models: a uniquely human disorder? *Biol. Psychiatry* 59, 1198–1207.

Rapoport, J. L., Addington, A. M., Frangou, S., and Psych, M. R. (2005). The neurodevelopmental model of schizophrenia: update 2005. *Mol. Psychiatry* 10, 434–449.

Sanders, A. R., Duan, J., Levinson, D. F., Shi, J., He, D., Hou, C., et al. (2008). No significant association of 14 candidate genes with schizophrenia in a large European ancestry sample: implications for psychiatric genetics. *Am. J. Psychiatry* 165, 497–506.

Sheehan, T. P., Chambers, R. A., and Russell, D. S. (2004). Regulation of affect by the lateral septum: implications for neuropsychiatry. *Brain Res. Brain Res. Rev.* 46, 71–117.

Spencer, J. R., Darbyshire, K. M., Boucher, A. A., Kashem, M. A., Long, L. E., McGregor, I. S., et al. (2013). Novel molecular changes induced by Nrg1 hypomorphism and Nrg1-cannabinoid interaction in adolescence: a hippocampal proteomic study in mice. *Front. Cell. Neurosci.* 7:15. doi: 10.3389/fncel.2013.00015

Stadelmann, A. M., Roser, P., Arning, L., Gallinat, J., Epplen, J. T., and Juckel, G. (2010). Acute effects of delta9-tetrahydrocannabinol on the auditory evoked mismatch negativity are modulated by the NRG1 gene. *Pharmacopsychiatry* 43, 194–195.

Stefansson, H., Sigurdsson, E., Steinthorsdottir, V., Bjornsdottir, S., Sigmundsson, T., Ghosh, S., et al. (2002). Neuregulin 1 and susceptibility to schizophrenia. *Am. J. Hum. Genet.* 71, 877–892.

Sumner, B. E., Cruise, L. A., Slattery, D. A., Hill, D. R., Shahid, M., and Henry, B. (2004). Testing the validity of c-fos expression profiling to aid the therapeutic classification of psychoactive drugs. *Psychopharmacology (Berl.)* 171, 306–321.

Taylor, S. B., Markham, J. A., Taylor, A. R., Kanaskie, B. Z., and Koenig, J. I. (2011). Sex-specific neuroendocrine and behavioral phenotypes in hypomorphic Type II Neuregulin 1 rats. *Behav. Brain Res.* 224, 223–232.

Tosato, S., Dazzan, P., and Collier, D. (2005). Association between the neuregulin 1 gene and schizophrenia: a systematic review. *Schizophr. Bull.* 31, 613–617.

Van Den Buuse, M., Wischhof, L., Lee, R. X., Martin, S., and Karl, T. (2009). Neuregulin 1 hypomorphic mutant mice: enhanced baseline locomotor activity but normal psychotropic drug-induced hyperlocomotion and prepulse inhibition regulation. *Int. J. Neuropsychopharmacol.* 12, 1383–1393.

Van Os, J., Kenis, G., and Rutten, B. P. (2010). The environment and schizophrenia. *Nature* 468, 203–212.

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BDNF deficiency and young-adult methamphetamine induce sex-specific effects on prepulse inhibition regulation

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Brain-derived neurotrophic factor (BDNF) has been implicated in the pathophysiology of schizophrenia, yet its role in the development of specific symptoms is unclear. Methamphetamine (METH) users have an increased risk of psychosis and schizophrenia, and METH-treated animals have been used extensively as a model to study the positive symptoms of schizophrenia. We investigated whether METH treatment in BDNF heterozygous (HET) mutant mice has cumulative effects on sensorimotor gating, including the disruptive effects of psychotropic drugs. BDNF HETs and wildtype (WT) littermates were treated during young adulthood with METH and, following a 2-week break, prepulse inhibition (PPI) was examined. At baseline, BDNF HETs showed reduced PPI compared to WT mice irrespective of METH pre-treatment. An acute challenge with amphetamine (AMPH) disrupted PPI but male BDNF HETs were more sensitive to this effect, irrespective of METH pre-treatment. In contrast, female mice treated with METH were less sensitive to the disruptive effects of AMPH, and there were no effects of BDNF genotype. Similar changes were not observed in the response to an acute apomorphine (APO) or MK-801 challenge. These results show that genetically-induced reduction of BDNF caused changes in a behavioral endophenotype relevant to the positive symptoms of schizophrenia. However, major sex differences were observed in the effects of a psychotropic drug challenge on this behavior. These findings suggest sex differences in the effects of BDNF depletion and METH treatment on the monoamine signaling pathways that regulate PPI. Given that these same pathways are thought to contribute to the expression of positive symptoms in schizophrenia, this work suggests that there may be significant sex differences in the pathophysiology underlying these symptoms. Elucidating these sex differences may be important for our understanding of the neurobiology of schizophrenia and developing better treatments strategies for the disorder.

Keywords: methamphetamine, prepulse inhibition, schizophrenia, BDNF

INTRODUCTION

Schizophrenia is a debilitating neuropsychiatric disease, and despite numerous factors being associated with disease risk and pathophysiology, the etiology of the illness is poorly understood. This gap in our knowledge has hindered the development of new treatment strategies that target the positive, negative and cognitive symptoms on the illness. Altered brain-derived neurotrophic factor (BDNF) signaling has been associated with schizophrenia through post-mortem, blood biomarker and genetic association studies (Angelucci et al., 2005). Post-mortem studies have consistently shown reduced BDNF expression in the hippocampus and dorso-lateral prefrontal cortex (DLPFC) of patients with schizophrenia, two brain regions that are highly implicated in the pathophysiology of the disorder (Takahashi et al., 2000; Weickert et al., 2003; Hashimoto et al., 2005; Wong et al., 2010; Thompson Ray et al., 2011). These findings have lead to numerous studies aiming to asses peripheral BDNF as a biomarker for the illness, and a recent meta-analysis found a moderate decrease in blood BDNF levels in drug-naïve and medicated schizophrenia patients (Green et al., 2011). Others have shown an association between the BDNF gene single nucleotide polymorphism (SNP) *val66met* and schizophrenia in some populations (Neves-Pereira et al., 2005; Gratacos et al., 2007). Although this has not been replicated in other population and genome-wide association studies, other groups have shown associations in patients with schizophrenia between the SNP and age of onset (Numata et al., 2006; Chao et al., 2008; Zhou et al., 2010), cognitive performance (Ho et al., 2006; Rybakowski et al., 2006; Kebir et al., 2009; Lu et al., 2012), and neuroimaging measures (Szeszko et al., 2005; Koolschijn et al., 2010; Smith et al., 2012). Interestingly, there are major sex-differences in the age of onset of schizophrenia and also symptom severity, with males showing earlier onset of disease and greater cognitive deficits (Hafner et al., 1993; Goldstein et al., 1998). It has recently been proposed that altered BDNF signaling in the disorder may contribute to these sex differences (Hill, 2012).

BDNF is important for neuronal differentiation and survival during early brain development, and has further roles in synaptic plasticity in the adult brain, however it is unclear how altered BDNF signaling may contribute to disruption of behavior related to the symptoms observed in schizophrenia. BDNF knock-out mice have a severe phenotype and typically do not survive beyond 3 weeks of age, whereas the BDNF heterozygous (HET) mice exhibit a more subtle phenotype and normal survival. We have previously shown that BDNF protein levels are reduced by a third to a half of WT expression levels throughout the brain in BDNF HETs (Hill and van den Buuse, 2011). This is similar to the reduction of BDNF protein observed in schizophrenia (Weickert et al., 2003), therefore these animals can be used as a model to examine the role of BDNF in behaviors related to schizophrenia. Indeed, this strain has been extensively characterized during the last decade in behavioral endophenotypes related to psychiatric disorders, with most studies showing little disturbance in baseline behavior, making them appropriate for experiments examining the effects of additional environmental manipulations. We have recently shown that male BDNF HETs show an increased vulnerability to the effects of young-adult corticosterone treatment, which caused a deficit in short-term spatial memory in a Y-maze task with parallel alterations in hippocampal NMDA receptor subunit expression (Klug et al., 2012). Others have demonstrated that BDNF HETs show altered sensitivity to the effects of stress and antidepressant treatments in paradigms testing anxiety and depressive-like behavior (Saarelainen et al., 2003; Duman et al., 2007, 2008; Ibarguen-Vargas et al., 2009). These findings support the notion that reduced BDNF expression, as is observed in schizophrenia, may alter an individual's sensitivity to the effects of other environmental risk factors (Caspi and Moffitt, 2006).

Drug abuse is one environmental factor that has been associated with increased risk of neuropsychiatric illnesses (Gururajan et al., 2012), and methamphetamine (METH) users have an increased risk of psychosis and schizophrenia (McKetin et al., 2010; Callaghan et al., 2012). While not all users will experience long-lasting psychosis, those who do tend to have an earlier age of first METH use, and increased familial risk of schizophrenia (Chen et al., 2003, 2005). These findings suggest that young adulthood may be a period of particular vulnerability, and that genetic factors also contribute to an individual's risk. One group has shown that the BDNF *val66met* SNP is associated with psychosis in Chinese METH users but not those from other ethnicities (Sim et al., 2010). Interestingly, this study found that METH dependence was associated with the *val* allele, while psychosis in METH users was associated with the *met* allele that has previously been linked with schizophrenia risk and disease outcomes. In animal models, BDNF expression has been shown to change following acute and repeated treatment with amphetamines (Le Foll et al., 2005; Angelucci et al., 2007), and rodent models with genetically modified expression of BDNF, including BDNF HETs, show altered sensitivity to a number of stimulant drugs (Hall et al., 2003; Bahi et al., 2008; Saylor and McGinty, 2008).

The aim of the current study was to examine the long-term effects of chronic METH treatment in BDNF HETs during young-adulthood on a behavioral endophenotype related to the positive symptoms of schizophrenia, prepulse inhibition (PPI) of the acoustic startle reflex. We predicted that METH administration in an escalating dosing regime during young adulthood would cause a disruption of PPI in adulthood, and given that BDNF has been implicated in the actions of METH previously, that BDNF HETs may respond to this treatment differentially. Given that BDNF levels are reduced in schizophrenia, BDNF HETs may be more likely to show a disturbance of PPI following METH exposure. PPI is a measure of sensorimotor gating, a form of information processing that is disrupted in patients with schizophrenia. Reduced PPI in schizophrenia is associated with thought disorder and functional impairment (Perry and Braff, 1994; Swerdlow et al., 2006), and deficits in PPI are considered to be a valid endophenotype related to the positive symptoms of the illness. PPI is one of the most widely used behavioral tests in mouse models related to schizophrenia, due to its high construct validity across species compared to other behavioral measures relevant to the disorder (Powell et al., 2009). The neuropharmacology of PPI has been characterized extensively in humans and rodent models, implicating dopaminergic, serotonergic, and glutamatergic signaling pathways (Geyer et al., 2001). Acute challenge with psychotropic drugs that target these neurotransmitter systems causes a disruption of PPI similar to what is observed in schizophrenia (van den Buuse, 2010). Our laboratory and others routinely use the psychotropic drugs amphetamine (AMPH), apomorphine (APO), and MK-801 to investigate the neuropharmacology of behavioral endophenotypes relevant to the positive symptoms of schizophrenia (Chavez et al., 2009). Acute AMPH can mimic behavioral changes observed in schizophrenia, largely due to increasing subcortical release of dopamine, similar to what is thought to cause psychotic episodes in schizophrenia (Abi-Dargham et al., 1998). Challenge with the D1/D2 receptor agonist APO can also be used to further investigate dopaminergic regulation of PPI. In contrast, the NMDA receptor antagonist MK-801 is used as an acute drug challenge model of the "glutamate hypofunction" hypothesis of schizophrenia (Lahti et al., 1995). By examining PPI during adulthood at baseline and following challenge with AMPH, APO and MK-801, we aimed to understand how BDNF depletion and METH treatment during young adulthood may interact to affect the regulation of this information processing mechanism, and whether neurotransmitter systems related to the pathophysiology of schizophrenia may be involved in their effects.

RESULTS

EFFECTS OF CHRONIC METH TREATMENT ON BODY WEIGHT AND LOCOMOTOR ACTIVITY DO NOT DIFFER BETWEEN BDNF HETs AND WT LITTERMATES

BDNF HETs of both sexes were heavier than their wildtype (WT) littermates throughout the treatment and behavioral testing periods [**Figure 1B**, main effect genotype $F_{(1,\ 66)} = 25.6, p < 0.001$]. In addition, during the treatment period female mice treated with METH did not show the same bodyweight increase as females treated with saline [first day of treatment vs. last day of treatment: sex × METH treatment × time-point interaction: $F_{(1,\ 66)} = 4.9, p = 0.031$; females only: METH treatment × time-point $F_{(1,\ 33)} = 5.7, p = 0.023$]. METH-treated female mice showed significant recovery of bodyweight gain during the weeks

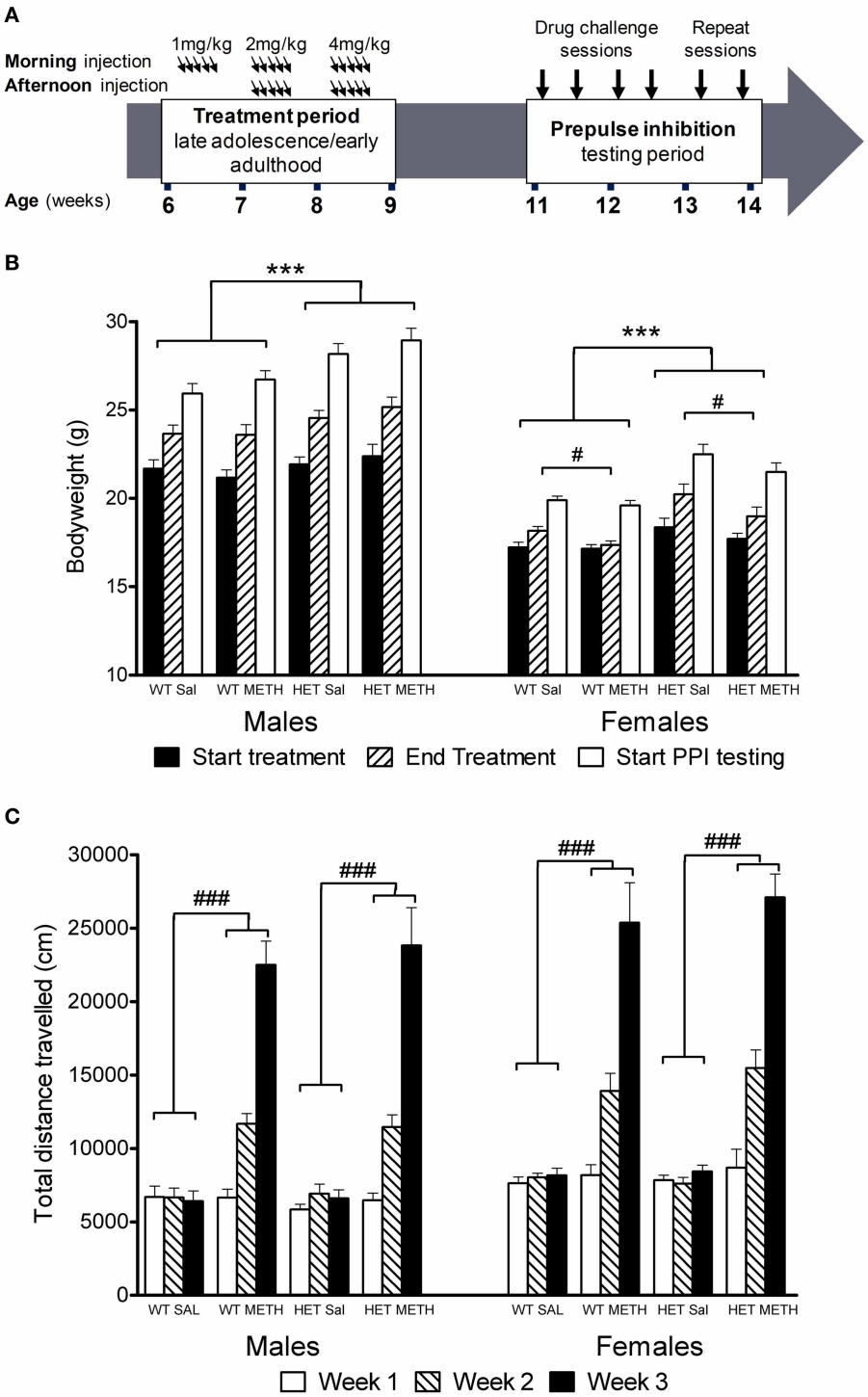

FIGURE 1 | Experimental timeline, bodyweight change, and locomotor activity during the treatment period. (A) Mice were treated with saline or an escalating dosing regime of METH from 6 to 9 weeks of age, i.e., during late adolescence/early adulthood. Following a 2-week break, the animals were tested in four PPI sessions following challenge treatment with either saline, AMPH, APO, or MK-801 in pseudorandomized order. **(B)** Bodyweight at the start or end of the chronic METH/saline treatment and at the start of PPI testing. BDNF HETs were significantly heavier than their WT littermates throughout the experiment. At the end of the chronic treatment period, METH-treated females had reduced bodyweight compared to saline-treated mice. **(C)** Locomotor activity recorded at the end of each subsequent treatment week following morning injections. METH-treated mice showed significant hyperactivity compared to saline-treated mice, which increased during the treatment period with increasing doses of METH administration. This effect did not differ between the genotypes. ***Signifies genotype effect $p < 0.001$, #signifies METH × time-point interaction $p < 0.05$, ###signifies METH × week interaction $p < 0.001$.

following the end of the treatment period despite their bodyweight remaining lower than saline-treated female mice during the remainder of the experimental period [weekly bodyweight in the 5 weeks following the end of the treatment period, sex × METH treatment interaction $F_{(1, 66)} = 4.6$, $p = 0.037$]. There was no body weight gain delay in male BDNF HETs. Importantly, METH treatment did not result in bodyweight *loss* in any of the groups.

METH administration during the 3-week treatment period caused significant hyperactivity [**Figure 1C**, main effect of METH: $F_{(1, 53)} = 208.0$, $p < 0.001$] which became more prominent over the escalating dosing period [METH × treatment week interaction: $F_{(2, 106)} = 189.7$, $p < 0.001$]. At the end of the first treatment week, there was no effect of the 1 mg/kg dose on locomotor activity. Subsequently, however, the animals showed increasing levels of hyperactivity in response to the higher doses of METH administered during the second and third weeks of the treatment period. There were no differences between male and female mice or between BDNF HETs and WT mice in the extent of METH-induced hyperactivity during the treatment period (**Figure 1C**).

REDUCED BASELINE PPI AND INCREASED STARTLE REACTIVITY IN BDNF HETs

Baseline PPI, obtained after injection of saline, was significantly lower in BDNF HET mice compared to WT [**Figure 2A**, main effect of genotype at 30 ms inter-stimulus interval (ISI) $F_{(1, 66)} = 13.1$, $p = 0.001$; 100 ms ISI: $F_{(1, 66)} = 8.8$, $p = 0.004$]. This effect was independent of METH treatment or the sex of the animals (**Figure 2A**).

Average startle across the saline session was significantly higher in male mice compared to female mice [**Figure 2B**, main effect

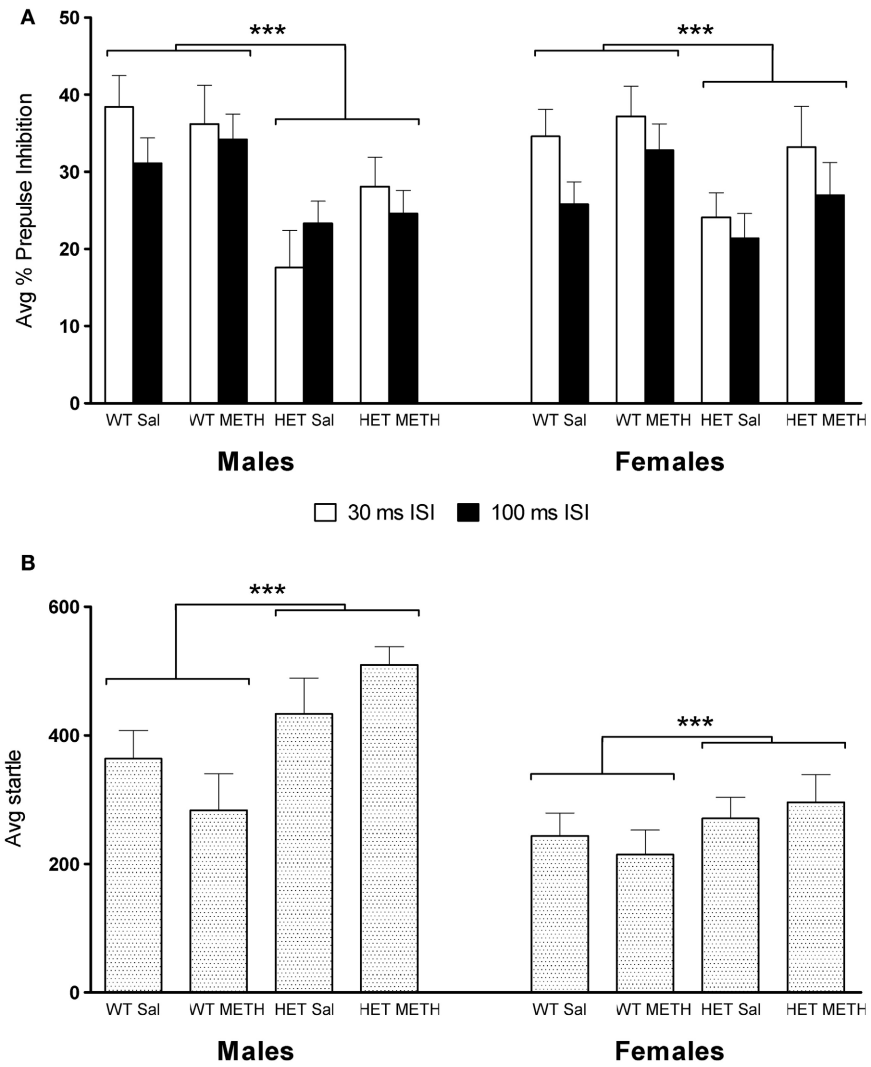

FIGURE 2 | PPI and startle reflex at baseline during saline challenge sessions. (A) Average PPI across all four PP intensities was significantly lower in BDNF HETs compared to WT controls. This effect was seen at both the 30 and 100 ms ISI and in both sexes. **(B)** Average startle responses across all four startle blocks were significantly increased in BDNF HETs of both sexes compared to WT controls. ***Signifies genotype effect $p < 0.005$.

of sex: $F_{(1, 66)} = 21.6$, $p < 0.001$]. In addition, baseline startle was significantly higher in BDNF HETs compared to WT controls [main effect of genotype $F_{(1, 66)} = 11.1, p = 0.001$]. In male mice, but not female mice, habituation of startle across the 4 blocks of stimulus-only pulses was slightly accelerated in BDNF HETs compared to WT controls [data not shown; startle block × genotype × sex interaction: $F_{(3, 198)} = 4.7, p = 0.005$].

SEX-DEPENDENT CHANGES IN THE EFFECT OF AMPH ON PPI AND STARTLE REACTIVITY

AMPH administration caused a significant disruption of PPI at both ISIs [**Figures 3A–D**, main effect, 30 ms ISI: $F_{(1, 66)} = 124.0$, $p < 0.001$, 100 ms ISI: $F_{(1, 66)} = 87.8$, $p < 0.001$]. However, at the 30ms ISI, there were significant sex-dependent effects of genotype and METH treatment on the response to AMPH challenge [AMPH × genotype × sex × prepulse (PP) interaction: $F_{(3, 198)} = 3.5$, $p = 0.026$] and further analysis was therefore done on the data separated by the sex of the animals. In male BDNF HET, irrespective of METH pre-treatment, PPI was disrupted to a greater extent by an acute AMPH challenge than in male WT, particularly at higher PP intensities [**Figure 3A**, AMPH × genotype × PP interaction: $F_{(3, 99)} = 4.3$, $p = 0.013$, see also **Table 1** for PPI at each PP intensity]. In contrast, female mice treated with METH showed a reduced sensitivity to the disruptive effects of an acute AMPH challenge, irrespective of the genotype [**Figure 3B**, interaction of AMPH × METH treatment: $F_{(1, 33)} = 4.6, p = 0.039$, see also **Table 2** for PPI at each PP intensity]. At the 100 ms ISI, there was a strong trend for reduced sensitivity to the disruptive effects of AMPH in METH-treated mice of both sexes (**Figures 3C,D**, AMPH × METH treatment interaction $p = 0.063$).

AMPH administration caused a reduction in startle amplitude compared to saline [**Figures 3E,F**, $F_{(1, 66)} = 29.9$, $p < 0.001$]. However, this effect of AMPH was reduced by prior METH treatment in a genotype-specific manner [AMPH × genotype × METH treatment interaction $F_{(1, 66)} = 4.8$, $p = 0.031$]. Irrespective of the sex of the animals, WT mice treated with METH did not show a reduction of startle in response to acute AMPH [WT only: AMPH × METH treatment interaction, $F_{(1, 36)} = 4.5$, $p = 0.042$]. This effect of METH pre-treatment was not observed in BDNF HETs.

NO MAJOR CHANGES IN THE EFFECTS OF APO IN BDNF HETs OR FOLLOWING METH TREATMENT

Given that the major effects of AMPH on PPI in mice are thought to be due to indirect activation of dopaminergic receptors, we were interested to examine whether the D1/D2 receptor agonist APO would show similar effects to AMPH. APO caused a significant disruption of PPI [**Figures 4A–D**, at both ISIs: main effect of APO challenge: $F_{(1, 66)} > 85.0, p < 0.001$]. In contrast to the sex-dependent effects of genotype and METH treatment that were observed in the sensitivity to AMPH, similar effects were not observed in the response to APO. A minor, but significant change in APO sensitivity was found as a PP-dependent genotype effect in male mice, where BDNF HETs were slightly less sensitive to the effects of APO, but only at the lowest PP intensity examined [30 ms ISI: APO challenge × genotype × sex × PP interaction $F_{(3, 198)} = 3.1, p = 0.037$; **Table 1**, PP2dB only: APO challenge × genotype × sex interaction $F_{(1, 66)} = 5.0, p = 0.029$].

APO also caused a significant reduction in startle response amplitude (**Figures 4E,F**). However, this effect was significantly larger in BDNF HETs [main effect of APO challenge: $F_{(1, 66)} = 65.9, p < 0.001$; APO × genotype interaction: $F_{(1, 66)} = 6.5, p = 0.013$], and this effect acted to normalize their startle response to that of WT animals treated with APO (APO only: main effect genotype $p = 0.154$).

NO MAJOR CHANGES IN THE EFFECTS OF MK-801 IN BDNF HETs OR FOLLOWING METH TREATMENT

MK-801 caused a significant disruption of PPI [at both ISIs: main effect of MK-801: $F_{(1, 66)} > 38.0, p < 0.001$]. There were no significant changes in the response to MK-801 in BDNF HETs or following METH treatment (**Figures 5A–D**). There was no significant effect of MK-801 administration on startle amplitudes (**Figures 5E,F**).

DISCUSSION

The key findings of the current study were that (1) compared to WT controls, BDNF HETs showed reduced baseline PPI, irrespective of METH pre-treatment or the sex of the animals; (2) male BDNF HETs showed greater sensitivity to the disruptive effects of an AMPH challenge on PPI at the 30 ms ISI, irrespective of whether they had been pre-treated with METH; (3) female mice pre-treated with METH showed a relative tolerance to the effects of AMPH on PPI at the 30 ms ISI, irrespective of their genotype. Thus, there were no interactions between METH treatment and BDNF depletion on PPI, and no changes were observed in the PPI-disrupting effects of APO and MK-801 in this model. Startle response amplitude was higher in BDNF HETs compared to WT controls, which may be in part due to their increased bodyweight. AMPH and APO reduced startle reactivity, and there were significant genotype effects. METH treatment attenuated the effects of AMPH on startle in WT mice but not BDNF HETs, while the effects of APO on startle amplitude were greater in BDNF HETs.

The main aim of the present study was to investigate whether BDNF deficiency, as present in BDNF HETs, would alter the long-term effects of chronic METH treatment on PPI. To this end, we developed a 3-week long escalating METH dosing regime, using a similar range of doses and frequency of injections to other escalating METH treatments in mice in the literature (Chao et al., 2012; Pogorelov et al., 2012). This METH treatment protocol caused significant hyperactivity during the treatment period. Unlike many previous studies of the effects of METH on PPI, behavior was examined after a 2-week break period, as we aimed to model the long term psychotic symptoms that occur in some METH users, rather than transient psychosis following METH which is probably less relevant to the pathophysiology of schizophrenia. For this reason, it is not surprising that our results differ from those of previous studies that assessed the immediate effects of METH on PPI. In experiments where PPI is tested following repeated pre-treatment with METH, this was found to disrupt PPI immediately following the final METH treatment. However METH pre-treated, non-drug challenged controls were not used in these experiments (Arai et al., 2008; Hadamitzky et al.,

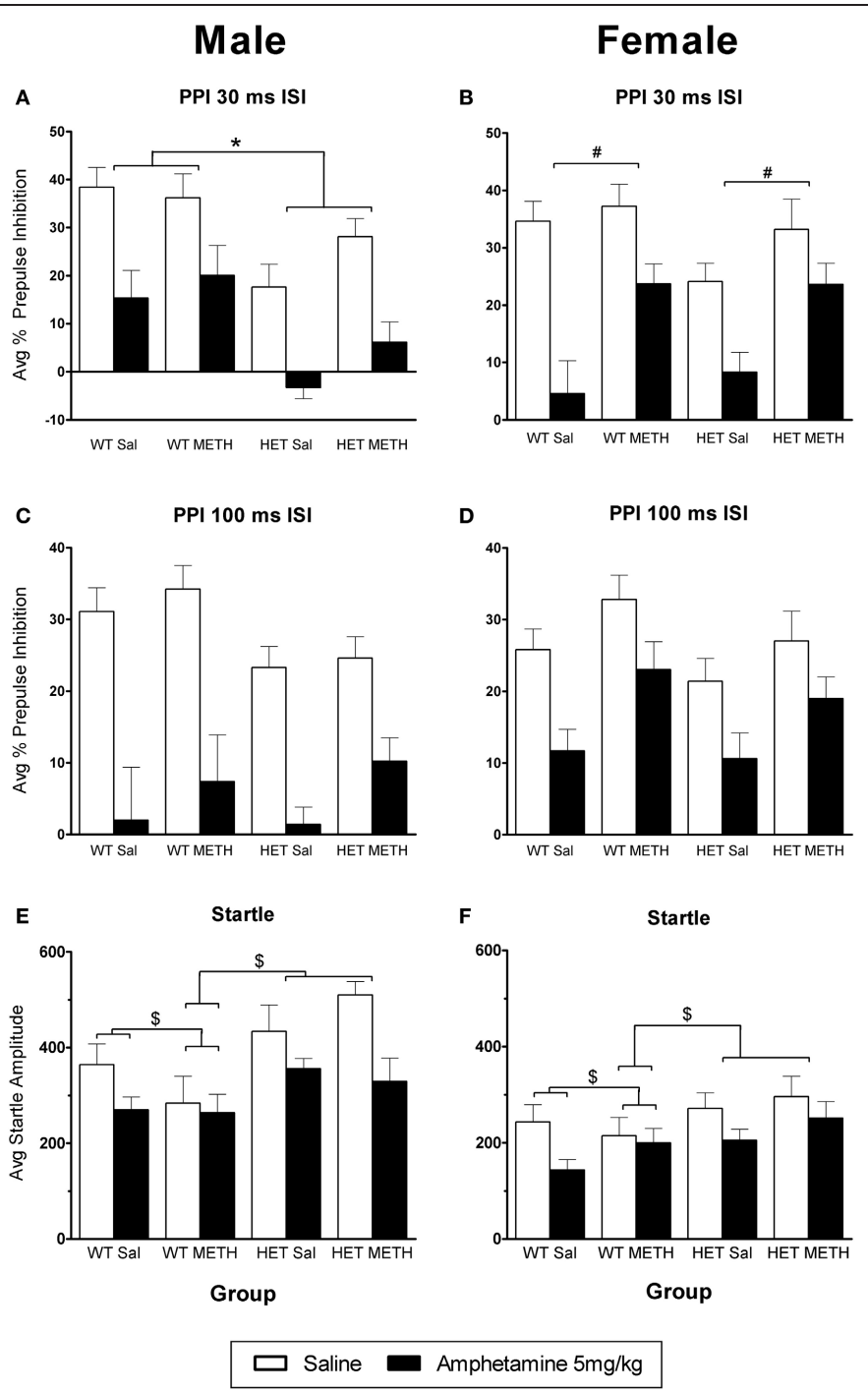

FIGURE 3 | AMPH-induced disruption of PPI. (A,B) At the 30 ms ISI, following an AMPH challenge, PPI was disrupted in all groups, however the effect of AMPH was enhanced in male BDNF HETs **(A)**, and was attenuated in female mice treated with METH **(B)**. **(C,D)** At the 100 ms ISI, following an AMPH challenge PPI was disrupted in all groups, however this was not significantly different between the treatment groups. **(E,F)** Startle responses following AMPH challenge were reduced, however this effect was not seen in WT mice treated with METH. *Signifies genotype × AMPH × PP interaction $p < 0.05$, #signifies METH treatment × AMPH interaction $p < 0.05$, $signifies AMPH × genotype × METH treatment interaction $p < 0.031$.

2011). One group has recently examined the effects of escalating METH treatment on PPI in mice after a break period, and found reduced PPI at baseline that could be rescued by electroconvulsive shock treatment (Chao et al., 2012). However there are major strain differences in PPI (Willott et al., 2003), and this study used CD-1 mice which may account for the differences in our findings. Additionally, while we calculated baseline PPI from saline challenge sessions, this previous study examined PPI

Table 1 | Mean PPI ± SEM at each PP intensity during challenge sessions with saline, AMPH, APO or MK-801 treatment in male WT and BDNF HET mice chronically pre-treated with saline or METH.

	30 ms ISI					100 ms ISI				
	PP2	PP4	PP8	PP16	Average	PP2	PP4	PP8	PP16	Average
MALE WT SALINE										
Saline	19.7 ± 7.5	21.4 ± 7.1	44.9 ± 5.5	67.6 ± 3.9	38.4 ± 4.1	24.8 ± 5.0	25.4 ± 4.9	23.9 ± 4.0	50.5 ± 3.8	31.1 ± 3.3
AMPH	−15.5 ± 8.8	1.5 ± 7.6	24.9 ±7.6	50.5 ± 5.1	15.3 ± 5.8	−4.9 ± 9.1	−2.7 ± 4.6	12.2 ± 3.8	31.4 ± 4.2	2.0 ± 7.4
APO	−28.8 ± 10.5	1.9 ± 5.7	22.8 ± 8.4	46.3 ± 4.9	10.5 ± 4.6	1.5 ± 3.3	−4.2 ± 6.3	13.1 ± 3.3	34.9 ± 4.3	11.3 ± 2.8
MK-801	0.5 ± 7.9	6.7 ± 4.7	23.7 ± 5.3	45.1 ± 4.4	19.0 ± 3.8	−5.6 ± 7.3	−15.4 ± 14.9	−2.4 ± 10.1	31.4 ± 4.2	2.0 ± 7.4
MALE WT METH										
Saline	5.1 ± 10.5	23.5 ± 5.9	44.7 ± 5.6	71.7 ± 2.4	36.2 ± 5.0	14.0 ± 6.3	27.9 ± 4.4	37.5 ± 4.1	57.6 ± 4.4	34.2 ± 3.3
AMPH	−20.4 ± 13.7	1.0 ± 10.6	38.1 ± 7.8	61.4 ± 4.6	20.0 ± 6.3	−10.8 ± 8.9	6.6 ± 7.5	28.1 ± 5.7	31.8 ± 6.4	7.4 ± 6.5
APO	−13.2 ± 12.7	2.5 ± 10.2	24.6 ± 12.6	45.3 ± 11.6	14.8 ± 10.2	−3.6 ± 8.1	4.4 ± 8.6	31.9 ± 8.1	41.1 ± 4.4	18.5 ± 5.8
MK-801	0.6 ± 8.8	15.4 ± 8.5	27.5 ± 7.8	42.6 ± 11.8	21.5 ± 8.2	−8.2 ± 8.5	−6.9 ± 10.2	12.9 ± 9.1	31.8 ± 6.4	7.4 ± 6.5
MALE BDNF HET SALINE										
Saline	4.6 ± 6.6	−1.6 ± 7.4	19.3 ± 6.5	48.0 ± 5.2	17.6 ± 4.8	15.5 ± 3.6	15.7 ± 2.9	20.5 ± 4.9	41.4 ± 6.2	23.3 ± 2.9
AMPH	−18.4 ± 7.5	−18.1 ± 4.4	−1.6 ± 6.4	24.9 ± 8.2	−3.3 ± 2.3	−8.8 ± 4.9	−9.1 ± 7.8	4.1 ± 5.2	19.7 ± 3.9	1.4 ± 2.4
APO	−1.4 ± 5.8	−6.8 ± 8.1	4.3 ± 10.0	21.4 ± 8.4	4.4 ± 6.9	0.7 ± 3.1	−2.5 ± 5.1	12.0 ± 3.6	21.1 ± 5.1	7.8 ± 3.5
MK-801	4.9 ± 6.1	3.1 ± 4.3	9.9 ± 5.5	35.2 ± 6.7	13.3 ± 3.8	−5.9 ± 8.5	3.9 ± 5.6	4.8 ± 9.4	28.3 ± 4.5	7.8 ± 5.1
MALE BDNF HET METH										
Saline	−3.5 ± 9.5	25.5 ± 4.9	32.2 ± 5.9	58.4 ± 4.4	28.1 ± 3.8	14.5 ± 3.3	23.3 ± 3.9	21.2 ± 4.1	39.3 ± 4.1	24.6 ± 3.0
AMPH	−7.3 ± 5.1	−0.9 ± 6.3	−4.2 ± 6.9	36.7 ± 6.5	6.1 ± 4.3	0.0 ± 3.3	3.1 ± 4.0	6.9 ± 5.7	30.9 ± 4.3	10.2 ± 3.3
APO	−14.8 ± 9.9	−3.7 ± 6.6	−0.5 ± 12.7	30.7 ± 5.2	2.9 ± 6.0	−5.1 ± 8.4	−6.5 ± 7.2	9.9 ± 6.6	30.8 ± 7.1	7.3 ± 4.5
MK-801	1.2 ± 7.0	−8.2 ± 8.0	4.9 ± 10.7	39.1 ± 5.8	9.3 ± 6.1	−24.2 ± 10.8	−17.5 ± 6.6	−0.2 ± 9.8	25.8 ± 6.4	−4.0 ± 6.5

Table 2 | Mean PPI ± SEM at each PP intensity during challenge sessions with saline, AMPH, APO, or MK-801 treatment in female WT and BDNF HET mice chronically pre-treated with saline or METH.

	30 ms ISI					100 ms ISI				
	PP2	PP4	PP8	PP16	Average	PP2	PP4	PP8	PP16	Average
FEMALE WT SALINE										
Saline	7.8 ± 7.8	28.8 ± 3.7	39.7 ± 5.8	62.0 ± 3.3	34.6 ± 3.5	6.3 ± 7.4	16.2 ± 4.3	31.1 ± 5.1	49.5 ± 3.6	25.8 ± 2.9
AMPH	−12.7 ± 7.3	−6.6 ± 5.0	8.5 ± 8.2	29.2 ± 7.6	4.6 ± 5.7	−7.1 ± 6.2	−4.4 ± 7.9	10.4 ± 8.6	27.8 ± 5.5	11.7 ± 3.0
APO	−17.1 ± 8.7	−9.5 ± 8.7	18.3 ± 8.8	33.0 ± 5.5	6.2 ± 5.5	−12.6 ± 3.6	2.2 ± 5.9	15.0 ± 5.7	25.5 ± 7.7	7.5 ± 4.1
MK-801	−1.0 ± 4.6	14.4 ± 3.7	16.3 ± 6.2	43.7 ± 5.6	18.4 ± 4.0	−3.5 ± 5.3	9.1 ± 3.3	13.5 ± 4.1	27.8 ± 5.5	11.7 ± 3.0
FEMALE WT METH										
Saline	18.4 ± 7.3	27.5 ± 4.5	39.8 ± 5.3	63.2 ± 2.6	37.2 ± 3.9	18.4 ± 4.7	26.8 ± 4.9	35.7 ± 3.5	50.1 ± 4.0	32.8 ± 3.4
AMPH	3.3 ± 6.4	5.9 ± 5.3	37.6 ± 4.2	48.0 ± 3.4	23.7 ± 3.5	3.5 ± 4.2	15.5 ± 5.4	25.3 ± 6.4	47.8 ± 4.5	23.0 ± 3.9
APO	5.4 ± 6.0	9.8 ± 8.1	26.5 ± 7.5	44.2 ± 6.3	21.5 ± 5.4	−3.5 ± 7.7	9.5 ± 3.2	16.9 ± 6.5	38.1 ± 4.7	15.3 ± 3.9
MK-801	11.5 ± 5.3	14.6 ± 6.4	29.8 ±5.4	50.4 ± 3.9	26.6 ± 3.8	3.0 ± 6.1	7.5 ± 4.0	24.9 ± 6.2	43.0 ± 5.2	19.6 ± 4.2
FEMALE BDNF HET SALINE										
Saline	13.3 ± 6.3	16.1 ± 4.4	26.0 ± 3.2	40.9 ± 4.9	24.1 ± 3.2	8.3 ± 4.9	18.9 ± 4.5	23.6 ± 3.9	34.8 ± 4.5	21.4 ± 3.2
AMPH	−3.5 ± 3.9	−0.1 ± 6.0	5.2 ± 6.3	31.6 ± 4.4	8.3 ± 3.5	5.5 ±3.8	1.5 ± 6.8	8.4 ± 4.4	26.9 ± 3.0	10.6 ± 3.6
APO	−25.4 ± 5.7	−12.4 ± 7.0	1.1 ± 8.2	20.2 ± 9.0	−4.1 ± 5.7	−15.0 ± 5.0	2.3 ± 6.7	10.1 ± 7.1	16.9 ± 6.7	3.6 ± 4.1
MK-801	0.4 ± 5.3	12.3 ± 7.6	26.4 ± 6.2	33.9 ± 8.6	18.2 ± 4.6	7.9 ± 3.6	5.5 ± 9.7	7.0 ± 9.2	28.3 ± 6.9	12.2 ± 6.0
FEMALE BDNF HET METH										
Saline	21.0 ± 9.5	23.7 ± 8.6	29.4 ± 4.4	58.5 ± 4.0	33.2 ± 5.3	17.0 ± 6.8	19.8 ± 4.7	29.8 ± 3.9	41.4 ± 5.0	27.0 ± 4.2
AMPH	3.7 ± 5.3	16.9 ± 8.2	28.1 ± 5.0	45.7 ± 5.6	23.6 ± 3.7	8.7 ± 4.1	7.9 ± 4.5	20.5 ± 4.8	38.8 ± 4.1	19.0 ± 3.0
APO	−8.1 ± 7.9	−6.8 ± 14.5	6.2 ± 8.5	35.0 ± 6.1	6.6 ± 8.2	−11.4 ± 8.5	1.8 ± 7.6	0.6 ± 5.6	20.8 ± 6.6	3.0 ± 6.0
MK-801	8.5 ± 8.5	7.8 ± 8.0	25.5 ± 5.9	44.3 ± 6.2	21.6 ± 5.5	5.5 ± 7.8	4.1 ± 6.5	20.1 ± 4.8	30.4 ± 6.2	15.0 ± 4.7

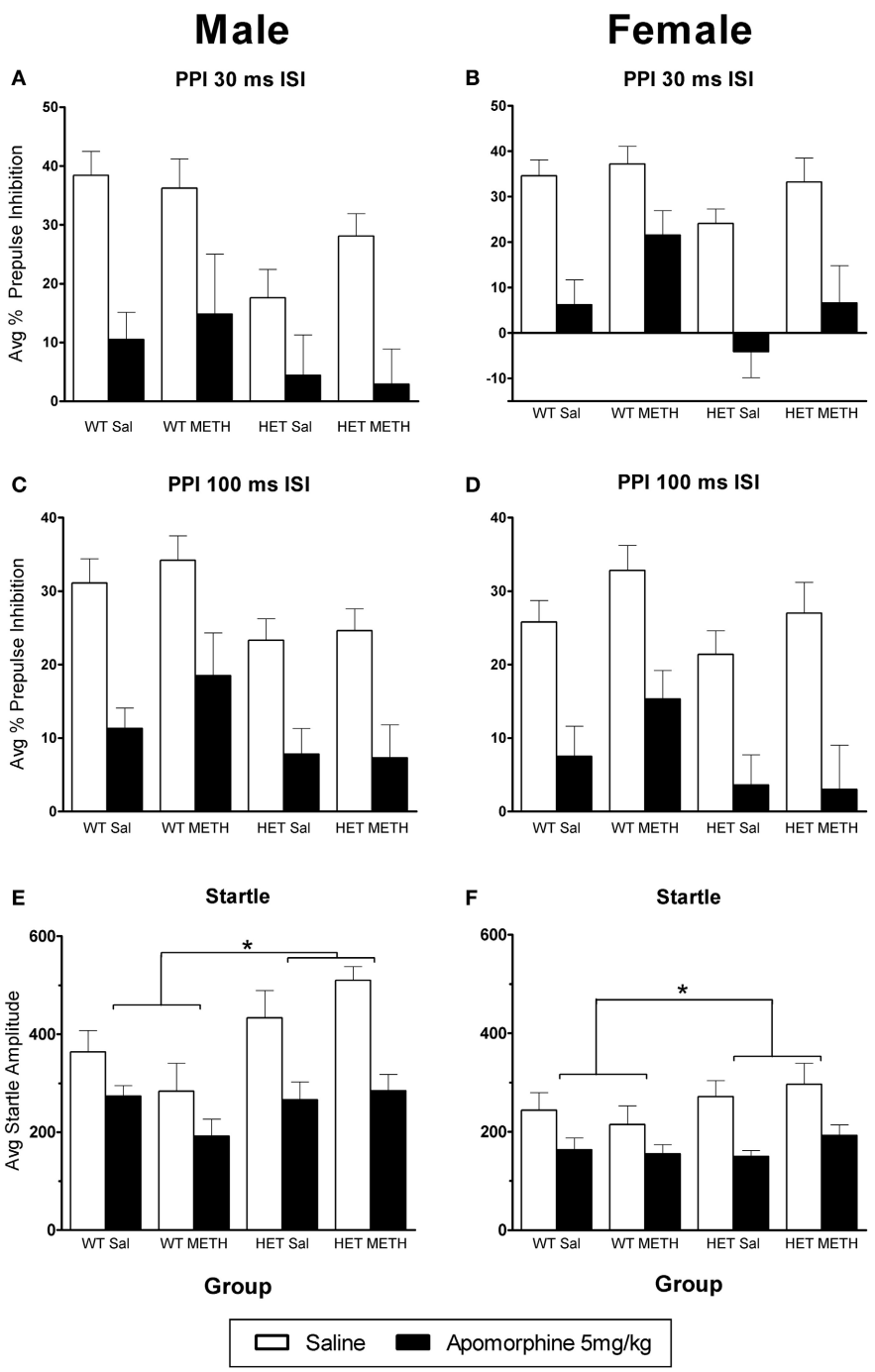

FIGURE 4 | APO-induced disruption of PPI. (A–D) PPI following an APO challenge in male **(A)** and female **(B)** mice at the 30 ms ISI and male **(C)** and female **(D)** mice at the 100 ms ISI. APO disrupted PPI in all groups, and this was unaffected by BDNF genotype or METH treatment. **(E,F)** Startle responses following an APO challenge were reduced; however this effect was greater in BDNF HETs. *Signifies genotype × APO interaction $p < 0.05$.

without challenge injections. The acute mild stress associated with saline injection has previously been shown to reduce PPI in some mouse strains (Wang et al., 2003), as increased stress hormone levels can alter PPI (van den Buuse et al., 2004). Given these differences between previous studies and our own, this highlights the importance of strain, timing following METH treatment and behavioral testing protocol on the effects of METH in rodent studies.

Our finding of reduced sensitivity or tolerance to a challenge dose of AMPH in METH pre-treated females is in contrast to a previous study, which showed that 1 week pre-treatment with low dose METH in male CD-1 mice enhanced the effects of

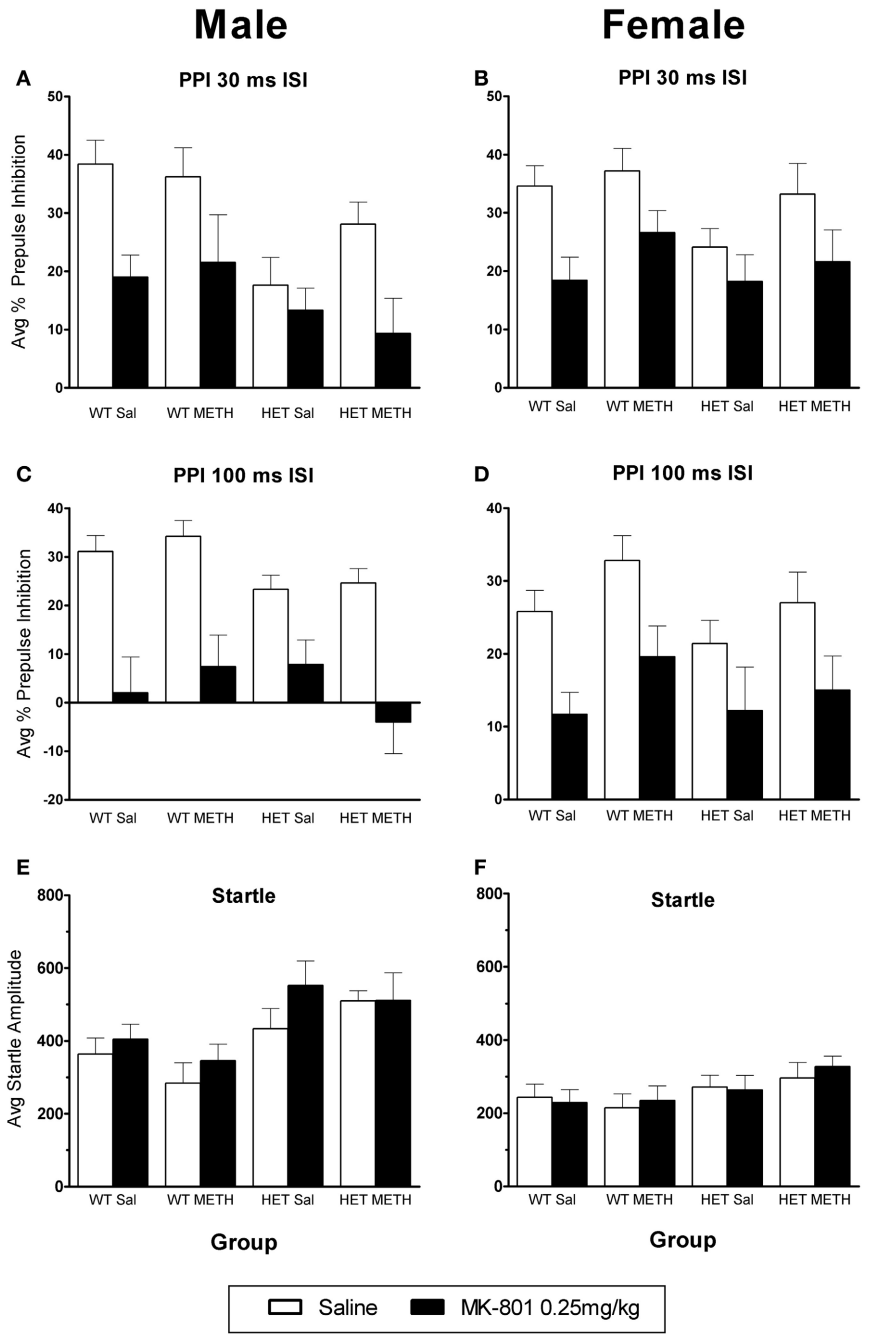

FIGURE 5 | MK-801-induced disruption of PPI. (A–D) PPI following an MK-801 challenge in male **(A)** and female **(B)** mice at the 30 ms ISI and male **(C)** and female **(D)** mice at the 100 ms ISI. MK-801 disrupted PPI in all groups, and this was unaffected by BDNF genotype or METH treatment. **(E,F)** Startle response following an MK-801 challenge. There was a trend toward increased startle reactivity following MK-801 challenge ($p = 0.081$).

a sub-threshold dose of METH (1 mg/kg) to disrupt PPI (Arai et al., 2008). However, given that our effects of METH were female-specific it is unclear how this previous result compares to our finding of tolerance. Furthermore we used a higher challenge dose of AMPH (5 mg/kg), and it may be of interest for future studies to examine sub-threshold doses of AMPH in our model. Another key difference between these studies was the inclusion of a break period in our own study, whereas "sensitization" was observed immediately followed the METH pre-treatment period (Arai et al., 2008). The aim of our study was to model behavioral changes accompanying persistent psychosis following METH abuse, whereas the findings of other studies which examine behavior immediately following METH exposure may be more reflective of what occurs during transient psychosis

following METH use (Sato et al., 1992). In contrast to our observation of tolerance to the effects of AMPH on PPI, in preliminary studies we have seen long-term locomotor sensitization to an AMPH challenge in this model (unpublished observations) which has also been shown previously by others following METH pre-treatment (Hall et al., 2008). This suggests that METH-induced plasticity occurring in the pathways regulating PPI is different to that which occurs in the pathways mediating locomotor sensitization. This may involve different contributions by monoamine neurotransmitters in these neural pathways.

The effects of BDNF depletion on PPI have also been examined previously. For example, in a previous study we did not observe baseline deficits in PPI in BDNF HETs (Klug et al., 2012). However, in that study, PPI was examined in animals that had not previously received chronic injections, and the response of BDNF HETs following a challenge drug may have been altered in the PPI paradigm immediately following that unfamiliar mild stressor. Forebrain restricted knockout of BDNF also resulted in no changes in baseline PPI in the absence of a challenge injection (Gorski et al., 2003). Our current observations of reduced baseline PPI in BDNF HETs irrespective of sex make this a novel model of sensorimotor gating deficits observed in schizophrenia, and future studies should aim to characterize the predictive validity of this finding by examining the response to current antipsychotic medications.

The significant effect of BDNF depletion on AMPH-disrupted PPI, but not on the effects of APO or MK-801, suggests differential changes in monoaminergic regulation of PPI pathways in BDNF HETs. Changes in the amount of dopamine release and reuptake following AMPH challenge, without changes in post-synaptic receptor expression, may explain the altered sensitivity to AMPH without major changes in the effects of the D1/D2 receptor agonist APO. Supporting this idea, dopamine levels (measured by HPLC and *in vivo* microdialysis) are increased in BDNF HETs in the caudate putamen, which is also associated with reduced dopamine transporter (DAT) function (Dluzen et al., 1999; Bosse et al., 2012). However these studies were conducted in male BDNF HETs, and further characterization of the dopaminergic system in both sexes may be necessary to understand the sex differences observed in our study. NMDA receptor-mediated regulation of PPI appears to be unaffected by BDNF depletion and METH treatment based on our findings of no changes in the sensitivity to the disruptive effects of MK-801.

It should be noted that, in addition to dopaminergic effects, changes in serotonergic activity may also play a role in altered sensitivity to the disruptive effects of AMPH on PPI in male BDNF HETs. Although the psychotropic actions of AMPH appear to be largely mediated by increasing subcortical dopamine release (Sulzer et al., 2005), one group has recently reported major contributions of both serotonergic and dopaminergic signaling in the effects of the active AMPH metabolite p-hydroxyamphetamine on PPI in mice (Onogi et al., 2010, 2011). Alterations in serotonin levels and transporter function have also been described in BDNF HETs in the CA3 region and ventral hippocampus (Daws et al., 2007; Deltheil et al., 2008; Guiard et al., 2008). The only study to assess sex differences in the serotonin system of BDNF HETs found that males, but not females, showed a deficit in serotonin transporter clearance rate in the CA3 at 2 months of age (Daws et al., 2007). Further characterization of the serotonin system in both sexes of BDNF HETs and in additional brain regions may clarify the involvement of serotonergic changes in the results that we have observed. We have previously shown that serotonergic lesions of the hippocampus in rats disrupt baseline PPI (Kusljic and van den Buuse, 2004), and it is possible that altered hippocampal serotonin system function in BDNF HETs may also contribute to changes in baseline PPI in both sexes.

Sex-differences in BDNF signaling have been reported extensively before (Wu et al., 2013), although information about sex-differences in behavior are somewhat lacking in the analysis of BDNF HETs. We previously demonstrated in male C57BL/6 mice that BDNF protein levels in the striatum and frontal cortex are correlated with serum testosterone levels during adolescent development, including a peak in BDNF protein levels at 8 weeks of age. In female mice, no such relationship was observed with serum estradiol levels (Hill et al., 2012). These findings suggest that BDNF is differentially involved in brain development during this period between the sexes, and may help to explain sex-specific changes in BDNF HETs. Specifically, if BDNF plays a differential role in the development of fronto-striatal circuits in male mice, this may result in altered monoaminergic regulation of PPI in male BDNF HETs, leaving these pathways spared in female BDNF HETs.

BDNF HETs also showed an increase in acoustic startle response. We observed increased bodyweight in these mice, which may have contributed to these effects. This has been described by others in BDNF HETs previously, and was associated with hyperphagia, hyperglycemia, insulin resistance, and hypoactivity (Lyons et al., 1999; Duan et al., 2003; Coppola and Tessarollo, 2004). In addition to these baseline startle differences, there were genotype effects on the action of AMPH and APO on startle response. Startle response in BDNF HETs was generally more affected by these drugs, with BDNF HETs being more sensitive to the effects of APO irrespective of METH pre-treatment, whereas only WT mice were insensitive to the effects of AMPH following METH treatment, and METH-treated HETs responded like saline pre-treated mice. All of these effects on startle were unaffected by sex of the animals. Therefore this is unlikely to have contributed to the changes in PPI that were observed, and in particular the changes in the sensitivity to the disruptive effects of AMPH which were sex-specific.

Our findings suggest that BDNF is not involved in the long term effects of METH treatment during young adulthood on PPI. In contrast, previous work has demonstrated that BDNF is involved in the acute neuronal response to METH and sensitization to cocaine (Narita et al., 2003; Bahi et al., 2008). These differences suggest that BDNF acts in discrete neural pathways following exposure to stimulants such as METH and cocaine, and plays a role in selective behavioral changes following these interventions. Interestingly there were large sex differences in the independent effects of both BDNF depletion and METH treatment during young adulthood. Sex differences in the role of BDNF during adolescent development of the forebrain may account for some of these effects (Hill et al., 2012). These findings highlight the need to study both sexes in preclinical research

into the effects of genetic and environmental factors on behavioral endophenotypes related to schizophrenia, something which is severely lacking in the current literature on the behavioral effects of METH. The current findings warrant further investigation into the sex-differences that result following BDNF depletion and METH treatment separately, but do not exclude the value of further research into the interactions between BDNF signaling and METH-induced dysfunction in other behavioral paradigms, such as those related to the negative and cognitive symptoms of schizophrenia.

In summary, we have demonstrated that BDNF depletion and METH treatment do not have interactive effects on PPI of the acoustic startle reflex, or its disruption by AMPH, APO, or MK-801. In contrast, there were sex-specific and independent effects of these two interventions, particularly on the sensitivity to the disruptive effects of AMPH. These findings suggest that METH users may show altered regulation of PPI, and that these effects may be female-specific and are unlikely to involve altered BDNF signaling. BDNF HETs of both sexes showed baseline changes in PPI and startle reactivity, but AMPH disrupted PPI was enhanced in male mice only. BDNF HETs may be a valuable model for further research into the effects of BDNF signaling in PPI brain circuits, and particularly sex-differences, which may contribute to sensorimotor gating deficits in patients with schizophrenia. Disrupted sensorimotor gating in schizophrenia is associated with a significant level of function impairment, and better understanding of the molecular pathology in neural circuits that cause this behavioral disturbance in schizophrenia should assist in the development of more efficacious therapies.

MATERIALS AND METHODS

ANIMALS

Male and female BDNF HETs and WT littermates were obtained from a breeding colony at the Florey Institute of Neuroscience and Mental Health. This colony was originally established with breeders from JAX Mice and Services (Bar Harbour, ME, USA) and maintained on a C57Bl/6 background. Experimental mice were weaned at 3 weeks of age and transported to the Mental Health Research Institute facility for all behavioral experiments. Mice were housed in individually ventilated cages (IVC, Tecniplast, Buguggiate, Italy) in same sex groups of 2–6 animals, with *ad libitum* access to rodent chow and tap water. The light period of the circadian cycle was from 07:00 a.m. to 07:00 p.m. and experiments were conducted during the light period. All experiments were done in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes set out by the National Health and Medical Research Council of Australia and were approved by the Florey Institute of Neuroscience and Mental Health Animal Experimentation Ethics Committee.

DRUG TREATMENT DURING ADOLESCENCE/EARLY ADULTHOOD

Groups of mice in separate cages were randomly allocated to receive METH [(±)-METH-HCl, National Measurement Institute, Pymble, NSW, Australia] or saline vehicle solution (0.9% sodium chloride, Baxter Healthcare, Old Toongabbie, NSW, Australia) treatments which commenced at 6 weeks of age. Numbers in each treatment group were as follows: Male WT Saline $n = 10$, Male WT METH $n = 9$, Male BDNF HET Saline $n = 9$, Male BDNF HET METH $n = 9$, Female WT Saline $n = 10$, Female WT METH $n = 10$, Female BDNF HET Saline $n = 8$, Female BDNF HET METH $n = 8$. Treatments were administered by intraperitoneal (IP) injection in the mouse housing room following an escalating dosing regime that has previously been used to mimic human patterns of abuse [Robinson and Camp (1987), **Figure 1A** shows treatment regime in experimental timeline]. During the first week of treatment, animals received one daily injection of 1 mg/kg METH in the morning (between 8 and 10 a.m) from Monday to Friday. During the second week of treatment, animals received two daily injections of 2 mg/kg METH, in the morning and afternoon (between 4 and 6 p.m.) from Monday to Friday. In the third week of treatment, animals received two daily injections of 4 mg/kg METH, in the morning and afternoon from Monday to Friday. Every Friday, the acute response of the animals to METH treatment was assessed in locomotor photocell cages. Vehicle injections matched the frequency of METH treatments throughout this 3-week period. In all cases, injection volumes of 10 ml/kg were administered using 30 g needles, and no injections were administered on weekends. Following the end of the treatment period, mice were left undisturbed for 2 weeks before commencement of PPI testing.

BEHAVIORAL TESTING DURING TREATMENT: LOCOMOTOR ACTIVITY

To measure the sensitivity of animals to METH administration during the treatment period, locomotor activity was measured immediately following treatment administration on Friday mornings during the 3 week treatment period. Locomotor photocell arenas [27 × 27 × 40 cm (l × w × h), TruScan, Coulbourn Instruments, Whitehall, PA, USA] were used to measure locomotor activity. Mice were habituated to the testing arena during two 1 h sessions in the 2 days prior to the first testing session. On Friday mornings during the treatment period, mice received their injections in the locomotor testing room and were immediately placed into photocell arenas. Horizontal activity was recorded for 1 h and was expressed as locomotor distance moved (cm) per 5 min interval.

BEHAVIORAL TESTING DURING ADULTHOOD: PREPULSE INHIBITION

Baseline and psychostimulant-induced disruption of PPI was assessed in mice using a pseudorandomized repeated-measures paradigm where mice were tested using all challenge drugs with 3–4 days washout between sessions. These drugs were chosen because they all disrupt PPI albeit via a differential pharmacological mechanism of action, i.e., acute dopamine release (AMPH), non-selective dopamine receptor agonism without effects on dopamine release (APO) and a non-dopaminergic mechanism involving NMDA receptor antagonism (MK-801) (Geyer et al., 2001; van den Buuse, 2010). AMPH (d-amphetamine sulfate, 5 mg/kg, Sigma, St. Louis, MO, USA), APO [R-(-)-APO hydrochloride hemihydrate, 5 mg/kg, Sigma] and MK-801 [(+)-MK-801 hydrogen maleate, 0.25 mg/kg, Sigma] were used as challenge drugs in the current study, and these doses have been used previously to produce a robust disruption of PPI in mice (Chavez et al., 2009). These were dissolved in saline, with saline

administered as the control solution. All drugs were administered IP 10 min before the start of the PPI session.

All mice were tested for startle, startle habituation and PPI of startle using previously published protocols (van den Buuse et al., 2009). Briefly, we used San Diego Instruments (San Diego, CA, USA) SRLab automated startle boxes and the PPI session consisted of 104 trials, taking approximately 36 min to complete. The protocol commenced with 3 min of 65 dB background noise. The session started and ended with a block of 8 trials delivering only a 115 dB pulse. Together with 16 pseudorandomly delivered pulse-alone stimuli during the main component of the session, responses to these startle pulses were used to construct startle habituation curves. PP-pulse trials consisted of a 115 dB pulse, preceded either by 30 or 100 ms by a PP of 2, 4, 8, or 16 dB over the 65 dB background noise (total SPL 67, 69, 73, or 81 dB). PPI was calculated as the difference between the median response to PP-pulse trials and the median response to pulse-alone trials, expressed as a percentage of the median response to the pulse-alone trials. The session also included 8 trials during which no sound stimulus was delivered, in order to check for non-specific movements.

STATISTICAL ANALYSES

All data were expressed as mean ± standard error of the mean (SEM). Differences within and between groups were analyzed by analysis of variance (ANOVA) with repeated measures (Systat, version 9, SPSS Inc., USA). For analysis of PPI data, PP intensity, startle block, ISI and acute drug challenge were included as within-group, repeated measures factors, while METH treatment, genotype, and sex were included as between-group factors. The effect of a drug challenge to disrupt PPI was considered significantly different when there was a drug challenge × between group factor interaction, using a repeated measures ANOVA that included data from saline and drug challenge sessions. For analysis of locomotor activity during the treatment period, week of treatment period and time during session were included as within-group, repeated measures factors, while METH treatment, genotype, and sex were included as between-group factors. When $p < 0.05$, differences were considered statistically significant. The Greenhouse-Geisser Epsilon correction was applied when multiple repeated measures were compared in the same ANOVA test.

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REFERENCES

Abi-Dargham, A., Gil, R., Krystal, J., Baldwin, R. M., Seibyl, J. P., Bowers, M., et al. (1998). Increased striatal dopamine transmission in schizophrenia: confirmation in a second cohort. *Am. J. Psychiatry* 155, 761–767.

Angelucci, F., Brene, S., and Mathe, A. A. (2005). BDNF in schizophrenia, depression and corresponding animal models. *Mol. Psychiatry* 10, 345–352. doi: 10.1038/sj.mp.4001637

Angelucci, F., Gruber, S. H., El Khoury, A., Tonali, P. A., and Mathe, A. A. (2007). Chronic amphetamine treatment reduces NGF and BDNF in the rat brain. *Eur. Neuropsychopharmacol.* 17, 756–762. doi: 10.1016/j.euroneuro.2007.03.002

Arai, S., Takuma, K., Mizoguchi, H., Ibi, D., Nagai, T., Takahashi, K., et al. (2008). Involvement of pallidotegmental neurons in methamphetamine- and MK-801-induced impairment of prepulse inhibition of the acoustic startle reflex in mice: reversal by $GABA_B$ receptor agonist baclofen. *Neuropsychopharmacology* 33, 3164–3175. doi: 10.1038/npp.2008.41

Bahi, A., Boyer, F., Chandrasekar, V., and Dreyer, J. L. (2008). Role of accumbens BDNF and TrkB in cocaine-induced psychomotor sensitization, conditioned-place preference, and reinstatement in rats. *Psychopharmacology (Berl.)* 199, 169–182. doi: 10.1007/s00213-008-1164-1

Bosse, K. E., Maina, F. K., Birbeck, J. A., France, M. M., Roberts, J. J., Colombo, M. L., et al. (2012). Aberrant striatal dopamine transmitter dynamics in brain-derived neurotrophic factor-deficient mice. *J. Neurochem.* 120, 385–395. doi: 10.1111/j.1471-4159.2011.07531.x

Callaghan, R. C., Cunningham, J. K., Allebeck, P., Arenovich, T., Sajeev, G., Remington, G., et al. (2012). Methamphetamine use and schizophrenia: a population-based cohort study in California. *Am. J. Psychiatry* 169, 389–396. doi: 10.1176/appi.ajp.2011.10070937

Caspi, A., and Moffitt, T. E. (2006). Gene-environment interactions in psychiatry: joining forces with neuroscience. *Nat. Rev. Neurosci.* 7, 583–590. doi: 10.1038/nrn1925

Chao, H. M., Kao, H. T., and Porton, B. (2008). BDNF Val66Met variant and age of onset in schizophrenia. *Am. J. Med. Genet. B Neuropsychiatr. Genet.* 147B, 505–506. doi: 10.1002/ajmg.b.30619

Chao, Y. L., Chen, H. H., and Chen, C. H. (2012). Effects of repeated electroconvulsive shock on methamphetamine-induced behavioral abnormalities in mice. *Brain Stimul.* 5, 393–401. doi: 10.1016/j.brs.2011.04.004

Chavez, C., Gogos, A., Jones, M. E., and van den Buuse, M. (2009). Psychotropic drug-induced locomotor hyperactivity and prepulse inhibition regulation in male and female aromatase knockout (ArKO) mice: role of dopamine D1 and D2 receptors and dopamine transporters. *Psychopharmacology (Berl.)* 206, 267–279. doi: 10.1007/s00213-009-1604-6

Chen, C. K., Lin, S. K., Sham, P. C., Ball, D., Loh El, W., and Murray, R. M. (2005). Morbid risk for psychiatric disorder among the relatives of methamphetamine users with and without psychosis. *Am. J. Med. Genet. B Neuropsychiatr. Genet.* 136B, 87–91. doi: 10.1002/ajmg.b.30187

Chen, C. K., Lin, S. K., Sham, P. C., Ball, D., Loh, E. W., Hsiao, C. C., et al. (2003). Pre-morbid characteristics and co-morbidity of methamphetamine users with and without psychosis. *Psychol. Med.* 33, 1407–1414. doi: 10.1017/S0033291703008353

Coppola, V., and Tessarollo, L. (2004). Control of hyperphagia prevents obesity in BDNF heterozygous mice. *Neuroreport* 15, 2665–2668. doi: 10.1097/00001756-200412030-00022

Daws, L. C., Munn, J. L., Valdez, M. F., Frosto-Burke, T., and Hensler, J. G. (2007). Serotonin transporter function, but not expression, is dependent on brain-derived neurotrophic factor (BDNF): *in vivo* studies in BDNF-deficient mice. *J. Neurochem.* 101, 641–651. doi: 10.1111/j.1471-4159.2006.04392.x

Deltheil, T., Guiard, B. P., Cerdan, J., David, D. J., Tanaka, K. F., Reperant, C., et al. (2008). Behavioral and serotonergic consequences of decreasing or increasing hippocampus brain-derived neurotrophic factor protein levels in mice. *Neuropharmacology* 55, 1006–1014. doi: 10.1016/j.neuropharm.2008.08.001

Dluzen, D. E., Story, G. M., Xu, K., Kucera, J., and Walro, J. M. (1999). Alterations in nigrostriatal dopaminergic function within BDNF mutant mice. *Exp. Neurol.* 160, 500–507. doi: 10.1006/exnr.1999.7225

Duan, W., Guo, Z., Jiang, H., Ware, M., and Mattson, M. P. (2003). Reversal of behavioral and metabolic abnormalities, and insulin resistance syndrome, by dietary restriction in mice deficient in brain-derived neurotrophic factor. *Endocrinology* 144,

2446–2453. doi: 10.1210/en.2002-0113

Duman, C. H., Schlesinger, L., Kodama, M., Russell, D. S., and Duman, R. S. (2007). A role for MAP kinase signaling in behavioral models of depression and antidepressant treatment. *Biol. Psychiatry* 61, 661–670. doi: 10.1016/j.biopsych.2006.05.047

Duman, C. H., Schlesinger, L., Russell, D. S., and Duman, R. S. (2008). Voluntary exercise produces antidepressant and anxiolytic behavioral effects in mice. *Brain Res.* 1199, 148–158. doi: 10.1016/j.brainres.2007.12.047

Geyer, M. A., Krebs-Thomson, K., Braff, D. L., and Swerdlow, N. R. (2001). Pharmacological studies of prepulse inhibition models of sensorimotor gating deficits in schizophrenia: a decade in review. *Psychopharmacology* 156, 117–154. doi: 10.1007/s002130100811

Goldstein, J. M., Seidman, L. J., Goodman, J. M., Koren, D., Lee, H., Weintraub, S., et al. (1998). Are there sex differences in neuropsychological functions among patients with schizophrenia? *Am. J. Psychiatry* 155, 1358–1364.

Gorski, J. A., Balogh, S. A., Wehner, J. M., and Jones, K. R. (2003). Learning deficits in forebrain-restricted brain-derived neurotrophic factor mutant mice. *Neuroscience* 121, 341–354. doi: 10.1016/S0306-4522(03)00426-3

Gratacos, M., Gonzalez, J. R., Mercader, J. M., De Cid, R., Urretavizcaya, M., and Estivill, X. (2007). Brain-derived neurotrophic factor Val66Met and psychiatric disorders: meta-analysis of case-control studies confirm association to substance-related disorders, eating disorders, and schizophrenia. *Biol. Psychiatry* 61, 911–922. doi: 10.1016/j.biopsych.2006.08.025

Green, M. J., Matheson, S. L., Shepherd, A., Weickert, C. S., and Carr, V. J. (2011). Brain-derived neurotrophic factor levels in schizophrenia: a systematic review with meta-analysis. *Mol. Psychiatry* 16, 960–972. doi: 10.1038/mp.2010.88

Guiard, B. P., David, D. J., Deltheil, T., Chenu, F., Le Maitre, E., Renoir, T., et al. (2008). Brain-derived neurotrophic factor-deficient mice exhibit a hippocampal hyperserotonergic phenotype. *Int. J. Neuropsychopharmacol.* 11, 79–92. doi: 10.1017/S1461145707007857

Gururajan, A., Manning, E. E., Klug, M., and van den Buuse, M. (2012). Drugs of abuse and increased risk of psychosis development. *Aust. N. Z. J. Psychiatry* 46, 1120–1135. doi: 10.1177/0004867412455232

Hadamitzky, M., Markou, A., and Kuczenski, R. (2011). Extended access to methamphetamine self-administration affects sensorimotor gating in rats. *Behav. Brain Res.* 217, 386–390. doi: 10.1016/j.bbr.2010.11.009

Hafner, H., Maurer, K., Loffler, W., and Riecher-Rossler, A. (1993). The influence of age and sex on the onset and early course of schizophrenia. *Br. J. Psychiatry* 162, 80–86. doi: 10.1192/bjp.162.1.80

Hall, D. A., Stanis, J. J., Marquez Avila, H., and Gulley, J. M. (2008). A comparison of amphetamine- and methamphetamine-induced locomotor activity in rats: evidence for qualitative differences in behavior. *Psychopharmacology (Berl.)* 195, 469–478. doi: 10.1007/s00213-007-0923-8

Hall, F. S., Drgonova, J., Goeb, M., and Uhl, G. R. (2003). Reduced behavioral effects of cocaine in heterozygous brain-derived neurotrophic factor (BDNF) knockout mice. *Neuropsychopharmacol* 28, 1485–1490. doi: 10.1038/sj.npp.1300192

Hashimoto, T., Bergen, S. E., Nguyen, Q. L., Xu, B., Monteggia, L. M., Pierri, J. N., et al. (2005). Relationship of brain-derived neurotrophic factor and its receptor TrkB to altered inhibitory prefrontal circuitry in schizophrenia. *J. Neurosci.* 25, 372–383. doi: 10.1523/JNEUROSCI.4035-04.2005

Hill, R. A. (2012). Interaction of sex steroid hormones and brain-derived neurotrophic factor-tyrosine kinase B signalling: relevance to schizophrenia and depression. *J. Neuroendocrinol.* 24, 1553–1561. doi: 10.1111/j.1365-2826.2012.02365.x

Hill, R. A., and van den Buuse, M. (2011). Sex-dependent and region-specific changes in TrkB signaling in BDNF heterozygous mice. *Brain Res.* 1384, 51–60. doi: 10.1016/j.brainres.2011.01.060

Hill, R. A., Wu, Y. W., Kwek, P., and van den Buuse, M. (2012). Modulatory effects of sex steroid hormones on brain-derived neurotrophic factor-tyrosine kinase B expression during adolescent development in C57Bl/6 mice. *J. Neuroendocrinol.* 24, 774–788. doi: 10.1111/j.1365-2826.2012.02277.x

Ho, B. C., Milev, P., O'Leary, D. S., Librant, A., Andreasen, N. C., and Wassink, T. H. (2006). Cognitive and magnetic resonance imaging brain morphometric correlates of brain-derived neurotrophic factor Val66Met gene polymorphism in patients with schizophrenia and healthy volunteers. *Arch. Gen. Psychiatry* 63, 731–740. doi: 10.1001/archpsyc.63.7.731

Ibarguen-Vargas, Y., Surget, A., Vourc'h, P., Leman, S., Andres, C. R., Gardier, A. M., et al. (2009). Deficit in BDNF does not increase vulnerability to stress but dampens antidepressant-like effects in the unpredictable chronic mild stress. *Behav. Brain Res.* 202, 245–251. doi: 10.1016/j.bbr.2009.03.040

Kebir, O., Mouaffak, F., Chayet, M., Leroy, S., Tordjman, S., Amado, I., et al. (2009). Semantic but not phonological verbal fluency associated with BDNF Val66Met polymorphism in schizophrenia. *Am. J. Med. Genet. B Neuropsychiatr. Genet.* 150B, 441–442. doi: 10.1002/ajmg.b.30794

Klug, M., Hill, R. A., Choy, K. H., Kyrios, M., Hannan, A. J., and van den Buuse, M. (2012). Long-term behavioral and NMDA receptor effects of young-adult corticosterone treatment in BDNF heterozygous mice. *Neurobiol. Dis.* 46, 722–731. doi: 10.1016/j.nbd.2012.03.015

Koolschijn, P. C., Van Haren, N. E., Bakker, S. C., Hoogendoorn, M. L., Hulshoff Pol, H. E., and Kahn, R. S. (2010). Effects of brain-derived neurotrophic factor Val66Met polymorphism on hippocampal volume change in schizophrenia. *Hippocampus* 20, 1010–1017. doi: 10.1002/hipo.20699

Kusljic, S., and van den Buuse, M. (2004). Functional dissociation between serotonergic pathways in dorsal and ventral hippocampus in psychotomimetic drug-induced locomotor hyperactivity and prepulse inhibition in rats. *Eur. J. Neurosci.* 20, 3424–3432. doi: 10.1111/j.1460-9568.2004.03804.x

Lahti, A. C., Koffel, B., Laporte, D., and Tamminga, C. A. (1995). Subanesthetic doses of ketamine stimulate psychosis in schizophrenia. *Neuropsychopharmacology* 13, 9–19. doi: 10.1016/0893-133X(94)00131-I

Le Foll, B., Diaz, J., and Sokoloff, P. (2005). A single cocaine exposure increases BDNF and D3 receptor expression: implications for drug-conditioning. *Neuroreport* 16, 175–178. doi: 10.1097/00001756-200502080-00022

Lu, W., Zhang, C., Yi, Z., Li, Z., Wu, Z., and Fang, Y. (2012). Association between BDNF Val66Met polymorphism and cognitive performance in antipsychotic-naive patients with Schizophrenia. *J. Mol. Neurosci.* 47, 505–510. doi: 10.1007/s12031-012-9750-4

Lyons, W. E., Mamounas, L. A., Ricaurte, G. A., Coppola, V., Reid, S. W., Bora, S. H., et al. (1999). Brain-derived neurotrophic factor-deficient mice develop aggressiveness and hyperphagia in conjunction with brain serotonergic abnormalities. *Proc. Natl. Acad. Sci. U.S.A.* 96, 15239–15244. doi: 10.1073/pnas.96.26.15239

McKetin, R., Hickey, K., Devlin, K., and Lawrence, K. (2010). The risk of psychotic symptoms associated with recreational methamphetamine use. *Drug Alcohol Rev.* 29, 358–363. doi: 10.1111/j.1465-3362.2009.00160.x

Narita, M., Aoki, K., Takagi, M., Yajima, Y., and Suzuki, T. (2003). Implication of brain-derived neurotrophic factor in the release of dopamine and dopamine-related behaviors induced by methamphetamine. *Neuroscience* 119, 767–775. doi: 10.1016/S0306-4522(03)00099-X

Neves-Pereira, M., Cheung, J. K., Pasdar, A., Zhang, F., Breen, G., Yates, P., et al. (2005). BDNF gene is a risk factor for schizophrenia in a Scottish population. *Mol. Psychiatry* 10, 208–212. doi: 10.1038/sj.mp.4001575

Numata, S., Ueno, S., Iga, J., Yamauchi, K., Hongwei, S., Ohta, K., et al. (2006). Brain-derived neurotrophic factor (BDNF) Val66Met polymorphism in schizophrenia is associated with age at onset and symptoms. *Neurosci. Lett.* 401, 1–5. doi: 10.1016/j.neulet.2006.02.054

Onogi, H., Nakagawasai, O., Sato, A., Mitazaki, S., Nakaya, K., Watanabe, K., et al. (2011). p-Hydroxyamphetamine causes prepulse inhibition disruption in mice: contribution of serotonin neurotransmission. *Behav. Brain Res.* 224, 159–165. doi: 10.1016/j.bbr.2011.06.002

Onogi, H., Nakagawasai, O., Tan-No, K., Mitazaki, S., Sato, A., Nakaya, K., et al. (2010). p-Hydroxyamphetamine causes prepulse inhibition disruptions in mice: contribution of dopamine neurotransmission. *Behav. Brain Res.* 214, 349–356. doi: 10.1016/j.bbr.2010.06.005

Perry, W., and Braff, D. L. (1994). Information-processing deficits and thought disorder in schizophrenia. *Am. J. Psychiatry* 151, 363–367.

Pogorelov, V. M., Nomura, J., Kim, J., Kannan, G., Ayhan, Y., Yang,

C., et al. (2012). Mutant DISC1 affects methamphetamine-induced sensitization and conditioned place preference: a comorbidity model. *Neuropharmacology* 62, 1242–1251. doi: 10.1016/j.neuropharm.2011.02.003

Powell, S. B., Zhou, X., and Geyer, M. A. (2009). Prepulse inhibition and genetic mouse models of schizophrenia. *Behav. Brain Res.* 204, 282–294. doi: 10.1016/j.bbr.2009.04.021

Robinson, T. E., and Camp, D. M. (1987). Long-lasting effects of escalating doses of d-amphetamine on brain monoamines, amphetamine-induced stereotyped behavior and spontaneous nocturnal locomotion. *Pharmacol. Biochem. Behav.* 26, 821–827. doi: 10.1016/0091-3057(87)90616-2

Rybakowski, J. K., Borkowska, A., Skibinska, M., Szczepankiewicz, A., Kapelski, P., Leszczynska-Rodziewicz, A., et al. (2006). Prefrontal cognition in schizophrenia and bipolar illness in relation to Val66Met polymorphism of the brain-derived neurotrophic factor gene. *Psychiatry Clin. Neurosci.* 60, 70–76. doi: 10.1111/j.1440-1819.2006.01462.x

Saarelainen, T., Hendolin, P., Lucas, G., Koponen, E., Sairanen, M., Macdonald, E., et al. (2003). Activation of the TrkB neurotrophin receptor is induced by antidepressant drugs and is required for antidepressant-induced behavioral effects. *J. Neurosci.* 23, 349–357.

Sato, M., Numachi, Y., and Hamamura, T. (1992). Relapse of paranoid psychotic state in methamphetamine model of schizophrenia. *Schizophr. Bull.* 18, 115–122. doi: 10.1093/schbul/18.1.115

Saylor, A. J., and McGinty, J. F. (2008). Amphetamine-induced locomotion and gene expression are altered in BDNF heterozygous mice. *Genes Brain Behav.* 7, 906–914. doi: 10.1111/j.1601-183X.2008.00430.x

Sim, M. S., Mohamed, Z., Hatim, A., Rajagopal, V. L., and Habil, M. H. (2010). Association of brain-derived neurotrophic factor (Val66Met) genetic polymorphism with methamphetamine dependence in a Malaysian population. *Brain Res.* 1357, 91–96. doi: 10.1016/j.brainres.2010.08.053

Smith, G. N., Thornton, A. E., Lang, D. J., Macewan, G. W., Ehmann, T. S., Kopala, L. C., et al. (2012). Hippocampal volume and the brain-derived neurotrophic factor Val66Met polymorphism in first episode psychosis. *Schizophr. Res.* 134, 253–259. doi: 10.1016/j.schres.2011.11.022

Sulzer, D., Sonders, M. S., Poulsen, N. W., and Galli, A. (2005). Mechanisms of neurotransmitter release by amphetamines: a review. *Prog. Neurobiol.* 75, 406–433. doi: 10.1016/j.pneurobio.2005.04.003

Swerdlow, N. R., Light, G. A., Cadenhead, K. S., Sprock, J., Hsieh, M. H., and Braff, D. L. (2006). Startle gating deficits in a large cohort of patients with schizophrenia: relationship to medications, symptoms, neurocognition, and level of function. *Arch. Gen. Psychiatry* 63, 1325–1335. doi: 10.1001/archpsyc.63.12.1325

Szeszko, P. R., Lipsky, R., Mentschel, C., Robinson, D., Gunduz-Bruce, H., Sevy, S., et al. (2005). Brain-derived neurotrophic factor val66met polymorphism and volume of the hippocampal formation. *Mol. Psychiatry* 10, 631–636. doi: 10.1038/sj.mp.4001656

Takahashi, M., Shirakawa, O., Toyooka, K., Kitamura, N., Hashimoto, T., Maeda, K., et al. (2000). Abnormal expression of brain-derived neurotrophic factor and its receptor in the corticolimbic system of schizophrenic patients. *Mol. Psychiatry* 5, 293–300. doi: 10.1038/sj.mp.4000718

Thompson Ray, M., Weickert, C. S., Wyatt, E., and Webster, M. J. (2011). Decreased BDNF, trkB-TK+ and GAD67 mRNA expression in the hippocampus of individuals with schizophrenia and mood disorders. *J. Psychiatry. Neurosci.* 36, 195–203. doi: 10.1503/jpn.100048

van den Buuse, M. (2010). Modeling the positive symptoms of schizophrenia in genetically modified mice: pharmacology and methodology aspects. *Schizophr. Bull.* 36, 246–270. doi: 10.1093/schbul/sbp132

van den Buuse, M., Morris, M., Chavez, C., Martin, S., and Wang, J. (2004). Effect of adrenalectomy and corticosterone replacement on prepulse inhibition and locomotor activity in mice. *Br. J. Pharmacol.* 142, 543–550. doi: 10.1038/sj.bjp.0705511

van den Buuse, M., Wischhof, L., Lee, R. X., Martin, S., and Karl, T. (2009). Neuregulin 1 hypomorphic mutant mice: enhanced baseline locomotor activity but normal psychotropic drug-induced hyperlocomotion and prepulse inhibition regulation. *Int. J. Neuropsychopharmacol.* 12, 1383–1393. doi: 10.1017/S1461145709000388

Wang, J. H., Short, J., Ledent, C., Lawrence, A. J., and van den Buuse, M. (2003). Reduced startle habituation and prepulse inhibition in mice lacking the adenosine A2A receptor. *Behav. Brain Res.* 143, 201–207. doi: 10.1016/S0166-4328(03)00036-6

Weickert, C. S., Hyde, T. M., Lipska, B. K., Herman, M. M., Weinberger, D. R., and Kleinman, J. E. (2003). Reduced brain-derived neurotrophic factor in prefrontal cortex of patients with schizophrenia. *Mol. Psychiatry* 8, 592–610. doi: 10.1038/sj.mp.4001308

Willott, J. F., Tanner, L., O'Steen, J., Johnson, K. R., Bogue, M. A., and Gagnon, L. (2003). Acoustic startle and prepulse inhibition in 40 inbred strains of mice. *Behav. Neurosci.* 117, 716–727. doi: 10.1037/0735-7044.117.4.716

Wong, J., Hyde, T. M., Cassano, H. L., Deep-Soboslay, A., Kleinman, J. E., and Weickert, C. S. (2010). Promoter specific alterations of brain-derived neurotrophic factor mRNA in schizophrenia. *Neuroscience* 169, 1071–1084. doi: 10.1016/j.neuroscience.2010.05.037

Wu, Y. W., Hill, R. A., Gogos, A., and van den Buuse, M. (2013). Sex differences and the role of estrogen in animal models of schizophrenia: interaction with BDNF. *Neuroscience* 239, 67–83. doi: 10.1016/j.neuroscience.2012.10.024

Zhou, D. H., Yan, Q. Z., Yan, X. M., Li, C. B., Fang, H., Zheng, Y. L., et al. (2010). The study of BDNF Val66Met polymorphism in Chinese schizophrenic patients. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 34, 930–933. doi: 10.1016/j.pnpbp.2010.04.019

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Altered dopamine ontogeny in the developmentally vitamin D deficient rat and its relevance to schizophrenia

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Schizophrenia is a heterogeneous group of disorders with unknown etiology. Although abnormalities in multiple neurotransmitter systems have been linked to schizophrenia, alterations in dopamine (DA) neurotransmission remain central to the treatment of this disorder. Given that schizophrenia is considered a neurodevelopmental disorder we have hypothesized that abnormal DA signaling in the adult patient may result from altered DA signaling during fetal brain development. Environmental and genetic risk factors can be modeled in rodents to allow for the investigation of early neurodevelopmental pathogenesis that may lead to clues into the etiology of schizophrenia. To address this we created an animal model of one such risk factor, developmental vitamin D (DVD) deficiency. DVD-deficient adult rats display an altered behavioral profile in response to DA releasing and blocking agents that are reminiscent of that seen in schizophrenia patients. Furthermore, developmental studies revealed that DVD deficiency also altered cell proliferation, apoptosis, and neurotransmission across the embryonic brain. In particular, DVD deficiency reduces the expression of crucial dopaminergic specification factors and alters DA metabolism in the developing brain. We speculate such alterations in fetal brain development may change the trajectory of DA neuron ontogeny to induce the behavioral abnormalities observed in adult offspring. The widespread evidence that both dopaminergic and structural changes are present in people who develop schizophrenia prior to onset also suggest that early alterations in development are central to the disease. Taken together, early alterations in DA ontogeny may represent a core feature in the pathology of schizophrenia. Such a mechanism could bring together evidence from multiple risk factors and genetic vulnerabilities to form a convergent pathway in disease pathophysiology.

Keywords: amphetamine, MK-801, dopamine, behavior, differentiation, development

INTRODUCTION

Schizophrenia is a severe and chronic psychiatric disorder consisting of a heterogeneous group of symptoms and cognitive impairments. On the basis of the convergent evidence from the fields of epidemiology, imaging and post-mortem analysis, the neurodevelopmental hypothesis and the dopamine (DA) hypothesis have become two major theories of schizophrenia. The developmental hypothesis proposes that genetic or environmental factors during critical early periods of brain development adversely impact on adult mental health (Murray and Lewis, 1987; Weinberger, 1987). The DA hypothesis proposes that DA dysfunction is central to the pathogenesis of schizophrenia (Carlsson and Lindqvist, 1963; Seeman and Lee, 1975; Angrist and Vankammen, 1984; Davis et al., 1991; Laruelle et al., 1996; Abi-Dargham et al., 1998). Recently, these two theories were revised and integrated through the substantial evidence that all the developmental risk factors which increase the risk of schizophrenia, appear to share a common endpoint or "final common pathway" of DA dysfunction (Di Forti et al., 2007; Murray et al., 2008; Howes and Kapur, 2009).

Clinical studies provide strong evidence of DA dysfunction in patients. Patients with schizophrenia show increased amphetamine-induced DA release in the striatum (Breier et al., 1997; Laruelle and Abi-Dargham, 1999; Laruelle et al., 1999; Abi-Dargham et al., 2009) and have altered presynaptic DA function, specifically increased DA synthesis capacity (Howes et al., 2012; Fusar-Poli and Meyer-Lindenberg, 2013). These factors are highly associated with psychosis (Howes et al., 2011a) and are generally not evident in stable schizophrenia patients that are not acutely experiencing a psychotic episode (Laruelle et al., 1999; Shotbolt et al., 2011). However, increased presynaptic DA function can also be observed during the prodromal phase of the disease (Howes et al., 2009) and in ultra-high risk subjects who then go on to develop psychosis (Howes et al., 2011b). Thus, it would appear that alterations in presynaptic DA function precede the onset of frank psychosis, suggesting that intervention prior to symptom onset may even offer the potential for preventing disease onset.

Substantial evidence from animal models indicates that fetal or perinatal factors can result in long-term alterations in dopaminergic function. For example, animal models designed to examine obstetric complications, such as fetal or neonatal hypoxia, resulted in increased DA-mediated behavioral responses, increased DA

release and elevated basal DA in subcortical regions (Bjelke et al., 1991; Bernert et al., 2003; Boksa and El-Khodor, 2003; Decker et al., 2003). Rodent models with prenatal exposure to virus-like agents (e.g., the synthetic double-stranded RNA, Poly I:C) that explore the neurobiological correlates of maternal infection (Meyer et al., 2009) also exhibit increased levels of DA and DA metabolites and enhanced striatal DA turnover (Ozawa et al., 2006; Winter et al., 2009). In addition, heuristic evidence that early dopaminergic alterations can lead to impaired cognition and behavior later in life has also been described. For example, Kellendonk et al. (2006) have shown persistent deficits in cognition and altered DA function in a mouse model that transiently overexpresses DA 2 receptors in the striatum during development. Thus, abnormal DA signaling early in development would appear to produce long lasting impairments in brain function.

Evidence continues to mount from both epidemiological and pre-clinical studies to indicate that developmental vitamin D (DVD) deficiency may be also an important developmental risk factor for schizophrenia. Over the past decade, our studies on the DVD-deficient rat model firmly established that DVD deficiency affects brain cell proliferation, differentiation, and gross brain structure, it also produces long-lasting cellular changes and alterations in behavior in the adult offspring. In particular, the DVD-deficient adult offspring display enhanced DA-related behavioral responses and alterations in DA signaling. Understanding the mechanism of action linking DVD deficiency with altered DA signaling could provide clues to shared pathways underpinning the pathogenesis of schizophrenia.

In this article, we integrate findings derived from the DVD-deficient rat model and schizophrenia to propose that developmental DA dysfunction may be a core factor in the susceptibility and/or development of schizophrenia (**Box 1**). We begin with a concise summary of the epidemiological clues that suggest altered prenatal/perinatal vitamin D levels increase the risk of developing schizophrenia. Subsequently we introduce the DVD-deficient rat, discussing evidence from both early development and in adult offspring suggesting alterations in DA development and function. Furthermore, the multiple signaling pathways that may lead to such DA abnormalities in the DVD-deficient rat are also discussed. The final section reviews how DVD deficiency could lead to long-lasting neuroanatomical, neurochemical, and behavioral changes that are relevant to schizophrenia, and in particular, DA dysfunction.

BOX 1 | Salient points.

- *Schizophrenia*

Neurodevelopmental disorder associated with altered dopamine function both prior to and during disease onset

- *Developmental vitamin D (DVD) deficiency*

Associated with increased susceptibility to schizophrenia

- *DVD rat model*

DVD-deficiency in the rat leads to a pattern of altered developmental and adult dopaminergic function

- *Vitamin D and dopamine*

Early vitamin D signaling is intrinsically linked with the developing dopamine system

- *Animal models of schizophrenia*

Multiple animal models of schizophrenia show alterations in dopamine development prior to post-adolescent alterations in behavior

- *Dopamine and schizophrenia*

Although focus remains tied to dopamine as a common endpoint in schizophrenia, understanding common dopaminergic origins may be

THE EPIDEMIOLOGY OF VITAMIN D AND SCHIZOPHRENIA

Numerous pieces of epidemiological evidence implicate low levels of maternal vitamin D as a potential risk factor for schizophrenia. Firstly, one of the most replicated findings is that people born in the winter and spring months of the year have an increased risk of developing schizophrenia later in life (Torrey et al., 1997b; McGrath, 1999; Davies et al., 2003), and this risk is larger at high latitudes that feature greater seasonal fluctuations (Davies et al., 2003). Secondly, people born in urban areas in comparison with those born in rural environments have an increased risk of developing schizophrenia (Torrey et al., 1997a; McGrath, 1999). Finally, the incidence of schizophrenia is significantly higher in the second generation of dark-skinned migrants to cold countries compared to native-born individuals (Cantor-Graae and Selten, 2005). Moreover, first generation migrants who arrive as babies or infants also have an increased risk of schizophrenia (Veling et al., 2011). This risk decreases with the increasing age of the migrant suggesting early life environmental conditions are critical. Given that vitamin D deficiency is common (1) during winter and spring, (2) at high latitudes (Holick et al., 1995), (3) in urban environments (McGrath et al., 2001) and (4) in dark skinned individuals (Clemens et al., 1982; Holick et al., 1995), these ecological data led to the hypothesis that low maternal vitamin D could be a modified risk factor for schizophrenia.

The direct analytical evidence in support of this hypothesis has also now been demonstrated. Schizophrenia has been shown to be ameliorated by vitamin D supplementation in the 1 year of life (McGrath et al., 2004). Most recently, and most importantly, a Danish population-based case-control study (423 cases and 423 control) that directly assessed vitamin D levels in blood spots from new born infants provided solid evidence showing that low prenatal vitamin D levels are associated with an increased risk of schizophrenia (McGrath et al., 2010). Taken together, these data support the hypothesis that an absence of vitamin D during development may lead to an increased risk of schizophrenia. To establish the biological plausibility of whether DVD deficiency could be related to schizophrenia, our group established a DVD-deficient rat model which has shown that low prenatal vitamin D adversely affects brain development and adult behavior, especially DA development and DA-related behaviors.

THE DVD-DEFICIENT RAT MODEL OF SCHIZOPHRENIA

DVD-deficient offspring are produced by feeding female Sprague-Dawley rats a diet that lacks vitamin D but contains normal calcium and phosphorous. Rats are maintained on this diet for 6 weeks, after which and prior to mating, serum vitamin D_3 depletion is confirmed by measuring the stable vitamin D metabolite 25 hydroxy-vitamin D ($25(OH)D_3$) as <0.34 ng/ml (Eyles et al., 2009). Vitamin D deficient dams are maintained on the vitamin D depleted diet until the birth of pups. Control animals are kept under identical conditions but are supplied with standard rat chow containing vitamin D_3. All dams (both control and depleted) are kept under standard housing conditions (control rat chow) after giving birth. Although vitamin D_3–depleted dams and offspring remain normocalcemic, increased parathyroid hormone levels have been observed in both the dams and pups (Cui et al., 2010; Burne et al., 2011). In this model the exposure to vitamin D_3 depletion is only transient as all dams are returned to a normal vitamin D containing diet ate birth. This is sufficient to replete DVD-deficient offspring to normal vitamin D_3levels by two weeks of age. Importantly, calcium levels in vitamin D deficient dams and DVD-deficient pups are not altered by this protocol (Eyles et al., 2006; O'Loan et al., 2007). The acute effects of DVD deficiency including abnormal brain development, changes in gross brain structure and altered neurochemistry in addition to persistent alterations in behavior will be discussed in the following sections.

BRAIN DEVELOPMENT IN THE DVD-DEFICIENT RAT

The idea that DA dysfunction represents the "final common pathway" in schizophrenia (Di Forti et al., 2007; Murray et al., 2008; Howes and Kapur, 2009) is supported by strong evidence of abnormal DA signaling in the *adult patient*, particularly at the presynaptic level (Howes et al., 2012; Fusar-Poli and Meyer-Lindenberg, 2013). However, we know little about the up-stream *developmental alterations* in DA physiology that may underpin these effects. For example, early life risk factors associated with schizophrenia may change the way DA systems develop. Thus, increases in presynaptic DA function in individuals who progress to clinical schizophrenia may result from abnormalities in the early ontogeny of DA systems. Animal models, such as the DVD-deficient rat, allow for more thorough investigations into early developing neurotransmitter systems and early developmental alterations that lead to behavioral and neurochemical abnormalities in the adult.

EARLY DOPAMINERGIC ABNORMALITIES

Embryonic DA neuron development is a dynamic process with multiple factors responsible for normal function. In the rat, differentiation of monoamine cells in the substantia nigra (located in the midbrain) begins as early as embryonic day (E) 11 with the peak period of DA neuron birth occurring at E12(Lauder and Bloom, 1974; Gates et al., 2006). Subsequent innervation of the striatum in the basal ganglia from midbrain monoamine neurons occurs from E14-17(Voorn et al., 1988). DVD deficiency has been shown to alter the gene expression of key DA specification factors (i.e., factors involved in the phenotypic development of DA neurons) at both of these crucial time-points in DA development. At E12, coinciding with monoamine cell differentiation, expression of Nurr1 and p57Kip2 were decreased in DVD-deficient rats (Cui et al., 2010). Tyrosine hydroxylase (TH; the rate limiting enzyme in DA synthesis and a reliable marker of DA neurons) also appeared to be reduced in DVD-deficient embryos at this same time point. Nurr1 expression was also decreased at E15. This represents a period when dopaminergic innervation of the striatum begins to occur. Nurr1 (also known as NR4A2), an orphan nuclear receptor, is an essential factor in DA neuron development and maturation (Wallen et al., 2001) and p75Kip2 cooperates with Nurr1 during DA cell development (Joseph et al., 2003). Nurr1-deficient mice show complete DA neuron agenesis (Zetterstrom et al., 1997) and Nurr1 has been shown to directly activate the TH promoter gene in cell cultures (Sakurada et al., 1999; Iwawaki et al., 2000; Kim et al., 2003). Therefore, decreased Nurr1 expression coupled with a trend for reduced TH expression strongly suggests decreased or delayed DA cell differentiation in DVD-deficient rats.

These alterations in DA specification factors during development in the DVD-deficient rat compliment alterations in DA turnover identified at birth. Under normal conditions, the majority of DA metabolism is through intra-neural oxidative deamination via monoamine oxidase (MAO) to produce dihydroxyphenylacetic acid (DOPAC). This is followed by a subsequent extra-neural *O*-methylation via catechol-*O*-methyl transferase (COMT) to form homovanillic acid (HVA; Westerink, 1985). DVD-deficient pups show a 45% reduction in brain COMT levels at birth (Kesby et al., 2009). Moreover, this reduction in COMT is associated with an increased ratio of DOPAC to HVA, suggesting altered DA turnover. COMT remains an interesting target for schizophrenia research with less efficient isoforms increasing the risk of schizophrenia when coupled with adolescent marijuana use (Howes and Kapur, 2009). Thus, DVD deficiency directly impacts on factors that are essential in early DA neuron development and embryonic DA turnover.

GROSS BRAIN ANATOMY, MITOSIS AND APOPTOSIS

The initial absence of vitamin D also affects other, more general, aspects of brain development that do not directly relate to DA neurons. For example, gross brain architecture is different in that DVD-deficient pups have cerebral hemispheres that are longer but not wider than control pups (Eyles et al., 2003). Furthermore, when corrections were made for the altered shape of these brains, the lateral ventricles were larger but the neocortex thinner than in control pups. Enlarged lateral ventricles have been observed in patients with schizophrenia and represent one of the more replicated neuroanatomical findings in schizophrenia (Chua et al., 2007; Nakamura et al., 2007; Pagsberg et al., 2007). These brain anatomical changes were also associated with altered rates of cellular proliferation.

Vitamin D is known to be involved in the modulation of cellular proliferation and apoptosis in many tissues (Banerjee and Chatterjee, 2003; Dusso et al., 2005). In the developing brain, levels of vitamin D receptor (VDR) expression coincide with increasing levels of apoptosis and decreasing levels of mitosis (Burket et al., 2003) suggesting similar actions to that seen in peripheral tissue. Conversely, the absence of vitamin D in the embryonic

brain results in increased levels of mitosis and decreased levels of apoptosis (Eyles et al., 2003; Ko et al., 2004). DVD deficiency also altered gene expression profiles regulating mitosis and apoptosis in the brain (Ko et al., 2004). Furthermore, neurosphere cultures derived from DVD-deficient rat pups result in a greater number of neurospheres than cultures from control rat pups (Cui et al., 2007), also suggesting increased cellular proliferation. Thus at both the cellular and transcriptional levels, vitamin D appears fundamentally involved in the rate of proliferation and cell death in the brain. These early alterations in both DA signaling, brain structure and proliferation in DVD-deficient offspring appear to produce associated abnormal neurochemistry and behavior in adulthood.

ALTERATIONS IN ADULT DVD-DEFICIENT OFFSPRING

DOPAMINE-BASED ALTERATIONS

Subcortical DA function is an essential factor with regard to novelty-induced behavioral activation (Hooks and Kalivas, 1995) and both novelty and stress (i.e., handling etc.) result in increased DA release in the prefrontal cortex (PFC; Feenstra et al., 1995). Moreover, enhanced responsiveness to novelty is associated with an increased response to agents that enhance synaptic DA levels (Chefer et al., 2003). Adult DVD-deficient rats show enhanced novelty-induced locomotion on a range of tasks including the hole board and elevated plus maze (Burne et al., 2004; Kesby et al., 2006). Interestingly, this enhanced response can be attenuated in DVD-deficient rats with handling procedures and injections (Burne et al., 2006; Kesby et al., 2006).

Amphetamine has been shown to induce psychotic-like phenotypes in non-psychotic individuals and schizophrenia patients show enhanced DA release and positive symptoms relative to healthy individuals after exposure to low doses (Janowsky et al., 1973; Lieberman et al., 1987; Laruelle et al., 1999). Amphetamine-induced behaviors in rodents are therefore considered a model of the psychotic symptoms seen in schizophrenia. Amphetamine induces DA release in the brain primarily due to actions at the DA transporter (DAT; Sulzer et al., 1993; Wieczorek and Kruk, 1994; Jones et al., 1998). Female DVD-deficient rats show an increased sensitivity to amphetamine-induced locomotion as adults but not juveniles (Kesby et al., 2010). Although male DVD-deficient rats do not show an enhanced response after an acute dose of amphetamine, a similar sensitivity to amphetamine appears to occur after multiple doses (Kesby et al., 2010). Adult female DVD-deficient rats also have increased levels of DAT in the caudate putamen (CPu) and increased affinity for DAT ligands in the nucleus accumbens (Acb; Kesby et al., 2010) suggesting alterations in DAT function may mediate the enhanced response to amphetamine.

DVD-deficient rats also show increased sensitivity to the antipsychotic haloperidol (Kesby et al., 2006). Haloperidol is a typical antipsychotic used to treat the positive symptoms of schizophrenia and its antipsychotic potency is directly related to the blockade of DA 2 receptors (Seeman and Lee, 1975; Creese et al., 1976). DA 2 receptors however, do not appear to be altered in DVD-deficient rats (Kesby et al., 2010) indicating the behavioral response to haloperidol in DVD-deficient rats is more complex than a simple change in receptor density. Overexpression of DA 2 receptors in schizophrenia appears to have only a small effect size (Laruelle, 1998; Seeman and Kapur, 2000) and as such is not necessarily a key feature of the disease even though all antipsychotic drugs target these receptors. Thus, DVD deficiency induces persistent post-adolescent sensitivity to the behavioral effects of dopaminergic drugs that appears to mirror the post-adolescent onset of frank psychotic symptoms in schizophrenia patients and these sensitivities can be attenuated with the use of antipsychotic drugs.

Aspects of learning and memory are also affected in DVD-deficient rats. Latent inhibition refers to a learning phenomenon describing how it takes longer to associate relevance to a familiar stimulus than a novel stimulus. DVD-deficient rats have impaired latent inhibition (Becker et al., 2005) suggesting a deficit in the ability to attend selectively to relevant stimuli. Acutely psychotic patients also show impairments in latent inhibition (Gray et al., 1991; Lubow and Gewirtz, 1995) and DA agonists have been shown to decrease latent inhibition in healthy adult males (Swerdlow et al., 2003). Moreover, DVD-deficient rats show increased impulsivity and a lack of inhibitory control when assessed on the 5-choice continuous performance task (Turner et al., 2013). The increased impulsivity in DVD-deficient rats can also be attenuated with the atypical antipsychotic, clozapine. Impulsivity in healthy humans has been associated with the availability of the DAT (Costa et al., 2012) and in rats; DA receptors in the medial PFC also appear to be critical (Pardey et al., 2012). Thus multiple DA-based behavioral alterations are present in the adult DVD-deficient rat. However, other neurotransmitter systems, closely linked to the DA system, also appear to be affected in DVD-deficient rats.

ALTERNATIVE NEUROTRANSMITTER SYSTEM ALTERATIONS

The use of N-methyl-D-aspartic acid (NMDA) receptor antagonists such as PCP, ketamine and MK-801 in animal models has become more widespread because the symptoms elicited in healthy people are more similar to those seen in people with schizophrenia than those observed after amphetamine (Krystal et al., 1994; Lahti et al., 2001). As a result, NMDA receptor hypofunction models of schizophrenia have been proposed and are also widely studied (Olney and Farber, 1995). DVD-deficient rats show a consistently enhanced locomotor response to MK-801(Kesby et al., 2006; O'Loan et al., 2007; Kesby et al., 2011). Importantly, this behavioral sensitivity is heavily dependent on the timing of vitamin D deficiency. Vitamin D deficiency in the later portion of gestation is required to elicit this behavioral sensitivity whereas vitamin D deficiency during the early portion of gestation has no impact (O'Loan et al., 2007). Coincidently, the later portion of gestation includes active DA neuron migration, differentiation and innervation in the embryonic brain. Although it is fairly clear that DA release is not required for the effects of MK-801 on locomotion (Carlsson and Carlsson, 1989), DA receptor antagonists have been shown to attenuate MK-801-induced behavior (Criswell et al., 1993; Willins et al., 1993; Andine et al., 1999; Kesby et al., 2006). Consistent with this, the enhanced locomotor response to MK-801 in DVD-deficient rats is selectively blocked by pretreatment with the DA 2 receptor antagonist haloperidol, at a dose that does not significantly attenuate MK-801-induced locomotion in control rats (Kesby et al., 2006). This suggests that abnormal

DA signaling remains a component of the enhanced response to MK-801 consistently observed in DVD-deficient rats.

In Summary, DVD deficiency results in multiple outcomes in the adult animal that suggest neurotransmission and neuron integrity may be compromised. In addition, there is strong evidence that the altered response to psychomimetic drugs in adult DVD-deficient rats appears closely linked to DA function. The mechanism for how the developmental absence of vitamin D may influence DA signaling in the adult remains unknown. However given the multiple pieces of evidence indicating early alterations in the ontogeny of DA systems in this model we suspect this may hold the key.

LINKING DOPAMINE ABNORMALITIES TO DVD DEFICIENCY

EARLY VITAMIN D SIGNALING AND DOPAMINE

Vitamin D is a nuclear steroid. Its signaling is via a single nuclear receptor called the VDR which is expressed widely throughout the human (Sutherland et al., 1992; Zehnder et al., 2001; Eyles et al., 2005) and rat brain (Clemens et al., 1988; Fu et al., 1997; Prufer et al., 1999). The VDR shares structural characteristics with other nuclear steroid receptors (Mangelsdorf et al., 1995). After ligand binding the VDR forms a heterodimer with the retinoid X receptor (RXR). This complex binds to vitamin D response elements (VDRE) in the promoters of a number of genes; to regulate their transcription (Christakos et al., 2003). Expression of the VDR begins early in development (Fu et al., 1997; Veenstra et al., 1998; Erben et al., 2002; Burket et al., 2003; Cui et al., 2013) and increasing levels of VDR coincide with increasing levels of apoptosis and decreasing levels of mitosis (Fu et al., 1997; Veenstra et al., 1998; Erben et al., 2002; Burket et al., 2003). However, it is the coincident expression of the VDR within developing DA neurons (Cui et al., 2013) and projections in the brain that suggest an important role for vitamin D in the developing DA system.

Effects of Vitamin D on dopamine differentiation and innervation

Expression of the VDR can be found as early as E12 in the neuroepithelium (Veenstra et al., 1998) coinciding with the peak differentiation of monoamine cells in the substantia nigra; the primary source of midbrain dopaminergic projections to the basal ganglia (Lauder and Bloom, 1974; Gates et al., 2006). As dividing mesencephalic DA progenitor cells stop proliferating, they immediately begin to express specification factors [initially Nurr1 (Joseph et al., 2003) with p57Kip2 expressed soon after (Wallen et al., 1999)] that help to establish the neurotransmitter phenotype of these cells. DVD-deficient E12 embryos show decreased gene expression of Nurr1, p57Kip2 and TH (Cui et al., 2010) suggesting altered vitamin D signaling affects early monoamine cell development, perhaps even prior to E12. Not surprisingly, all three of these factors are linked and it would appear that Nurr1 is the upstream effector that results in altered p57Kip2 and TH levels. For example, Nurr1 has been shown to activate the expression of p57kip2 which then cooperates with Nurr1 in the maintenance of DA neurons (Joseph et al., 2003). Moreover, Nurr1 has been shown to regulate important proteins in DA synthesis and function including TH, vesicular monoamine transporter 2 (VMAT2) and DAT (Smidt and Burbach, 2007). Thus a decrease in Nurr1 expression would be expected to result in decreased p57Kip2 and TH as found in the DVD-deficient embryo.

Monoaminergic striatal innervation occurs from E14-17 (Voorn et al., 1988) with functional release observed at E18 (Nomura et al., 1981). Consistent with the premise that vitamin D plays a role in dopaminergic cell development, VDR expression in the differentiating field of the midbrain and basal ganglia can be observed by E15 (Veenstra et al., 1998). Furthermore, DVD-deficient embryos show decreased expression of Nurr1 at E15 (Cui et al., 2010). Thus, the appearance of the nuclear expression of the VDR in the mesencephalon at the peak period of DA neuron differentiation raises the possibility that the absence of vitamin D at this point may lead to changes consistent with the absence of this ligand. Namely, increased rates of DA neuron proliferation and delayed differentiation. This is consistent with the reduction in the expression of post-mitotic specification factors such as Nurr1. Interestingly, although Nurr1 gene expression in the mesencephalon peaks from E13 to E15 (Volpicelli et al., 2004) the levels of Nurr1 in the developing rat cortex show a different temporal window of expression with peak protein levels occurring later at P1 (Li et al., 2011). Whether the levels of cortical Nurr1 are decreased or delayed as observed in the mesencephalon of DVD-deficient rats is currently unknown.

How the absence (or presence) of vitamin D could alter Nurr1 levels remains unknown. However, retinoid function and specifically the interactions of retinoid receptors and Nurr1 have led researchers to suggest that retinoid signaling may be one link between the genetic and environmental susceptibility to schizophrenia (Palha and Goodman, 2006). Both Nurr1 and the VDR form heterodimers with the RXR (Mangelsdorf et al., 1995; Perlmann and Jansson, 1995; Aarnisalo et al., 2002). Indeed, signaling through the RXR-Nurr1 heterodimer is involved in the neuroprotective actions of Nurr1 in DA neurons (Wallen-Mackenzie et al., 2003). However, in rat neural precursor cells the RXR-Nurr1 heterodimer has been shown to *reduce* Nurr1 activity in DA neuron generation and *reduce* TH promoter activity (Yoon et al., 2010). It is important to note that levels of the VDR are unaltered in DVD-deficient pups (Eyles et al., 2003) allowing for ligand-independent actions. The interactions between, and functions of, the VDR and RXR appear to be extremely dependent on the presence of vitamin D. For example, the VDR-RXR heterodimer with no ligand acts as a weak transcriptional repressor (Tagami et al., 1998). Furthermore, when no ligand is present the RXR increases the nuclear accumulation of VDR by slowing nuclear export whereas, when bound to vitamin D, the VDR regulates the import of the RXR into the nucleus (Prufer and Barsony, 2002). Thus the non-ligand bound VDR may lead to decreased levels of cytosolic VDR and reduced competition for RXR compared with Nurr1. This may lead to an increase in the inhibitory functions of the RXR-Nurr1 heterodimer on DA neuron generation (Yoon et al., 2010). Decreased DA neuron generation would subsequently lead to reduced levels of Nurr1 and TH as found in DVD-deficient embryos. The presence and interaction of the VDR (minus ligand) on the availability or function of the RXR-Nurr1 heterodimer is unknown but this remains an intriguing target.

In addition, recent work also suggests that Nurr1 expression induces the expression of the glial derived neurotrophic factor

(GDNF) receptor, Ret, in adult nigral DA neurons (Decressac et al., 2012). The decreased Nurr1 levels may therefore decrease GDNF signaling in DVD-deficient embryos. Moreover, evidence continues to accumulate indicating vitamin D positively regulates GDNF levels in the developing mesencephalon (Orme et al., 2013). GDNF is an important factor involved in DA neuron development, survival and function (Lin et al., 1993; Chun et al., 2002; Kholodilov et al., 2004). Thus, the combined local expression these three factors (VDR, RXR, and Nurr1) in addition to their cooperative signaling capabilities may be causal for the downstream effects of DVD-deficiency on DA neuron development.

Effects of Vitamin D on postnatal dopamine events

Developing DA neurons undergo two postnatal phases of natural cell death (Oo and Burke, 1997); the first peak is around postnatal day (P) 2 and the second peak occurs around P14. The combination of these two phases of cell death determines the number of DA cells within the adult brain. There are various regulatory factors involved in this process. For example, the first of these stages is heavily regulated by GDNF levels (Oo et al., 2003) and interactions with striatal targets (Burke, 2004). The second stage is less well understood but the dependence on striatal targets appears to remain (Burke, 2004). These processes establish DA neuron number and functional connectivity in the juvenile animal. Vitamin D has been shown to increase GDNF synthesis in the brain (Wang et al., 2000). Given the importance of GDNF in the postnatal programmed cell death of DA neurons in addition to its roles in DA neuron development, survival and function (Lin et al., 1993; Chun et al., 2002; Kholodilov et al., 2004); this represents a potential factor that could permanently alter key dopaminergic regions that contribute to adult behavior. We assume the levels of Nurr1 in DVD-deficient rats would have returned to control levels by this stage given the trend toward this in embryonic development (Cui et al., 2010). However, any consequence of earlier Nurr1 signaling deficits on the expression of the GDNF receptor, Ret, may persevere. Subsequently, this may lead to reductions in GDNF signaling during these crucial phases of DA cell death in DVD-deficient rats.

These DA cell death events are also dependent on the functional interaction of DA neurons and their striatal targets (Burke, 2004). Therefore, compromised neuronal signaling after DVD deficiency could potentially impact the survival of DA neurons. For example, silencing the VDR in cortical cultures from E16 embryos disrupts L-type voltage-sensitive calcium channels which are involved in calcium homeostasis and neuronal integrity (Gezen-Ak et al., 2011). Furthermore, DVD-deficient pups also show altered DA turnover as a result of decreased levels of COMT (Kesby et al., 2009). Both of these vitamin D-induced alterations could potentially lead to altered signaling in DA neurons. Thus altered DA signaling at birth suggests that the early postnatal period may represent an early developmental window whereby permanent alterations in DA function may lead to altered adult behavior.

AN ALTERED DEVELOPMENTAL TRAJECTORY

Schizophrenia

One key facet of schizophrenia is the post-adolescent onset of "psychosis" or psychotic symptoms (Delisi, 1992). The wealth of epidemiological evidence for a neurodevelopmental pathogenesis as described throughout this review needs to be considered in the light of the post-adolescent onset of disease (Andreasen, 1995). The brain undergoes a high level of reorganization throughout adolescence (Spear, 2000; Andersen, 2003; Adriani and Laviola, 2004). Thus, the normal processes involved in brain maturation may be compromised such that the refinement of projections and signaling pathways uncovers or reveals an underlying dysfunction, such as altered genetic architecture (Lee et al., 2012) or subtle changes in neurochemical function (Howes et al., 2006; Howes et al., 2009).

The period of adolescent maturation is of particular importance to the clinical onset of psychotic disorders such as schizophrenia. For example, phencyclidine and ketamine (i.e., NMDA receptor antagonists) fail to produce hallucinations in prepubertal children, however they routinely do in adults (Hirsch et al., 1997). Thus the underlying connectivity/neurotransmission required for these drugs to elicit psychosis analogous to that seen in schizophrenia may not be functional until after adolescence. Obviously the presence of early onset schizophrenia suggests that these same processes *can* be present prior to adolescence but the fact remains that subtle alterations in cytoarchitecture, neurotransmission or brain connectivity may not yield a psychotic phenotype until these maturational processes are established. Importantly, the prodromal phase of schizophrenia that overlaps with this period of maturation is now being linked to dopaminergic abnormalities. For example, individuals at ultra-high risk of developing schizophrenia show increased striatal DA synthesis (Howes et al., 2006, 2009). There is also both behavioral and structural evidence that brain development is altered in people prior to disease onset. For example, behavioral abnormalities, IQ and social deficits have been described in children who later develop the disease (Aylward et al., 1984; Done et al., 1994; Ellenbroek and Cools, 1998; Murray and Fearon, 1999; Cannon et al., 2002, 2006). Moreover, at the onset of psychosis there are already changes in the gross anatomy of patients' brains. For example, the lateral ventricles are increased in size (Chua et al., 2007; Nakamura et al., 2007; Pagsberg et al., 2007) and the cortex of schizophrenia patients frequently has decreased white and gray matter volume (Gur et al., 2000; Pantelis et al., 2003; Chua et al., 2007; Nakamura et al., 2007; Pagsberg et al., 2007). More recently two studies have shown that decreases in the gray matter volume of the parietal cortex and hippocampus precede the onset of psychosis in prodromal patients (Mechelli et al., 2011; Dazzan et al., 2012). Thus, there are a number of changes reflecting altered brain development and these appear to be present well before the onset of the symptoms required for clinical diagnosis.

Although cases of early onset schizophrenia suggest these adolescent maturational processes are not "key" per se, they still seem to play a significant role. For example, the decreases in cortical thickness found in early onset schizophrenia become more localized and more akin to those seen in adult onset schizophrenia when these patients reach adulthood (Greenstein et al., 2006) suggesting a role for adolescent maturation even in those with early onset schizophrenia. Furthermore, these examples also support a hierarchy of susceptibility in that highly susceptible individuals may be compromised earlier in life whereas others may require further extended environmental or developmental stressors to

elicit frank symptoms. The fact that the enlarged lateral ventricles in DVD-deficient rats only persist to adulthood when the period of vitamin D deficiency is extended to weaning (Eyles et al., 2003; Feron et al., 2005) supports a titratable approach to brain susceptibility. In addition, it also suggests that further study on the temporal window of vitamin D deficiency would be extremely informative.

Rodent analogs

There are notable similarities between adolescent/sexual maturation in rodents and humans. For example, the course of sexual maturation in rodents is preceded by the overproduction of synapses and accompanied by their subsequent elimination (Andersen et al., 1997). These dynamic changes in receptor density are thought to reflect the focussing and strengthening of synaptic connections required for adult life. This also occurs in humans with an estimated loss of almost one-half of the average number of synapses per cortical neuron over the adolescent period (Rakic et al., 1994). This period therefore represents a window whereby external influences prior to, rather than after, can differentially impact on adult brain function in both rodents and humans (Spear, 2000; Andersen, 2003; Adriani and Laviola, 2004). Of particular interest to both DVD deficiency and schizophrenia are the dynamic changes observed in the DA system over this period. For example, in the rat the density of both D1 and D2 receptors increase in the striatum prior to puberty, followed by their decline during puberty (Andersen et al., 1997). However, it is important to note that the development and maturation of the DA system is a dynamic process with behavioral and neurochemical responses continuing to change in rats for years after birth (Hebert and Gerhardt, 1999; Rutz et al., 2009).

Akin to drug sensitivities and the psychotic symptoms observed in schizophrenia, postpubertal psychomotor sensitivities to drugs such as amphetamine and MK-801 have been found in developmental animal models after DVD deficiency (Kesby et al., 2010), gestational disruptions in neurogenesis (Flagstad et al., 2004), neonatal ventral hippocampal lesions (Al-Amin et al., 2001) and prenatal Poly I:C administration (Meyer et al., 2008). Taken together these suggest a myriad of interventions can result in psychotic-like drug sensitivities that become observable after adolescence. Perhaps the most important aspect of these models is that they do not include any additional "stressor" during the adolescent period and are the sole results of early life interventions that alter normal brain development. Early intervention with antipsychotic treatment has already been shown to attenuate structural and behavioral abnormalities after prenatal Poly I:C administration (Piontkewitz et al., 2012) emphasizing that the developmental cascade prior to adolescence is critical to the "schizophrenia phenotype." Furthermore, these models also provide the ability to investigate developmental abnormalities induced by a range of interventions and identify the convergent etiological pathways that result in similar adult behavioral phenotypes.

DOPAMINE: A COMMON ENDPOINT OR A COMMON BEGINNING

The premise that no single genetic vulnerability or molecular factor "causes" schizophrenia is well accepted amongst the research community and is confirmed by both the heterogeneity of the symptom profile and the lack of a diagnostic marker. A common endpoint, that includes aspects of DA dysfunction (Di Forti et al., 2007; Murray et al., 2008; Howes and Kapur, 2009), remains highly supported by the clinical evidence and allows for a specific outcome to investigate the etiology of the disease. However, we postulate that a common DA endpoint may arise precisely because it is central to the developmental pathology (**Figure 1**). The dopaminergic system is one of the most organized neurotransmitter systems in the brain and is fundamental for a range of functions involved in cognition, motivation and reward (Smith and Kieval, 2000; Aldridge et al., 2004; Nicola, 2007). Furthermore, alterations in DA signaling have a range of cascading effects on other neurotransmitter systems such as glutamate (e.g., NMDA) and GABA. Therefore, even small alterations in DA function or organization have the potential to lead to complex cognitive outcomes when coupled with other general and variable insults such as altered genetic architecture, drug use, stress or adolescent maturation. In addition, these secondary stressors may individually produce differing phenotypes and thus heterogeneity in symptom profile.

Our work the in DVD-deficient rat model suggests that the developmental absence of this ligand produces discrete alterations in how DA systems develop. Alterations in DA specification factors (Cui et al., 2010) and DA metabolism (Kesby et al., 2009) induced by DVD deficiency could have lasting influences on the DA signaling. Additionally, these animals show behavioral sensitivities to psychomimetic drugs at adulthood (Kesby et al., 2006, 2010, 2011; O'Loan et al., 2007) which model at least the positive symptoms of schizophrenia (Laruelle and Abi-Dargham, 1999; Lahti et al., 2001). Moreover, another developmental model, that utilizes PolyI:C to mimic prenatal infection, also shows alterations in Nurr 1, and similar behavioral sensitivities to these psychomimetic drugs in adulthood (Meyer et al., 2008; Vuillermot et al., 2010). Taken together this suggests that vastly differing developmental insults can cause similar phenotypes and perhaps even converge on common early mechanisms (Eyles et al., 2012). Thus a network of small communication errors, perhaps via the interaction of a variety of key receptors (RXR, VDR, Nurr1, Ret) result in an altered developmental landscape. This may lead to an altered number of DA neurons after perinatal cell death events or altered DA functionality/connectivity.

The data amassed in the DVD-deficient rat suggest that subtle developmental alterations in DA function can lead to an altered adult behavioral phenotype. Moreover, utilizing differing developmental insults, animal models have demonstrated similar developmental dopaminergic abnormalities and adult behavioral phenotypes. Therefore, schizophrenia may be a disorder occurring via genetic or environmental factors that features a common early disruption in DA development. The emerging data suggesting that altered DA function precedes the onset of psychosis further suggests that dopaminergic abnormalities are not a byproduct of psychosis but rather a latent signal of altered DA development. Thus, altered dopaminergic function in schizophrenia may represent a potential biological marker and even a target for intervention.

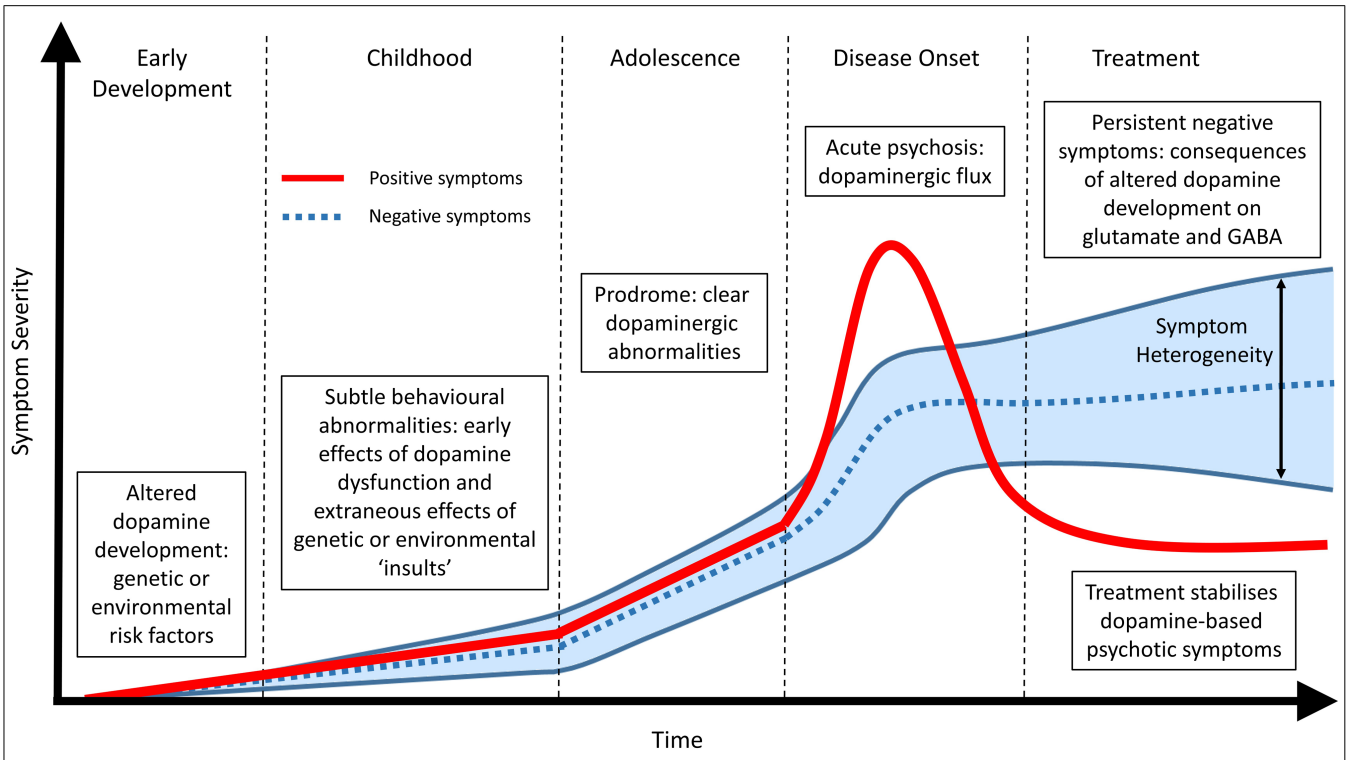

FIGURE 1 | Temporal profile of developing schizophrenia symptoms. Early alterations in dopamine development due to genetic, environmental or a combination of both lead to abnormalities in dopamine function (positive symptoms) and subsequent alterations in other neurotransmitter systems (negative symptoms). During adolescence and the prodromal phase of the disease clear changes in dopamine function can be observed. Frank psychosis and disease onset are directly related to dopaminergic function and can be effectively treated. The lack of effect of antipsychotic treatment on persisting negative symptoms suggest they are not directly related to dopamine function. Rather, they represent the downstream consequence of early dopamine dysfunction or the extraneous effects of specific genetic and environmental risk factors on other neurotransmitter systems such as glutamate and GABA. These non-specific actions, outside of the core schizophrenia etiology, result in a large heterogeneous profile of negative symptoms in patients.

CONCLUSION

Schizophrenia is an extremely complex disorder. We are not suggesting that future therapeutic interventions be limited to a simple "fix DA early and fix schizophrenia" interpretation. However, observations in the DVD-deficient rat, alternative animal models and existing clinical evidence suggests that a core/common feature may be early DA dysfunction. That this could originate from an early alteration in DA development is not a radical premise but the concept itself is extremely hard to assess in humans for a variety of obvious temporal and ethical reasons. However, understanding how the potential cascade of events after early alterations in DA neuron development influence other neurotransmitter systems in animal models, such as the DVD-deficient rat, may increase our understanding of the actual etiology of schizophrenia. Furthermore, subtle alterations to DA development may provide a platform to learn and understand the variable outcomes associated with a range of "second-hit" targets. The future of basic schizophrenia research should be focused on early dopaminergic development with the goal of further understanding existing and underlying abnormalities in schizophrenia patients that may, in turn, direct treatment solutions in the clinic.

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REFERENCES

Aarnisalo, P., Kim, C. H., Lee, J. W., and Perlmann, T. (2002). Defining requirements for heterodimerization between the retinoid X receptor and the orphan nuclear receptor Nurr1. *J. Biol. Chem.* 277, 35118–35123. doi: 10.1074/jbc.M201707200

Abi-Dargham, A., Gil, R., Krystal, J., Baldwin, R. M., Seibyl, J. P., Bowers, M., et al. (1998). Increased striatal dopamine transmission in schizophrenia: confirmation in a second cohort. *Am. J. Psychiat.* 155, 761–767.

Abi-Dargham, A., van de Giessen, E., Slifstein, M., Kegeles, L. S., and Laruelle, M. (2009). Baseline and amphetamine-stimulated dopamine activity are related in drug-naive schizophrenic subjects. *Biol. Psychiatry* 65, 1091–1093. doi: 10.1016/j.biopsych.2008.12.007

Adriani, W., and Laviola, G. (2004). Windows of vulnerability to psychopathology and therapeutic strategy in the adolescent rodent model. *Behav. Pharmacol.* 15, 341–352. doi: 10.1097/00008877-200409000-00005

Al-Amin, H. A., Weickert, C. S., Weinberger, D. R., and Lipska, B. K. (2001). Delayed onset of enhanced MK-801-induced motor hyperactivity after neonatal lesions of the rat ventral hippocampus. *Biol. Psychiatry* 49, 528–539. doi: 10.1016/S0006-3223(00)00968-9

Aldridge, J. W., Berridge, K. C., and Rosen, A. R. (2004). Basal

ganglia neural mechanisms of natural movement sequences. *Can. J. Physiol. Pharmacol.* 82, 732–739. doi: 10.1139/y04-061

Andersen, S. L. (2003). Trajectories of brain development: point of vulnerability or window of opportunity? *Neurosci. Biobehav. Rev.* 27, 3–18. doi: 10.1016/S0149-7634(03)00005-8

Andersen, S. L., Rutstein, M., Benzo, J. M., Hostetter, J. C., and Teicher, M. H. (1997). Sex differences in dopamine receptor overproduction and elimination. *Neuroreport* 8, 1495–1498. doi: 10.1097/00001756-199704140-00034

Andine, P., Widermark, N., Axelsson, R., Nyberg, G., Olofsson, U., Martensson, E., et al. (1999). Characterization of MK-801-induced behavior as a putative rat model of psychosis. *J. Pharmacol. Exp. Ther.* 290, 1393–1408.

Andreasen, N. C. (1995). Symptoms, signs, and diagnosis of schizophrenia. *Lancet* 346, 477–481. doi: 10.1016/S0140-6736(95)91325-4

Angrist, B., and Vankammen, D. P. (1984). Cns stimulants as tools in the study of schizophrenia. *Trends Neurosci.* 7, 388–390. doi: 10.1016/S0166-2236(84)80062-4

Aylward, E., Walker, E., and Bettes, B. (1984). Intelligence in schizophrenia: meta-analysis of the research. *Schizophr. Bull.* 10, 430–459. doi: 10.1093/schbul/10.3.430

Banerjee, P., and Chatterjee, M. (2003). Antiproliferative role of vitamin D and its analogs – a brief overview. *Mol. Cell. Biochem.* 253, 247–254. doi: 10.1023/A:1026072118217

Becker, A., Eyles, D. W., McGrath, J. J., and Grecksch, G. (2005). Transient prenatal vitamin D deficiency is associated with subtle alterations in learning and memory functions in adult rats. *Behav. Brain Res.* 161, 306–312. doi: 10.1016/j.bbr.2005.02.015

Bernert, G., Hoeger, H., Mosgoeller, W., Stolzlechner, D., and Lubec, B. (2003). Neurodegeneration, neuronal loss, and neurotransmitter changes in the adult guinea pig with perinatal asphyxia. *Pediatr. Res.* 54, 523–528. doi: 10.1203/01.PDR.0000081760.48331.7A

Bjelke, B., Andersson, K., Ogren, S. O., and Bolme, P. (1991). Asphyctic Lesion – Proliferation of tyrosine hydroxylase-immunoreactive nerve-cell bodies in the rat substantia-nigra and functional-changes in dopamine neurotransmission. *Brain Res.* 543, 1–9. doi: 10.1016/0006-8993(91)91041-X

Boksa, P., and El-Khodor, B. F. (2003). Birth insult interacts with stress at adulthood to alter dopaminergic function in animal models: possible implications for schizophrenia and other disorders. *Neurosci. Biobehav. Rev.* 27, 91–101. doi: 10.1016/S0149-7634(03)00012-5

Breier, A., Su, T. P., Saunders, R., Carson, R. E., Kolachana, B. S., deBartolomeis, A., et al. (1997). Schizophrenia is associated with elevated amphetamine-induced synaptic dopamine concentrations: evidence from a novel positron emission tomography method. *Proc. Natl. Acad. Sci. U.S.A.* 94, 2569–2574. doi: 10.1073/pnas.94.6.2569

Burke, R. E. (2004). Ontogenic cell death in the nigrostriatal system. *Cell Tissue Res.* 318, 63–72. doi: 10.1007/s00441-004-0908-4

Burket, R., McGrath, J., and Eyles, D. (2003). Vitamin D receptor expression in the embryonic rat brain. *Neurosci. Res. Commun.* 33, 63–71. doi: 10.1002/nrc.10081

Burne, T. H. J., Becker, A., Brown, J., Eyles, D. W., Mackay-Sim, A., and McGrath, J. J. (2004). Transient prenatal Vitamin D deficiency is associated with hyperlocomotion in adult rats. *Behav. Brain Res.* 154, 549–555. doi: 10.1016/j.bbr.2004.03.023

Burne, T. H. J., O'Loan, J., McGrath, J. J., and Eyles, D. W. (2006). Hyperlocomotion associated with transient prenatal vitamin D deficiency is ameliorated by acute restraint. *Behav. Brain Res.* 174, 119–124. doi: 10.1016/j.bbr.2006.07.015

Burne, T. H. J., O'Loan, J., Splatt, K., Alexander, S., McGrath, J. J., and Eyles, D. W. (2011). Developmental vitamin D (DVD) deficiency alters pup-retrieval but not isolation-induced pup ultrasonic vocalizations in the rat. *Physiol. Behav.* 102, 201–204. doi: 10.1016/j.physbeh.2010.11.006

Cannon, M., Caspi, A., Moffitt, T. E., Harrington, H., Taylor, A., Murray, R. M., et al. (2002). Evidence for early-childhood, pan-developmental impairment specific to schizophreniform disorder – results from a longitudinal birth cohort. *Arch. Gen. Psychiatry* 59, 449–456. doi: 10.1001/archpsyc.59.5.449

Cannon, M., Moffitt, T. E., Caspi, A., Murray, R. M., Harrington, H., and Poulton, R. (2006). Neuropsychological performance at the age of 13 years and adult schizophreniform disorder – prospective birth cohort study. *Br. J. Psychiatry* 189, 463–464. doi: 10.1192/bjp.bp.105.020552

Cantor-Graae, E., and Selten, J. P. (2005). Schizophrenia and migration: a meta-analysis and review. *Am. J. Psychiatry* 162, 12–24. doi: 10.1176/appi.ajp.162.1.12

Carlsson, A., and Lindqvist, M. (1963). Effect of chlorpromazine or haloperidol on formation of 3methoxytyramine and normetanephrine in mouse brain. *Acta Pharmacol. Toxicol. (Copenh)* 20, 140–144. doi: 10.1111/j.1600-0773.1963.tb01730.x

Carlsson, M., and Carlsson, A. (1989). The NMDA antagonist MK-801 causes marked locomotor stimulation in monoamine-depleted mice. *J. Neural Transm.* 75, 221–226. doi: 10.1007/BF01258633

Chefer, V. I., Zakharova, I., and Shippenberg, T. S. (2003). Enhanced responsiveness to novelty and cocaine is associated with decreased basal dopamine uptake and release in the nucleus accumbens: quantitative microdialysis in rats under transient conditions. *J. Neurosci.* 23, 3076–3084.

Christakos, S., Dhawan, P., Liu, Y., Peng, X. R., and Porta, A. (2003). New insights into the mechanisms of vitamin D action. *J. Cell. Biochem.* 88, 695–705. doi: 10.1002/jcb.10423

Chua, S. E., Cheung, C., Cheung, V., Tsang, J. T. K., Chen, E. Y. H., Wong, J. C. H., et al. (2007). Cerebral grey, white matter and csf in never-medicated, first-episode schizophrenia. *Schizophr. Res.* 89, 12–21. doi: 10.1016/j.schres.2006.09.009

Chun, H. S., Yoo, M. S., DeGiorgio, L. A., Volpe, B. T., Peng, D., Baker, H., et al. (2002). Marked dopaminergic cell loss subsequent to developmental, intranigral expression of glial cell line-derived neurotrophic factor. *Exp. Neurol.* 173, 235–244. doi: 10.1006/exnr.2001.7842

Clemens, T. L., Adams, J. S., Henderson, S. L., and Holick, M. F. (1982). Increased skin pigment reduces the capacity of skin to synthesise vitamin D3. *Lancet* 1, 74–76. doi: 10.1016/S0140-6736(82)90214-8

Clemens, T. L., Garrett, K. P., Zhou, X. Y., Pike, J. W., Haussler, M. R., and Dempster, D. W. (1988). Immunocytochemical localization of the 1,25-dihydroxyvitamin D3 receptor in target cells. *Endocrinology* 122, 1224–1230. doi: 10.1210/endo-122-4-1224

Costa, A., la Fougere, C., Pogarell, O., Moller, H. J., Riedel, M., and Ettinger, U. (2012). Impulsivity is related to striatal dopamine transporter availability in healthy males. *Psychiatry Res.* 211, 251–256. doi: 10.1016/j.pscychresns.2012.07.011

Creese, I., Burt, D. R., and Snyder, S. H. (1976). Dopamine receptor binding predicts clinical and pharmacological potencies of antischizophrenic drugs. *Science* 192, 481–483. doi: 10.1126/science.3854

Criswell, H. E., Johnson, K. B., Mueller, R. A., and Breese, G. R. (1993). Evidence for involvement of brain dopamine and other mechanisms in the behavioral action of the *N*-Methyl-D-aspartic acid antagonist Mk-801 in control and 6-hydroxydopamine-lesioned rats. *J. Pharmacol. Exp. Ther.* 265, 1001–1010.

Cui, X., Pelekanos, M., Liu, P.-Y., Burne, T. H. J., McGrath, J. J., and Eyles, D. W. (2013). The vitamin D receptor in dopamine neurons; its presence in human substantia nigra and its ontogenesis in rat midbrain. *Neuroscience* 236, 77–87. doi: 10.1016/j.neuroscience.2013.01.035

Cui, X. Y., McGrath, J. J., Burne, T. H. J., Mackay-Sim, A., and Eyles, D. W. (2007). Maternal vitamin D depletion alters neurogenesis in the developing rat brain. *Int. J. Dev. Neurosci.* 25, 227–232. doi: 10.1016/j.ijdevneu.2007.03.006

Cui, X. Y., Pelekanos, M., Burne, T. H. J., McGrath, J. J., and Eyles, D. W. (2010). Maternal vitamin D deficiency alters the expression of genes involved in dopamine specification in the developing rat mesencephalon. *Neurosci. Lett.* 486, 220–223. doi: 10.1016/j.neulet.2010.09.057

Davies, G., Welham, J., Chant, D., Torrey, E. F., and McGrath, J. (2003). A systematic review and meta-analysis of Northern Hemisphere season of birth studies in schizophrenia. *Schizophr. Bull.* 29, 587–593. doi: 10.1093/oxfordjournals.schbul.a007030

Davis, K. L., Kahn, R. S., Ko, G., and Davidson, M. (1991). Dopamine in schizophrenia – a review and reconceptualization. *Am. J. Psychiatry* 148, 1474–1486.

Dazzan, P., Soulsby, B., Mechelli, A., Wood, S. J., Velakoulis, D., Phillips, L. J., et al. (2012). Volumetric abnormalities predating the onset of schizophrenia and affective psychoses: an MRI study in subjects at ultrahigh risk of psychosis. *Schizophr. Bull.* 38, 1083–1091. doi: 10.1093/schbul/sbr035

Decker, M. J., Hue, G. E., Caudle, W. M., Miller, G. W., Keating, G. L., and Rye, D. B. (2003). Episodic

neonatal hypoxia evokes executive dysfunction and regionally specific alterations in markers of dopamine signaling. *Neuroscience* 117, 417–425. doi: 10.1016/S0306-4522(02)00805-9

Decressac, M., Kadkhodaei, B., Mattsson, B., Laguna, A., Perlmann, T., and Bjorklund, A. (2012). α-synuclein-induced down-regulation of Nurr1 disrupts GDNF signaling in nigral dopamine neurons. *Sci. Transl. Med.* 4, 63ra156. doi: 10.1126/scitranslmed.3004676

Delisi, L. E. (1992). The significance of age of onset for schizophrenia. *Schizophr. Bull.* 18, 209–215. doi: 10.1093/schbul/18.2.209

Di Forti, M., Lappin, J. M., and Murray, R. M. (2007). Risk factors for schizophrenia – all roads lead to dopamine. *Eur. Neuropsychopharmology* 17, S101–S107. doi: 10.1016/j.euroneuro.2007.02.005

Done, D. J., Crow, T. J., Johnstone, E. C., and Sacker, A. (1994). Childhood antecedents of schizophrenia and affective illness: social adjustment at ages 7 and 11. *BMJ* 309, 699–703. doi: 10.1136/bmj.309.6956.699

Dusso, A. S., Brown, A. J., and Slatopolsky, E. (2005). Vitamin D. *Am. J. Physiol. Renal Physiol.* 289, F8–F28. doi: 10.1152/ajprenal.00336.2004

Ellenbroek, B. A., and Cools, A. R. (1998). The neurodevelopment hypothesis of schizophrenia: clinical evidence and animal models. *Neurosci. Res. Commun.* 22, 127–136. doi: 10.1002/(SICI)1520-6769(199805/06)22

Erben, R. G., Soegiarto, D. W., Weber, K., Zeitz, U., Lieberherr, M., Gniadecki, R., et al. (2002). Deletion of deoxyribonucleic acid binding domain of the vitamin D receptor abrogates genomic and nongenomic functions of vitamin D. *Mol. Endocrinol.* 16, 1524–1537. doi: 10.1210/me.16.7.1524

Eyles, D., Anderson, C., Ko, P., Jones, A., Thomas, A., Burne, T., et al. (2009). A sensitive LC/MS/MS assay of 250H vitamin D-3 and 250H vitamin D-2 in dried blood spots. *Clin. Chim. Acta* 403, 145–151. doi: 10.1016/j.cca.2009.02.005

Eyles, D., Brown, J., Mackay-Sim, A., McGrath, J., and Feron, F. (2003). Vitamin D-3 and brain development. *Neuroscience* 118, 641–653. doi: 10.1016/S0306-4522(03)00040-X

Eyles, D., Feldon, J., and Meyer, U. (2012). Schizophrenia: do all roads lead to dopamine or is this where they start? Evidence from two epidemiologically informed developmental rodent models. *Transl. Psychiatry* 2, e81. doi: 10.1038/tp.2012.6

Eyles, D. W., Smith, S., Kinobe, R., Hewison, M., and McGrath, J. J. (2005). Distribution of the vitamin D receptor and 1 alpha-hydroxylase in human brain. *J. Chem. Neuroanat.* 29, 21–30. doi: 10.1016/j.jchemneu.2004.08.006

Eyles, D. W., Rogers, F., Buller, K., McGrath, J. J., Ko, P., French, K., et al. (2006). Developmental vitamin D (DVD) deficiency in the rat alters adult behaviour independently of HPA function. *Psychoneuroendocrinology* 31, 958–964. doi: 10.1016/j.psyneuen.2006.05.006

Feenstra, M. G. P., Botterblom, M. H. A., and Vanuum, J. F. M. (1995). Novelty-induced increase in dopamine release in the rat prefrontal cortex in-vivo – inhibition by diazepam. *Neurosci. Lett.* 189, 81–84. doi: 10.1016/0304-3940(95)11456-7

Feron, F., Burne, T. H. J., Brown, J., Smith, E., McGrath, J. J., Mackay-Sim, A., et al. (2005). Developmental Vitamin D-3 deficiency alters the adult rat brain. *Brain Res. Bull.* 65, 141–148. doi: 10.1016/j.brainresbull.2004.12.007

Flagstad, P., Mork, A., Glenthoj, B. Y., van Beek, J., Michael-Titus, A. T., and Didriksen, M. (2004). Disruption of neurogenesis on gestational day 17 in the rat causes behavioral changes relevant to positive and negative schizophrenia symptoms and alters amphetamine-induced dopamine release in nucleus accumbens. *Neuropsychopharmacology* 29, 2052–2064. doi: 10.1038/sj.npp.1300516

Fu, G. K., Lin, D., Zhang, M. Y. H., Bikle, D. D., Shackleton, C. H. L., Miller, W. L., et al. (1997). Cloning of human 25-hydroxyvitamin D-1 alpha-hydroxylase and mutations causing vitamin D-dependent rickets type 1. *Mol. Endocrinol.* 11, 1961–1970. doi: 10.1210/me.11.13.1961

Fusar-Poli, P., and Meyer-Lindenberg, A. (2013). Striatal presynaptic dopamine in schizophrenia, Part II: meta-analysis of F-18/C-11 -DOPA PET studies. *Schizophr. Bull.* 39, 33–42. doi: 10.1093/schbul/sbr180

Gates, M. A., Torres, E. M., White, A., Fricker-Gates, R. A., and Dunnett, S. B. (2006). Re-examining the ontogeny of substantia nigra dopamine neurons. *Eur. J. Neurosci.* 23, 1384–1390. doi: 10.1111/j.1460-9568.2006.04637.x

Gezen-Ak, D., Dursun, E., and Yilmazer, S. (2011). The effects of vitamin D receptor silencing on the expression of LVSCC-A1C and LVSCC-A1D and the release of NGF in cortical neurons. *PLoS ONE* 6:e17553. doi: 10.1371/journal.pone.0017553

Gray, J. A., Feldon, J., Rawlins, J. N. P., Smith, A. D., and Hemsley, D. R. (1991). The neuropsychology of schizophrenia. *Behav. Brain Sci.* 14, 1–19. doi: 10.1017/S0140525X00065055

Greenstein, D., Lerch, J., Shaw, P., Clasen, L., Giedd, J., Gochman, P., et al. (2006). Childhood onset schizophrenia: cortical brain abnormalities as young adults. *J. Child Psychol. Psychiatry* 47, 1003–1012. doi: 10.1111/j.1469-7610.2006.01658.x

Gur, R. E., Turetsky, B. I., Cowell, P. E., Finkelman, C., Maany, V., Grossman, R. I., et al. (2000). Temporolimbic volume reductions in schizophrenia. *Arch. Gen. Psychiatry* 57, 769–775. doi: 10.1001/archpsyc.57.8.769

Hebert, M. A., and Gerhardt, G. A. (1999). Age-related changes in the capacity, rate, and modulation of dopamine uptake within the striatum and nucleus accumbens of Fischer 344 rats: an in vivo electrochemical study. *J. Pharmacol. Exp. Ther.* 288, 879–887.

Hirsch, S. R., Das, I., Garey, L. J., and deBelleroche, J. (1997). A pivotal role for glutamate in the pathogenesis of schizophrenia, and its cognitive dysfunction. *Pharmacol. Biochem. Behav.* 56, 797–802. doi: 10.1016/S0091-3057(96)00428-5

Holick, M. F., Matsuoka, L. Y., and Wortsman, J. (1995). Regular use of sunscreen on vitamin D levels. *Arch. Dermatol.* 131, 1337–1339. doi: 10.1001/archderm.131.11.1337

Hooks, M. S., and Kalivas, P. W. (1995). The Role of mesoaccumbens pallidal circuitry in novelty-induced behavioral activation. *Neuroscience* 64, 587–597. doi: 10.1016/0306-4522(94)00409-X

Howes, O., Bose, S., Turkheimer, F., Valli, I., Egerton, A., Stahl, D., et al. (2011a). Progressive increase in striatal dopamine synthesis capacity as patients develop psychosis: a PET study. *Mol. Psychiatry* 16, 885–886. doi: 10.1038/mp.2011.20

Howes, O. D., and Kapur, S. (2009). The dopamine hypothesis of schizophrenia: version III-025EFThe final common pathway. *Schizophr. Bull.* 35, 549–562. doi: 10.1093/schbul/sbp006

Howes, O. D., Montgomery, A. J., Asselin, M. C., Murray, R. M., Grasby, P. M., and McGuire, P. K. (2006). The pre-synaptic dopaminergic system before and after the onset of psychosis: initial results. *Eur. Neuropsychopharmacol.* 16, S177. doi: 10.1016/S0924-977X(06)70050-5

Howes, O. D., Kambeitz, J., Kim, E., Stahl, D., Slifstein, M., Abi-Dargham, A., et al. (2012). The nature of dopamine dysfunction in schizophrenia and what this means for treatment. *Arch. Gen. Psychiatry* 69, 776–786. doi: 10.1001/archgenpsychiatry.2012.169

Howes, O. D., Bose, S. K., Turkheimer, F., Valli, I., Egerton, A., Valmaggia, L. R., et al. (2011b). Dopamine synthesis capacity before onset of psychosis: a prospective (18)F -DOPA PET Imaging Study. *Am. J.. Psychiatry* 168, 1311–1317. doi: 10.1176/appi.ajp.2011.11010160

Howes, O. D., Montgomery, A. J., Asselin, M. C., Murray, R. M., Valli, I., Tabraham, P., et al. (2009). Elevated striatal dopamine function linked to prodromal signs of schizophrenia. *Arch. Gen. Psychiatry* 66, 13–20. doi: 10.1001/archgenpsychiatry.2008.514

Iwawaki, T., Kohno, K., and Kobayashi, K. (2000). Identification of a potential Nurr1 response element that activates the tyrosine hydroxylase gene promoter in cultured cells. *Biochem. Biophys. Res. Commun.* 274, 590–595. doi: 10.1006/bbrc.2000.3204

Janowsky, D. S., Elyousef, M. K., Davis, J. M., and Sekerke, H. J. (1973). Provocation of schizophrenic symptoms by intravenous administration of methylphenidate. *Arch. Gen. Psychiatry* 28, 185–191. doi: 10.1001/archpsyc.1973.01750320023004

Jones, S. R., Gainetdinov, R. R., Wightman, R. M., and Caron, M. G. (1998). Mechanisms of amphetamine action revealed in mice lacking the dopamine transporter. *J. Neurosci.* 18, 1979–1986.

Joseph, B., Wallen-Mackenzie, A., Benoit, G., Murata, T., Joodmardi, E., Okret, S., et al. (2003). p57(Kip2) cooperates with Nurr1 in developing dopamine cells. *Proc. Natl. Acad. Sci. U.S.A.* 100, 15619–15624. doi: 10.1073/pnas.2635658100

Kellendonk, C., Simpson, E. H., Polan, H. J., Malleret, G., Vronskaya, S., Winiger, V., et al. (2006). Transient and selective overexpression of dopamine D2 receptors in the striatum causes persistent abnormalities in prefrontal cortex functioning. *Neuron* 49, 603–615. doi: 10.1016/j.neuron.2006.01.023

Kesby, J. P., Burne, T. H. J., McGrath, J. J., and Eyles, D. W. (2006). Developmental vitamin D deficiency alters MK 801-induced hyperlocomotion in the adult rat: an animal model of schizophrenia.

Biol. Psychiatry 60, 591–596. doi: 10.1016/j.biopsych.2006.02.033

Kesby, J. P., Cui, X., Ko, P., McGrath, J. J., Burne, T. H. J., and Eyles, D. W. (2009). Developmental vitamin D deficiency alters dopamine turnover in neonatal rat forebrain. *Neurosci. Lett.* 461, 155–158. doi: 10.1016/j.neulet.2009.05.070

Kesby, J. P., Cui, X., O'Loan, J., McGrath, J. J., Burne, T. H. J., and Eyles, D. W. (2010). Developmental vitamin D deficiency alters dopamine-mediated behaviors and dopamine transporter function in adult female rats. *Psychopharmacology* 159–168. doi: 10.1007/s00213-009-1717-y

Kesby, J. P., O'Loan, J. C., Alexander, S., Deng, C., Huang, X. F., McGrath, J. J., et al. (2011). Developmental vitamin D deficiency alters MK-801-induced behaviours in adult offspring. *Psychopharmacology (Berl.)* 220, 455–463. doi: 10.1007/s00213-011-2492-0

Kholodilov, N., Yarygina, O., Oo, T. F., Zhang, H., Sulzer, D., Dauer, W., et al. (2004). Regulation of the development of mesencephalic dopaminergic systems by the selective expression of glial cell line-derived neurotrophic factor in their targets. *J. Neurosci.* 24, 3136–3146. doi: 10.1523/JNEUROSCI.4506-03.2004

Kim, K. S., Kim, C. H., Hwang, D. Y., Seo, H., Chung, S. M., Hong, S. J., et al. (2003). Orphan nuclear receptor Nurr1 directly transactivates the promoter activity of the tyrosine hydroxylase gene in a cell-specific manner. *J. Neurochem.* 85, 622–634. doi: 10.1046/j.1471-4159.2003.01671.x

Ko, P., Burkert, R., McGrath, J., and Eyles, D. (2004). Matemal vitamin D-3 deprivation and the regulation of apoptosis and cell cycle during rat brain development. *Brain Res. Dev. Brain Res.* 153, 61–68. doi: 10.1016/j.devbrainres.2004.07.013

Krystal, J. H., Karper, L. P., Seibyl, J. P., Freeman, G. K., Delaney, R., Bremner, J. D., et al. (1994). Subanesthetic effects of the noncompetitive NMDA antagonist, ketamine, in humans. Psychotomimetic, perceptual, cognitive, and neuroendocrine responses. *Arch. Gen.Psychiatry* 51, 199–214. doi: 10.1001/archpsyc.1994.03950030035004

Lahti, A. C., Weiler, M. A., Tamara Michaelidis, B. A., Parwani, A., and Tamminga, C. A. (2001). Effects of ketamine in normal and schizophrenic volunteers. *Neuropsychopharmacology* 25, 455–467. doi: 10.1016/S0893-133X(01)00243-3

Laruelle, M. (1998). Imaging dopamine transmission in schizophrenia. A review and meta-analysis. *Q. J. Nucl. Med.* 42, 211–221.

Laruelle, M., and Abi-Dargham, P. (1999). Dopamine as the wind of the psychotic fire: new evidence from brain imaging studies. *J. Psychopharmacol.* 13, 358–371. doi: 10.1177/026988119901300405

Laruelle, M., Abi-Dargham, A., Gil, R., Kegeles, L., and Innis, R. (1999). Increased dopamine transmission in schizophrenia: relationship to illness phases. *Biol. Psychiatry* 46, 56–72. doi: 10.1016/S0006-3223(99)00067-0

Laruelle, M., Abi-Dargham, A., van Dyck, C. H., Gil, R., D'Souza, C. D., Erdos, J., et al. (1996). Single photon emission computerized tomography imaging of amphetamine-induced dopamine release in drug-free schizophrenic subjects. *Proc. Natl. Acad. Sci. U.S.A.* 93, 9235–9240. doi: 10.1073/pnas.93.17.9235

Lauder, J. M., and Bloom, F. E. (1974). Ontogeny of monoamine neurons in locus coeruleus, raphe nuclei and substantia nigra of rat.1. Cell-Differentiation. *J. Comp. Neurol.* 155, 469–481. doi: 10.1002/cne.901550407

Lee, S. H., DeCandia, T. R., Ripke, S., Yang, J., Sullivan, P. F., Goddard, M. E., et al. (2012). Estimating the proportion of variation in susceptibility to schizophrenia captured by common SNPs. *Nat. Genet.* 44, U235–U247. doi: 10.1038/ng.1108

Li, Y. M., Cong, B., Ma, C. L., Qi, Q. A., Fu, L. H., Zhang, G. Z., et al. (2011). Expression of Nurr1 during rat brain and spinal cord development. *Neurosci. Lett.* 488, 49–54. doi: 10.1016/j.neulet.2010.10.078

Lieberman, J. A., Kane, J. M., and Alvir, J. (1987). Provocative tests with psychostimulant drugs in schizophrenia. *Psychopharmacology (Berl.)* 91, 415–433. doi: 10.1007/BF00216006

Lin, L. F. H., Doherty, D. H., Lile, J. D., Bektesh, S., and Collins, F. (1993). Gdnf – a glial-cell line derived neurotrophic factor for midbrain dopaminergic-neurons. *Science* 260, 1130–1132. doi: 10.1126/science.8493557

Lubow, R. E., and Gewirtz, J. C. (1995). Latent inhibition in humans – data, theory, and implications for schizophrenia. *Psychol. Bull.* 117, 87–103. doi: 10.1037/0033-2909.117.1.87

Mangelsdorf, D. J., Thummel, C., Beato, M., Herrlich, P., Schutz, G., Umesono, K., et al. (1995). The nuclear receptor superfamily – the 2nd decade. *Cell* 83, 835–839. doi: 10.1016/0092-8674(95)90199-X

McGrath, J. (1999). Hypotheses: is low prenatal vitamin D a risk-modifying factor for schizophrenia? *Schizophr. Res.* 40, 173–177. doi: 10.1016/S0920-9964(99)00052-3

McGrath, J. J., Kimlin, M. G., Saha, S., Eyles, D. W., and Parisi, A. V. (2001). Vitamin D insufficiency in south-east Queensland. *Med. J. Aust.* 174, 150–151.

McGrath, J. J., Saari, K., Hakko, H., Jokelainen, J., Jones, P. B., Jarvelin, M. R., et al. (2004). Vitamin D supplementation during the first year of life and risk of schizophrenia: a Finnish birth-cohort study. *Schizophr. Res.* 67, 16–16. doi: 10.1016/j.schres.2003.08.005

McGrath, J. J., Eyles, D. W., Pedersen, C. B., Anderson, C., Ko, P., Burne, T. H., et al. (2010). Neonatal vitamin D status and risk of schizophrenia: a population-based case-control study. *Arch. Gen. Psychiatry* 67, 889–894. doi: 10.1001/archgenpsychiatry.2010.110

Mechelli, A., Riecher-Rossler, A., Meisenzahl, E. M., Tognin, S., Wood, S. J., Borgwardt, S. J., et al. (2011). Neuroanatomical abnormalities that predate the onset of psychosis a multicenter study. *Arch. Gen. Psychiatry* 68, 489–495. doi: 10.1001/archgenpsychiatry.2011.42

Meyer, U., Feldon, J., and Fatemi, S. H. (2009) In-vivo rodent models for the experimental investigation of prenatal immune activation effects in neurodevelopmental brain disorders. *Neurosci .Biobehav. Rev.* 33, 1061–1079. doi: 10.1016/j.neubiorev.2009.05.001

Meyer, U., Nyffeler, M., Schwendener, S., Knuesel, I., Yee, B. K., and Feldon, J. (2008). Relative prenatal and postnatal maternal contributions to schizophrenia-related neurochemical dysfunction after in utero immune challenge. *Neuropsychopharmacology* 33, 441–456. doi: 10.1038/sj.npp.1301413

Murray, R. M., and Lewis, S. W. (1987). Is schizophrenia a neurodevelopmental disorder. *Br. Med. J.* 295, 681–682. doi: 10.1136/bmj.295.6600.681

Murray, R. M., and Fearon, P. (1999). The developmental 'risk factor' model of schizophrenia. *J. Psychiatr. Res.*33, 497–499. doi: 10.1016/S0022-3956(99)00032-1

Murray, R. M., Lappin, J., and Di Forti, M. (2008). Schizophrenia: from developmental deviance to dopamine dysregulation. *Eur. Neuropsychopharmacol.* 18, S129–S134. doi: 10.1016/j.euroneuro.2008.04.002

Nakamura, M., Salisbury, D. F., Hirayasu, Y., Bouix, S., Pohl, K. M., Yoshida, T., et al. (2007). Neocortical gray matter volume in first-episode schizophrenia and first-episode affective psychosis: a cross-sectional and longitudinal MRI study. *Biol. Psychiatry* 62, 773–783. doi: 10.1016/j.biopsych.2007.03.030

Nicola, S. M. (2007). The nucleus accumbens as part of a basal ganglia action selection circuit. *Psychopharmacology (Berl.)* 191, 521–550. doi: 10.1007/s00213-006-0510-4

Nomura, Y., Yotsumoto, I., and Segawa, T. (1981). Ontogenetic development of high potassium-induced and acetylcholine-induced release of dopamine from striatal slices of the rat. *Dev. Brain Res.* 1, 171–177. doi: 10.1016/0165-3806(81)90105-X

O'Loan, J., Eyles, D. W., Kesby, J., Ko, P., McGrath, J. J., and Burne, T. H. J. (2007). Vitamin D deficiency during various stages of pregnancy in the rat; its impact on development and behaviour in adult offspring. *Psychoneuroendocrinology* 32, 227–234. doi: 10.1016/j.psyneuen.2006.12.006

Olney, J. W., and Farber, N. B. (1995). Glutamate receptor dysfunction and schizophrenia. *Arch. Gen. Psychiatry* 52, 998–1007. doi: 10.1001/archpsyc.1995.03950240016004

Oo, T. F., and Burke, R. E. (1997). The time course of developmental cell death in phenotypically defined dopaminergic neurons of the substantia nigra. *Dev. Brain Res.* 98, 191–196. doi: 10.1016/S0165-3806(96)00173-3

Oo, T. F., Kholodilov, N., and Burke, R. E. (2003). Regulation of natural cell death in dopaminergic neurons of the substantia nigra by striatal glial cell line-derived neurotrophic factor in vivo. *J. Neurosci.* 23, 5141–5148.

Orme, R. P., Bhangal, M. S., and Fricker, R. A. (2013) Calcitriol imparts neuroprotection in vitro to midbrain dopaminergic neurons by upregulating GDNF expression. *PLoS ONE* 8:e62040. doi: 10.1371/journal.pone.0062040

Ozawa, K., Hashimoto, K., Kishimoto, T., Shimizu, E., Ishikura, H., and Iyo, M. (2006). Immune activation during pregnancy in mice leads to dopaminergic hyperfunction and cognitive impairment in the offspring: a neurodevelopmental animal model of schizophrenia. *Biol. Psychiatry* 59, 546–554. doi: 10.1016/j.biopsych.2005.07.031

Pagsberg, A. K., Baare, W. F. C., Christensen, A. M. R., Fagerlund,

B., Hansen, M. B., LaBianca, J., et al. (2007). Structural brain abnormalities in early onset first-episode psychosis. *J. Neural Transm.* 114, 489–498. doi: 10.1007/s00702-006-0573-8

Palha, J. A., and Goodman, A. B. (2006). Thyroid hormones and retinoids: a possible link between genes and environment in schizophrenia. *Brain Res. Rev.* 51, 61–71. doi: 10.1016/j.brainresrev.2005.10.001

Pantelis, C., Velakoulis, D., McGorry, P. D., Wood, S. J., Suckling, J., Phillips, L. J., et al. (2003). Neuroanatomical abnormalities before and after onset of psychosis: a cross-sectional and longitudinal MRI comparison. *Lancet* 361, 281–288. doi: 10.1016/S0140-6736(03)12323-9

Pardey, M. C., Kumar, N. N., Goodchild, A. K., and Cornish, J. L. (2012). Catecholamine receptors differentially mediate impulsive choice in the medial prefrontal and orbitofrontal cortex. *J. Psychopharmacol.* 27, 203–212. doi: 10.1177/0269881112465497

Perlmann, T., and Jansson, L. (1995). A novel pathway for vitamin-a signaling mediated by Rxr heterodimerization with Ngfi-B and Nurr1. *Gene Dev.* 9, 769–782. doi: 10.1101/gad.9.7.769

Piontkewitz, Y., Arad, M., and Weiner, I. (2012). Tracing the development of psychosis and its prevention: what can be learned from animal models. *Neuropharmacology* 62, 1273–1289. doi: 10.1016/j.neuropharm.2011.04.019

Prufer, K., and Barsony, J. (2002). Retinoid X receptor dominates the nuclear import and export of the unliganded vitamin D receptor. *Mol. Endocrinol.* 16, 1738–1751. doi: 10.1210/me.2001-0345

Prufer, K., Veenstra, T. D., Jirikowski, G. F., and Kumar, R. (1999). Distribution of 1,25-dihydroxyvitamin D3 receptor immunoreactivity in the rat brain and spinal cord. *J. Chem. Neuroanat.* 16, 135–145. doi: 10.1016/S0891-0618(99)00002-2

Rakic, P., Bourgeois, J.-P., and Goldman-Rakic, P. (1994) "Synaptic development of the cerebral cortex: implications for learning, memory, and mental illness," in *Progress in Brain Research, The Self-Organizing Brain: From Growth Cones to Functional Networks*, eds J. van Pelt M. Corner, H. Uylings, and F. Lopes da Silva (Amsterdam: Elsevier), 227–243. doi: 10.1016/S0079-6123(08)60543-9

Rutz, S., Majchrzak, M., Siedschlag, V., Barbelivien, A., Harati, H., Rothmaier, A. K., et al. (2009). The modulation of striatal dopamine release correlates with water-maze performance in aged rats. *Neurobiol. Aging* 30, 957–972. doi: 10.1016/j.neurobiolaging.2007.09.011

Sakurada, K., Ohshima-Sakurada, M., Palmer, T. D., and Gage, F. H. (1999). Nurr1, an orphan nuclear receptor, is a transcriptional activator of endogenous tyrosine hydroxylase in neural progenitor cells derived from the adult brain. *Development* 126, 4017–4026.

Seeman, P., and Kapur, S. (2000). Schizophrenia: more dopamine, more D2 receptors. *Proc. Natl. Acad. Sci. U.S.A.* 97, 7673–7675. doi: 10.1073/pnas.97.14.7673

Seeman, P., and Lee, T. (1975). Antipsychotic drugs: direct correlation between clinical potency and presynaptic action on dopamine neurons. *Science* 188, 1217–1219. doi: 10.1126/science.1145194

Shotbolt, P., Stokes, P. R., Owens, S. F., Toulopoulou, T., Picchioni, M. M., Bose, S. K., et al. (2011). Striatal dopamine synthesis capacity in twins discordant for schizophrenia. *Psychol. Med.* 41, 2331–2338. doi: 10.1017/S0033291711000341

Smidt, M. P., and Burbach, J. P. (2007). How to make a mesodiencephalic dopaminergic neuron. *Nat. Rev. Neurosci.* 8, 21–32. doi: 10.1038/nrn2039

Smith, Y., and Kieval, J. Z. (2000). Anatomy of the dopamine system in the basal ganglia. *Trends Neurosci.* 23, S28–S33. doi: 10.1016/S1471-1931(00)00023-9

Spear, L. P. (2000). The adolescent brain and age-related behavioral manifestations. *Neurosci. Biobehav. Rev.* 24, 417–463. doi: 10.1016/S0149-7634(00)00014-2

Sulzer, D., Maidment, N. T., and Rayport, S. (1993). Amphetamine and other weak bases act to promote reverse transport of dopamine in ventral midbrain neurons. *J. Neurochem.* 60, 527–535. doi: 10.1111/j.1471-4159.1993.tb03181.x

Sutherland, M. K., Somerville, M. J., Yoong, L. K., Bergeron, C., Haussler, M. R., and McLachlan, D. R. (1992). Reduction of vitamin D hormone receptor mRNA levels in Alzheimer as compared to Huntington hippocampus: correlation with calbindin-28k mRNA levels. *Brain Res. Mol. Brain Res.* 13, 239–250. doi: 10.1016/0169-328X(92)90032-7

Swerdlow, N. R., Stephany, N., Wasserman, L. C., Talledo, J., Sharp, R., and Auerbach, P. P. (2003). Dopamine agonists disrupt visual latent inhibition in normal males using a within-subject paradigm. *Psychopharmacology* 169, 314–320. doi: 10.1007/s00213-002-1325-6

Tagami, T., Lutz, W. H., Kumar, R., and Jameson, J. L. (1998). The interaction of the vitamin D receptor with nuclear receptor corepressors and coactivators. *Biochem. Biophys. Res. Commun.* 253, 358–363. doi: 10.1006/bbrc.1998.9799

Torrey, E. F., Bowler, A. E., and Clark, K. (1997a). Urban birth and residence as risk factors for psychoses: an analysis of 1880 data. *Schizophr. Res.* 25, 169–176. doi: 10.1016/S0920-9964(97)00020-0

Torrey, E. F., Miller, J., Rawlings, R., and Yolken, R. H. (1997b). Seasonality of births in schizophrenia and bipolar disorder: a review of the literature. *Schizophr. Res.* 28, 1–38. doi: 10.1016/S0920-9964(97)00092-3

Turner, K. M., Young, J. W., McGrath, J. J., Eyles, D. W., and Burne, T. H. J. (2013). Cognitive performance and response inhibition in developmentally vitamin D (DVD)-deficient rats. *Behav. Brain Res.* 242, 47–53. doi: 10.1016/j.bbr.2012.12.029

Veenstra, T. D., Prufer, K., Koenigsberger, C., Brimijoin, S. W., Grande, J. P., and Kumar, R. (1998). 1,25-Dihydroxyvitamin D-3 receptors in the central nervous system of the rat embryo. *Brain Res.* 804, 193–205. doi: 10.1016/S0006-8993(98)00565-4

Veling, W., Hoek, H. W., Selten, J. P., and Susser, E. (2011). Age at migration and future risk of psychotic disorders among immigrants in the Netherlands: a 7-Year incidence study. *Am. J. Psychiatry* 168, 1278–1285. doi: 10.1176/appi.ajp.2011.11010110

Volpicelli, F., Perrone-Capano, C., Da Pozzo, P., Colucci-D'Amato, L., and di Porzio, U. (2004). Modulation of nurr1 gene expression in mesencephalic dopaminergic neurones. *J. Neurochem.* 88, 1283–1294. doi: 10.1046/j.1471-4159.2003.02254.x

Voorn, P., Kalsbeek, A., Jorritsmabyham, B., and Groenewegen, H. J. (1988). The prenatal and postnatal-development of the dopaminergic cell groups in the ventral mesencephalon and the dopaminergic innervation of the striatum of the rat. *Neuroscience* 25, 857–887. doi: 10.1016/0306-4522(88)90041-3

Vuillermot, S., Weber, L., Feldon, J., and Meyer, U. (2010). A longitudinal examination of the neurodevelopmental impact of prenatal immune activation in mice reveals primary defects in dopaminergic development relevant to schizophrenia. *J. Neurosci.* 30, 1270–1287. doi: 10.1523/JNEUROSCI.5408-09.2010

Wallen-Mackenzie, A., de Urquiza, A. M., Petersson, S., Rodriguez, F. J., Friling, S., Wagner, J., et al. (2003). Nurr1-RXR heterodimers mediate RXR ligand-induced signaling in neuronal cells. *Gene Dev.* 17, 3036–3047. doi: 10.1101/gad.276003

Wallen, A., Zetterstrom, R. H., Solomin, L., Arvidsson, M., Olson, L., and Perlmann, T. (1999). Fate of mesencephalic AHD2-expressing dopamine progenitor cells in Nurr1 mutant mice. *Exp. Cell Res.* 253, 737–746. doi: 10.1006/excr.1999.4691

Wallen, A., Castro, D. S., Zetterstrom, R. H., Karlen, M., Olson, L., Ericson, J., et al. (2001). Orphan nuclear receptor Nurr1 is essential for Ret expression in midbrain dopamine neurons and in the brain stem. *Mol. Cell. Neurosci.* 18, 649–663. doi: 10.1006/mcne.2001.1057

Wang, Y., Chiang, Y. H., Su, T. P., Hayashi, T., Morales, M., Hoffer, B. J., et al. (2000). Vitamin D-3 attenuates cortical infarction induced by middle cerebral arterial ligation in rats. *Neuropharmacology* 39, 873–880. doi: 10.1016/S0028-3908(99)00255-5

Weinberger, D. R. (1987). Implications of normal brain-development for the pathogenesis of schizophrenia. *Arch. Gen. Psychiatry* 44, 660–669. doi: 10.1001/archpsyc.1987.01800190080012

Westerink, B. H. C. (1985). Sequence and significance of dopamine metabolism in the rat-brain. *Neurochem. Int.* 7, 221–227. doi: 10.1016/0197-0186(85)90108-1

Wieczorek, W. J., and Kruk, Z. L. (1994). Differential action of (+)-amphetamine on electrically-evoked dopamine overflow in rat-brain slices containing corpus striatum and nucleus-accumbens. *Brit. J. Pharmacol.* 111, 829–836. doi: 10.1111/j.1476-5381.1994.tb14813.x

Willins, D. L., Narayanan, S., Wallace, L. J., and Uretsky, N. J. (1993). The Role of dopamine and ampa kainate receptors in the nucleus-accumbens in the hypermotility response to Mk801. *Pharmacol. Biochem. Behav.* 46, 881–887. doi: 10.1016/0091-3057(93)90217-H

Winter, C., Djodari-Irani, A., Sohr, R., Morgenstern, R., Feldon, J., Juckel, G., et al. (2009). Prenatal immune activation leads to multiple changes in basal neurotransmitter levels in the adult brain: implications for brain disorders of neurodevelopmental origin such as schizophrenia. *Int. J.*

Neuropsychopharmacol. 12, 513–524. doi: 10.1017/S1461145708009206

Yoon, E. H., Lee, K. J., Kim, Y. S., Chang, M. S., Lee, S. H., and Park, C. H. (2010). Retinoid X receptor a acts as alpha negative regulator in Nurr1-induced dopaminergic differentiation in rat neural precursor cells. *Neuroreport* 21, 1162–1166. doi: 10.1097/WNR.0b013e328340ccf9

Zehnder, D., Bland, R., Williams, M. C., McNinch, R. W., Howie, A. J., Stewart, P. M., et al. (2001). Extrarenal expression of 25-hydroxyvitamin D-3-1 alpha-hydroxylase. *J. Clin. Endocrinol. Metab.* 86, 888–894. doi: 10.1210/jc.86.2.888

Zetterstrom, R. H., Solomin, L., Jansson, L., Hoffer, B. J., Olson, L., and Perlmann, T. (1997). Dopamine neuron agenesis in Nurr1-deficient mice. *Science* 276, 248–250. doi: 10.1126/science.276.5310.248

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Rethinking schizophrenia in the context of normal neurodevelopment

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The schizophrenia brain is differentiated from the normal brain by subtle changes, with significant overlap in measures between normal and disease states. For the past 25 years, schizophrenia has increasingly been considered a neurodevelopmental disorder. This frame of reference challenges biological researchers to consider how pathological changes identified in adult brain tissue can be accounted for by aberrant developmental processes occurring during fetal, childhood, or adolescent periods. To place schizophrenia neuropathology in a neurodevelopmental context requires solid, scrutinized evidence of changes occurring during normal development of the human brain, particularly in the cortex; however, too often data on normative developmental change are selectively referenced. This paper focuses on the development of the prefrontal cortex and charts major molecular, cellular, and behavioral events on a similar time line. We first consider the time at which human cognitive abilities such as selective attention, working memory, and inhibitory control mature, emphasizing that attainment of full adult potential is a process requiring decades. We review the timing of neurogenesis, neuronal migration, white matter changes (myelination), and synapse development. We consider how molecular changes in neurotransmitter signaling pathways are altered throughout life and how they may be concomitant with cellular and cognitive changes. We end with a consideration of how the response to drugs of abuse changes with age. We conclude that the concepts around the timing of cortical neuronal migration, interneuron maturation, and synaptic regression in humans may need revision and include greater emphasis on the protracted and dynamic changes occurring in adolescence. Updating our current understanding of post-natal neurodevelopment should aid researchers in interpreting gray matter changes and derailed neurodevelopmental processes that could underlie emergence of psychosis.

Keywords: cognition, neurogenesis, myelination, excitatory synapses, neural migration, NMDA receptor, GABA receptor, dopamine receptor

INTRODUCTION

A recent perspective titled "Rethinking Schizophrenia" (Insel, 2010) forecast that if researchers and clinicians approached schizophrenia as a neurodevelopmental disorder, with several discernible disease stages, then prevention focusing on the pre-psychotic illness phase would be a real possibility by 2030. The perspective (Insel, 2010) relied on the popular neurodevelopmental theory that the consequences of genetic predisposition and early adverse events, such as mid-gestational insults, would be latent throughout the first two decades of life and become evident as psychosis in early adulthood as first proposed by Weinberger (1987). Weinberger emphasized that the normative maturational changes "unmasked" an earlier insult. While we agree that adolescence is a critical period and that pre- and perinatal development are potential vulnerable time periods when schizophrenia susceptibility genes and environments may contribute to the future onset of schizophrenia, current data indicate human neurodevelopment is not confined to the womb, but is a protracted process that continues in post-natal life well into adolescence and early adulthood. Thus, there is opportunity for perturbation of developmental processes beyond the fetal and perinatal period and, we suggest that developmental disruption well after birth may also contribute to onset of schizophrenia. Our emphasis on the importance of post-natal events is similar to what another schizophrenia neurodevelopmental theorist, Feinberg, originally proposed (Feinberg, 1983). Feinberg

suggested that exuberant synaptic regression ("pruning") in adolescence may underlie schizophrenia, a theory that has dominated our thinking about abnormal neurodevelopment in adolescence in schizophrenia for over 25 years. One of the attractions of Feinberg's hypothesis is that it seemingly fits with the emerging consensus that progressive brain changes in adolescence or early adulthood, thought to be related to tissue loss, are associated with schizophrenia, at least in some individuals (Borgwardt et al., 2009). While we suggest that Feinberg's over-exuberant synaptic pruning theory of schizophrenia is potentially flawed, as it relies on the timing of normative synaptic pruning with inaccurately extrapolated synaptic densities that do not appear consistent with more recent studies, we do submit that charting normal adolescent brain changes is essential if we are to understand the processes underlying schizophrenia pathology. Indeed, a better understanding of changes occurring in the normal adolescent brain may serve to unite protagonists of neurodevelopmental and of neurodegenerative pathologies of schizophrenia. Also, many more molecular studies on human cortical growth and development in the post-natal periods have appeared since the 1980s and the results of these studies, which show diverse and dynamic post-natal changes, deserve to also be incorporated into our working models of the physical substrates of brain maturation in humans and into our theories about abnormal neurodevelopment in schizophrenia.

Here we review current normative neurodevelopmental studies and place them in the context of what is known about schizophrenia neuropathology. We start by considering when emergent behavioral properties of the human prefrontal cortex, like executive function, mature in humans; as prefrontal cortex function is greatly impacted in schizophrenia. Next we consider the timing of cognitive decline in people with schizophrenia, as this can implicate distinct neurobiological events in the pathophysiology. Then we review the temporal development of the "hardware" of the brain; the genesis of new neurons, their journey to the cortex, myelination of axons, and growth of dendritic arbors. Changes in excitatory and inhibitory synapses are reviewed next. We then consider how changes in neurotransmitter receptors and psychopharmacological responses to drugs across development might provide clues to the developmental processes gone astray in schizophrenia. Although there are well-documented differences in the average age of onset of schizophrenia in males (earlier) as compared to females, our review does not cover gender differences in neurodevelopment because our main vantage point is transcriptional and gender differences account for relatively little (130 transcripts = 8%) of the variance of gene transcript levels in a large microarray study of a developmental brain collection and typically occur early in life, not during adolescence (Weickert et al., 2009; Kang et al., 2011).

COGNITIVE DEVELOPMENT

Basic cognitive functions, such as selective attention and response selection are firmly established by early childhood. However, the development of complex executive functions are characterized by a protracted development well through adolescence (Levin et al., 1991; Anderson et al., 2001; Brocki and Bohlin, 2004). Executive functioning capabilities include keeping and manipulating information in short-term memory (also known as working memory), planning, ignoring irrelevant information (involving inhibition and cognitive control), solving problems, and applying existing information to novel situations to derive new solutions (also known as reasoning). Schizophrenia, a disease which typically has its onset during late adolescence or early adulthood, is coincident in time with cognitive maturation of the prefrontal and parietal cortices. This section will: (1) review the timing of the development of executive functions and relationship to measures of neural maturation; (2) place the prefrontal associated cognitive and neural dysfunction accompanying schizophrenia in a developmental perspective; and (3) consider the evidence regarding the timing of cognitive decline in schizophrenia.

NEURODEVELOPMENT OF EXECUTIVE FUNCTIONING

We will consider three aspects of executive processing (working memory, cognitive control, and reasoning), which are separate constructs with differing developmental trajectories and with both distinct and overlapping neural substrates (Brocki and Bohlin, 2004; Huizinga et al., 2006). During the attainment of increased working memory capacity as children get older the superior frontal sulcus (prefrontal cortex) and the intraparietal sulcus (parietal cortex) show a greater degree of task-related activation and have increased white matter connectivity (Klingberg et al., 2002; Kwon et al., 2002; Olesen et al., 2003; Nagy et al., 2004; Edin et al., 2007). When manipulation of information in working memory is required (such as repeating remembered information in reverse order), children perform more poorly and show less activation of the dorsolateral prefrontal cortex (DLPFC) and superior parietal cortices than adolescents and young adults during the same task (Crone et al., 2006). Conversely, other brain regions that are activated during working memory tasks in children, such as the ventromedial prefrontal cortex, show decreased activity in adolescence and early adulthood during working memory tests (Scherf et al., 2006). Thus, in some prefrontal (dorsal) and parietal regions children typically show less activity than adolescents and young adults during executive function/working memory tests; whereas in the ventral prefrontal cortex the pattern is reversed. This suggest that within the frontal lobes, there may be a preference to use more ventral areas to solve working memory problems initially, but this function may be taken over by more dorsal areas of the prefrontal cortex later in life.

Another aspect of executive function is cognitive control. Cognitive control relies on a broad group of mental abilities that work together in context to "allow information processing and behavior to vary adaptively from moment to moment depending on current goals, rather than remaining rigid and inflexible" and is thought to rely heavily on the prefrontal cortex (http://carterlab.ucdavis.edu/research/control.php, accessed 7 April 2013). Cognitive control increases as humans mature from childhood to adulthood and this increasing ability correlates with increases in myelination of white matter tracts between the frontal and parietal cortices (Fair et al., 2007; Spear, 2007). The Wisconsin Card Sorting test, a classic test of cognitive flexibility and control, which also relies on fronto-parietal function, shows

a developmental pattern of gradual performance improvement interspersed with periods of accelerated improvement throughout childhood to young adulthood (Heaton et al., 1993; **Figure 1**). Other aspects of cognitive control involve performance monitoring, which is associated with dorsal anterior cingulate activity in adults. Similar to under activation of the dorsal frontal cortical regions in working memory performance, children and adolescents display less dorsal anterior cingulate activity during performance monitoring (Velanova et al., 2008). The protracted development of mature cognition has been linked to relatively late maturation of several association cortices, as compared to other cortical regions such as the sensorimotor and occipital lobes (Giedd et al., 1999; Casey et al., 2000; Gogtay et al., 2004; Blakemore and Choudhury, 2006). We suggest that while a developmental increase in task-related activity of the dorsal prefrontal cortex may be critical to executive function that is typical of adults, this dorsal prefrontal cortex functional maturation co-occurs with functional maturation of other associative regions.

Reasoning (also referred to as a form of intelligence) is thought to rely to a large extent on "executive" processing again residing primarily in the frontal lobes. The two types of reasoning, crystallized and fluid, have somewhat different developmental trajectories. Crystallized intelligence refers to use of previously acquired knowledge to solve problems; whereas fluid intelligence refers to the ability to think logically and solve problems in novel situations independent of previously acquired knowledge. Fluid reasoning shows a rapid increase in ability from mid-childhood until early adulthood, whereas crystallized reasoning ability grows gradually, peaking in mid-life (Cattell, 1987; McArdle et al., 2002). The rostrolateral prefrontal cortex is consistently associated with logical reasoning ability (Prabhakaran et al., 1997; Christoff et al., 2001; Kroger et al., 2002), which also involves activation of other cortices that can vary depending on task demands (Bunge et al., 2005; Wright et al., 2008; Crone et al., 2009). While even toddlers (3-years old) can solve simple reasoning problems (Goswami, 1989), accurate performance on more difficult reasoning problems only occurs in later childhood to adolescence (Sternberg and Rifkin, 1979; Richland et al., 2006). During difficult reasoning problems rostral prefrontal cortical activity is delayed or fails to be sustained in children (Bunge et al., 2009; Crone et al., 2009). Thus, during difficult reasoning problems the timing and maintenance of activity increases as performance increases from early childhood to young adulthood. It is clear from these three examples of cognitive functional change during human development that most executive cognitive processes involve the ability to achieve and maintain activation of focal frontal regions in appropriate contexts. This suggests that molecular and cellular maturation in circuitry controlling the magnitude, extent and co-ordination of pyramidal neuron firing across association areas would be expected to increase during childhood and attain near adult levels during adolescence.

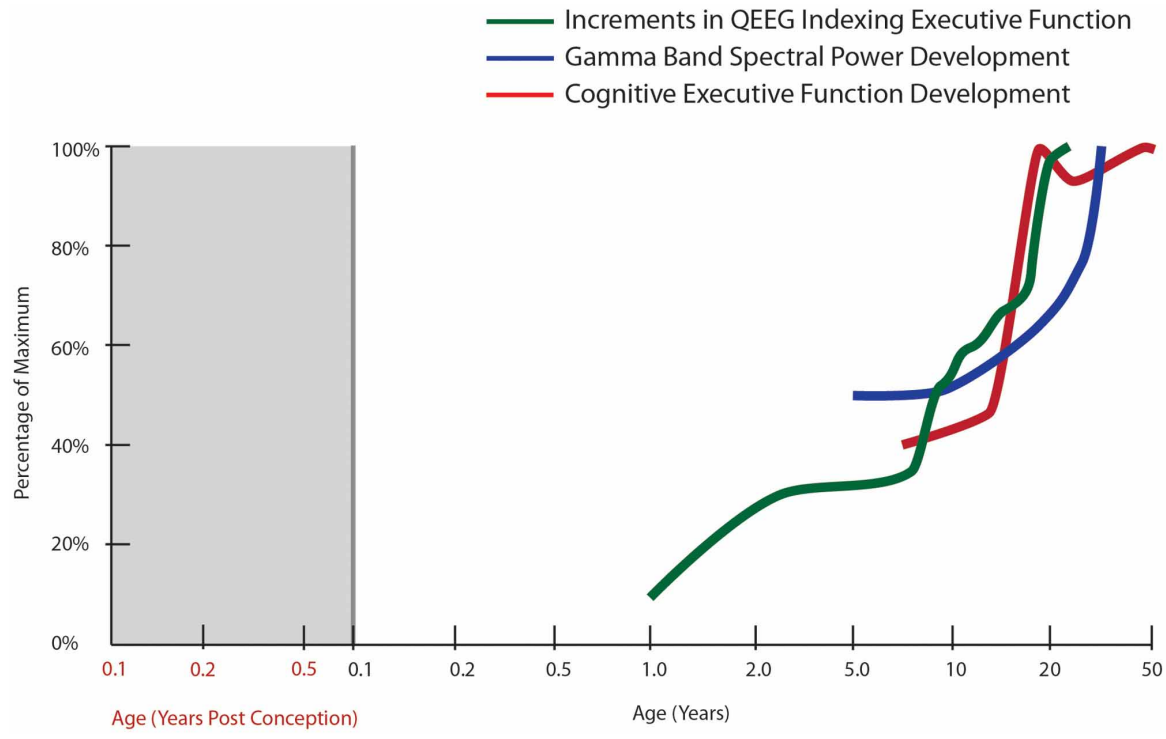

FIGURE 1 | Normal developmental trajectory of cognitive executive function. The trajectory of cognitive executive functioning development is derived from developmental tables in the Wisconsin Card Sorting Manual [modified from Heaton et al. (1993)]. The other trajectories correspond to the spectral power in gamma bands during a face recognition task (30–75 Hz, all electrodes; Uhlhaas et al., 2009) as well as cumulative increments in computer quantified changes in EEG frequency spectrum (QEEG) between fronto-temporal cortices, derived from Hudspeth and Pribram (1990).

Mature ability to meet executive challenges of the adult are associated with more efficient prefrontal cortical activity (Casey et al., 1997; Bunge et al., 2002; Tamm et al., 2002; Durston et al., 2006). The increasing ability of the brain to transfer information more efficiently across widely distributed neural networks is an important part of adolescent development and likely involves faster and more synchronized axonal firing across long distances. *In vivo* brain imaging findings of macro-level increases in white matter (Snook et al., 2005; Liston et al., 2006; Eluvathingal et al., 2007; Giorgio et al., 2008) are thought to reflect progressive myelination at the micro-level (see Myelination section). However, while increased activity in frontal-parietal regions is a general rule throughout development from child to adulthood, for some tasks such as those requiring response inhibition, the lateral prefrontal cortex may show decreased activity (reflecting increased neural efficiency) as development progresses from children to young adults (Fair et al., 2007).

In general, based on its rich connections with other cortical and subcortical structures, the prefrontal cortex is also ideally suited to the task of coordinating activity within the neural network to facilitate increased neural efficiency and improved executive function. Electroencephalography (EEG) data show age-related changes in neural oscillations and synchrony that support enhanced temporal coordination of distributed cortical processes throughout development (Uhlhaas et al., 2009; **Figure 1**). Interestingly, this work also suggests a period of destabilization during adolescence, followed by reorganization during young adulthood (18–21 years of age), which is characterized by increases in gamma-band power, theta and beta band synchrony. In fact, EEG has detected several region-specific growth spurts (brief periods of accelerated neural development): the first typically occurring in toddlers, a second in early school age children, a third during puberty and early adolescence, and a final growth spurt in young adulthood (Hudspeth and Pribram, 1990, 1992; **Figure 1**). Thus, important changes in physiological and structural parameters may occur by gradual changes interspersed by occasional rapid increases, two distinct patterns of change that can also be detected with molecular markers, especially for inhibitory interneurons (see later sections of this review). These physiological changes parallel the patterns found for cognitive development where gradual change can be interspersed with brief periods of accelerated cognitive development (Thatcher, 1991, 1992, 1994). The other major point to consider is that while task-related activity of the prefrontal cortex increases in development, it appears that this activity must be integrated and coordinated with other regions and that both an increase in focal prefrontal activity and synchrony of this region with other association cortices may occur during adolescence. Thus, adolescence is a critical window for the organization and functional adjustment of cortical circuitry rendering this time of life particularly sensitive to disruptive effects. Given that the typical emergence of schizophrenia is during late adolescence or early adulthood, these later developmental changes, which may represent vulnerable periods, become especially relevant for the pathophysiology of schizophrenia, where abnormal patterns of oscillatory brain activity, especially in the gamma range, are observed in patients (Uhlhaas et al., 2008).

EXECUTIVE FUNCTION AND PREFRONTAL CORTEX DEVELOPMENT IN SCHIZOPHRENIA

One of the most debilitating problems for people with schizophrenia are the enduring cognitive deficits (Green, 1996), which are often unresponsive to antipsychotic medication (Heinrichs and Zakzanis, 1998; Goldberg et al., 2007). In terms of cognitive dysfunction, the most consistent findings are within the domains of executive function, working memory, inhibitory control, and reasoning (Weinberger et al., 1986; Goldman-Rakic, 1994; Weickert et al., 2000a; Silver et al., 2003; Ravizza et al., 2010). Almost three decades of functional and structural neuroimaging studies in schizophrenia provide converging evidence of localized abnormal activity and connectivity of the prefrontal cortex (Weinberger et al., 1986; Andreasen et al., 1997; Manoach et al., 1999, 2000; Barch et al., 2001; Meyer-Lindenberg et al., 2001; Perlstein et al., 2001, 2003; Callicott et al., 2003; Tan et al., 2006; Potkin et al., 2009). The concomitant refinement of cognitive executive processes, the physical maturation of neural circuitry underlying executive function, and the onset of schizophrenia in adolescence or early adulthood suggests that a failure in these maturational processes may play a critical role in the pathophysiology of schizophrenia.

One of the key questions for our field is when precisely do cognitive problems begin in people with schizophrenia? This is thought to be important as it may help point to the culprit neurodevelopmental event gone awry in the disease. Population and birth cohort studies show that some premorbid individuals displayed lower cognitive ability during childhood and adolescence. Significant deficits in premorbid IQ can be evident by the age of 16 years in people who subsequently develop schizophrenia (Dickson et al., 2012) and may precede the prodromal period (Woodberry et al., 2008; Khandaker et al., 2011). Overall group deficiencies in cognitive ability may be detectable before 8 years of age in people with schizophrenia (Jones et al., 1994; Cannon et al., 2002a; Seidman et al., 2013), and there is a relationship between the size of the IQ decrement and risk of subsequent schizophrenia (Khandaker et al., 2011; Schulz et al., 2012). However, not all people with schizophrenia start off with lower cognitive ability as a child; there is evidence showing that it is adolescent cognitive development in particular that is subject to perturbation in at least 40% of men and women later diagnosed with schizophrenia (Reichenberg et al., 2005). Additionally, there is a well-established 10 point drop from fairly normal pre-morbid IQ estimates in many adults with schizophrenia (50%), which demonstrates that many people with schizophrenia experience healthy childhood cognitive development, with only about 25% having a low premorbid IQ (Weickert et al., 2000a). The nature of the post-childhood schizophrenia-related cognitive declines affecting prefrontal-related verbal and executive abilities in particular (David et al., 1997; Bhojraj et al., 2010; Maccabe et al., 2013) suggests a deviation in the normal adolescent neurodevelopmental processes that typically support improvements in cognitive ability and efficiency with increasing age. We suggest that there may be at least two different patterns of cognitive decline in people who are destined to develop schizophrenia. One pattern where cognitive decline is found early in life and intellectual impairment is widespread, implying that early developmental

processes occurring before 8 years of age may have gone awry (Weickert and Goldberg, 2000). However, the modal pattern of cognitive decline in people with schizophrenia is one of decline that is restricted to the second decade of life and may include more exaggerated loss of prefrontal associated cognitive functions (Weickert and Goldberg, 2000). Therefore, it may be informative to consider the underlying neurobiology in the context of alterations of developmental trajectories in later maturing executive functions in order to adequately address the risk factors for those people with schizophrenia demonstrating an adolescent IQ drop. There is some debate about whether premorbid cognitive changes represent liability- or disease-related processes. Whichever is the case, the disease-specific cognitive dysfunctions manifesting during adolescence (Heinrichs and Zakzanis, 1998) and prefrontal, temporal, and parietal cortical gray matter deficits associated with the prodrome (Wood et al., 2008) and schizophrenia itself (Cannon et al., 2002b) may be separate from or additional to the early childhood cognitive dysfunctions found in the subset of people who go onto develop schizophrenia.

The current literature suggests that brain changes in schizophrenia, if apparent premorbidly, are prominent in prefrontal and associative cortical regions and that they correlate with premorbid executive dysfunction. Structural brain changes present in adolescence or young adults during the early transition to illness provide further support for this view (Pantelis et al., 2003). The findings described above suggest a period of later development (e.g., adolescence) when neural perturbations may result in schizophrenia. Therefore, we suggest that schizophrenia should be considered in the context of alterations in the developmental trajectories of these executive functions and their underlying dynamic changes in neural substrates to adequately address the risk factors for aberrant neurodevelopment of schizophrenia. The remainder of this review will address the timing of normal prefrontal cortical development at the cellular and molecular level and its potential to inform us about the cause of neurobiological changes found in the cortex of people with schizophrenia.

EARLY EVENTS IN THE FORMATION OF THE HUMAN CORTEX

Appropriate genesis and migration of neurons are essential building blocks for a healthy brain. Relatively recent studies challenge dogma by suggesting that for some neuronal subtypes these processes may be ongoing throughout development and into adulthood (Gould et al., 1999; Weickert et al., 2000b; Bernier et al., 2002; Fung et al., 2010, 2011a; Wang et al., 2011). This has implications for adolescent-onset neurodevelopmental disorders, such as schizophrenia, as aberrations in these processes could be contributing to disease. Thus, while we begin with early events, we suggest that some of these early processes are not completely exhausted in post-natal life.

NEUROGENESIS AND NEURONAL MIGRATION

When the nervous system begins, pluripotent cells from the outer embryonic layer, the ectoderm, produce neuroepithelial cells of the neural plate that invaginates to form the neural tube. The most rostral region of this tube swells to form the telencephalon with the lumen eventually becoming the lateral ventricles of the cerebral hemispheres. The telencephalon forms the cerebral cortex, basal ganglia, and hippocampus (Kandel et al., 2000). One of the most important divisions of the telencephalon relevant to schizophrenia neuropathology is the distinction between the dorsal (pallium) and ventral (subpallium) neurogenic zones as they by and large give rise to the two different neuronal types of the cortex. These zones are not only defined by their position, but by expression of distinct molecules [reviewed by Corbin and Butt (2011)]. Progenitors from the dorsal telencephalon spawn all the cortical excitatory pyramidal neurons that migrate radially to the cortical layers (Rakic, 1972). The ventral neurogenic zone gives rise to most of the cortical inhibitory neurons (Anderson et al., 1997; Tamamaki et al., 1997; Ayala et al., 2007) that migrate tangentially to reach the cortex (see below). Recent studies suggest that some inhibitory (GABAergic) interneurons in the human and non-human primate cortex, particularly many of those expressing calretinin, could also arise from progenitors within the dorsopallial proliferative zone (Letinic et al., 2002; Rakic and Zecevic, 2003; Fertuzinhos et al., 2009; Petanjek et al., 2009a,b; Zecevic et al., 2011), although it is unclear if these "dorsal" progenitors may be ventral in origin, having migrated to the dorsal neurogenic zone at an earlier developmental stage.

Cells in both germinal zones initially undergo proliferative, symmetric division, giving rise to two daughter cells that both retain their apical process and progenitor identity, resulting in expansion of the progenitor population (Kosodo et al., 2004; Kosodo and Huttner, 2009). The greatly expanded neuronal number and surface area of the human cerebral cortex depends on repeated rounds of symmetric cell division followed by later asymmetric division resulting in committed neuronal precursors. Given that there are not gross abnormalities in the size or shape of the human brain (in most cases of schizophrenia) it is likely that these very early embryonic events of division and early neuronal differentiation are intact in most people who later go on to develop schizophrenia. However, neuronal precursors residing in the ventral subventricular zone (vSVZ: Haubensak et al., 2004) retain the ability to divide and generate neurons well into post-natal human life and into adulthood (see below), and more selective disruption of the processes controlling vSVZ neurogenesis could lead to much more subtle changes impacting development of cortical interneurons. Therefore, disruption of the vSVZ neurogenesis process would be consistent with the pathology of mental illnesses involving GABAergic interneurons and with a post-natal age of onset, like what is found in schizophrenia.

Indeed, a deficit in GABAergic inhibition is one of the most robust findings in schizophrenia neuropathology, so the timing of cortical interneuron development warrants further consideration. In the human, GABAergic interneuron birth in the (vSVZ) ganglionic eminence starts as early as 5 gestational weeks (gw: Zecevic et al., 2011) and neurons are found in the nascent human cortex by 5.5–6 gw (Zecevic, 2004). Radial glial cells in the dorsal VZ form around 17 gw. Proliferation of the calretinin positive cell can be detected in the human dorsal SVZ at 20 gw (Zecevic et al., 2011). At 23 gw in the human, proliferation in dorsal VZ proliferation is no longer detectable suggesting that most of the cortical pyramidal neurons are born in embryonic life (Zecevic, 2004; **Figure 2**). Markers of immature neurons are, however, still

abundant in the vSVZ (where cortical interneurons are born) during the first few post-natal years of life (Weickert et al., 2000b; Chong et al., 2008; Wang et al., 2011) and persist into adulthood in humans and monkeys (Fung et al., 2011a; Wang et al., 2011; **Figure 2**). Neurogenesis has been implicated in schizophrenia pathology by the involvement of disease-associated neurogenesis regulator genes such as DISC1 and neuregulin (Millar et al., 2000; Ghashghaei et al., 2006; Duan et al., 2007; Mao et al., 2009). Current studies using post-mortem material have reported reduced adult neurogenesis in the hippocampus (by PSA-NCAM and Ki67 immunoreactivity: Barbeau et al., 1995; Reif et al., 2006), however, neurogenesis in the vSVZ remains to be studied in schizophrenia.

RADIAL MIGRATION OF NEUROBLASTS FROM SVZ

After their birth, neurons travel either by radial (dorsal-born pyramidal neurons), or tangential (ventral-born GABAergic interneurons) migration to their final destination in the cortex. Radial migration, via radial glial cells, occurring around 20 gw in humans, is the main mode of migration for pyramidal neurons (Rakic, 1972; Nadarajah et al., 2001; Hatten, 2002; Nadarajah and Parnavelas, 2002; Nadarajah, 2003; **Figure 2**). Once radial migration of pyramidal neurons ceases, cortical maturation in the form of proper alignment of neuronal cells, synapse formation, and neurite outgrowth lasts from the sixteenth fetal week until the post-natal period (Sidman and Rakic, 1973; Lequin and Barkovich, 1999). One molecular regulator of radial migration and cortical layering, reelin, is an extracellular matrix protein produced by Cajal-Retzius cells in layer I of the cerebral cortex (Marin-Padilla, 1990; Del Rio et al., 1997). In mice lacking normal reelin function (reeler mice), inverted neocortical layers and abnormally dispersed cells are observed due to lack of neuronal ability to respond to migration signals in reeler mutants (Caviness, 1976; Goffinet, 1984; D'Arcangelo et al., 1995; Chai et al., 2009). Reelin is down-regulated in post-mortem brains from people with schizophrenia (Grayson et al., 2005), with significant (approximately 50%) reelin reductions in hippocampus, caudate nuclei, and cerebellum (Impagnatiello et al., 1998; Costa et al., 2002). However, the fact that cortical layering appears to be fairly intact in most cortical areas in schizophrenia, is not consistent with a major loss of function of the reelin gene early in development. Anomalous cortical development due to distorted distribution of neurons, especially in layer II of the entorhinal cortex from patients with schizophrenia has been reported (Jakob

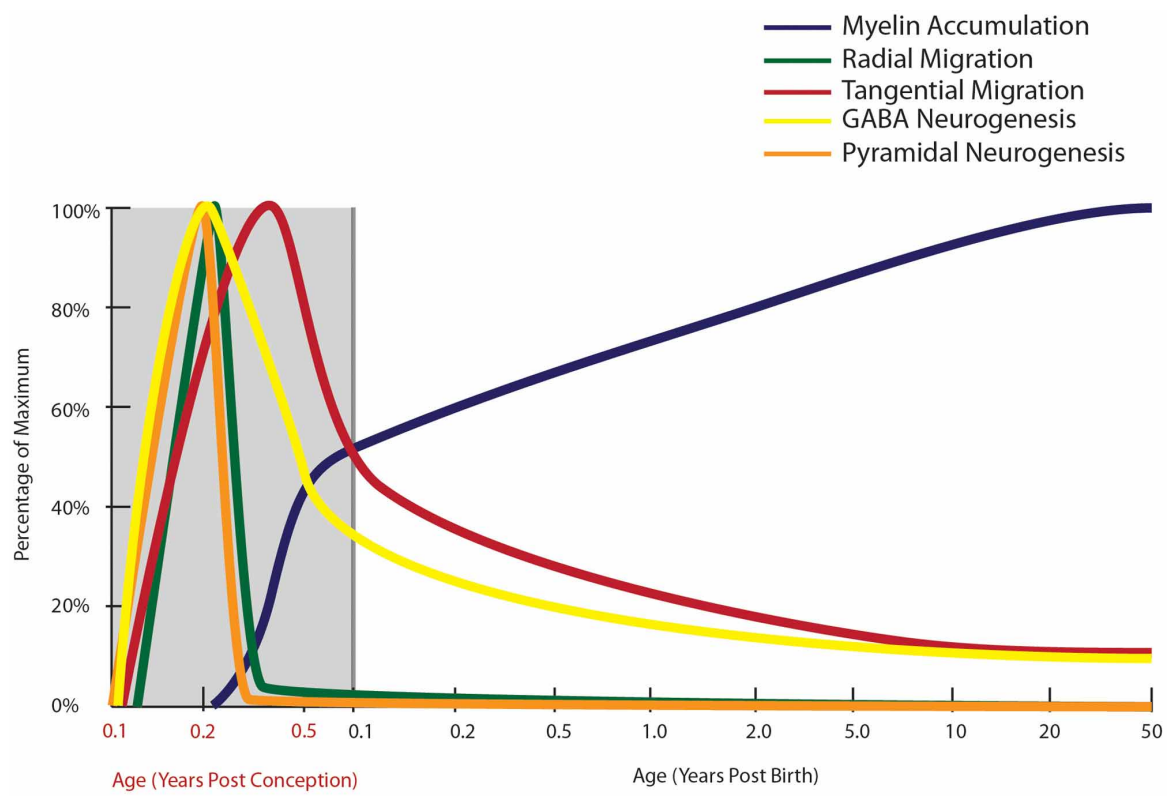

FIGURE 2 | Normal developmental trajectory of neurogenesis, neuronal migration, and myelination in the human. Neurogenesis and the subsequent migration of neurons to the cortex begin within a few weeks of gestation in the human (Zecevic et al., 2011). Pyramidal neurogenesis and migration of these cells to the cortex occurs by radial migration and is completed by mid-gestation (Nadarajah and Parnavelas, 2002), while genesis and migration of GABAergic interneurons continues into early post-natal life, with emerging evidence based on the presence of molecular markers of immature neurons suggesting that this process continues into adulthood in primates (Gould et al., 1999, 2001; Bernier et al., 2002; Fung et al., 2011a; Wang et al., 2011). Prefrontal myelination occurs predominantly in early post-natal life, still increasing through adolescence before reaching adult levels (Kang et al., 2011).

and Beckmann, 1986; Arnold et al., 1991, 1997; Falkai et al., 2000) at least in some cases, but not all (Jakob and Beckmann, 1986; Krimer et al., 1997; Bernstein et al., 1998), which could be consistent with more subtle alterations in reelin-mediated migration events than what is found in the reeler mouse.

TANGENTIAL MIGRATION OF INTERNEURONS

In recent decades, there has been an increased understanding of the origins and migration of cortical interneurons (Anderson et al., 1997; Nadarajah and Parnavelas, 2002; Zecevic et al., 2011). During development of the mammalian telencephalon, interneurons migrate tangentially from the ventral ganglionic eminences (vSVZ) to the developing cortex (Corbin et al., 2001; Marin and Rubenstein, 2003; Takemura, 2005). Classically, the migration of cortical interneurons was thought to be completed during fetal life (Sidman and Rakic, 1973; Korr and Schmitz, 1999; Zecevic et al., 2005) and thus the number of interneurons was believed to remain stable throughout life (Spalding et al., 2005; Bhardwaj et al., 2006). While migration of interneurons occurs in the human embryo (Sidman and Rakic, 1973; O'Rahilly and Muller, 1994; Meyer, 2001; Bayatti et al., 2008) and may peak in the midgestational period in humans (Meyer, 2001), recent evidence suggests that it may also continue years after birth. Since we and others find a high density of nascent neurons in their birthplace, the SVZ, and a high neuronal density just below the cortex in human infants (Chong et al., 2008; Fung et al., 2011a; Wang et al., 2011), this suggest that addition of new cortical interneurons may contribute to the large growth of the human brain evidenced by a quadrupling of brain weight (Dekaban and Sadowsky, 1978; Beltaifa et al., 2005) and a large increase in cortical gray matter volume from birth to about 5 years of age (Iwasaki et al., 1997; Shankle et al., 1998a,b; Durston et al., 2001; Lenroot et al., 2007). So, while regressive events have received the bulk of the focus in considering cortical development in the context of schizophrenia there is in fact much larger cortical growth events, which are occurring within the first few years after birth.

Measurement of biochemical markers representing different interneuron subtypes suggests that parvalbumin and cholecystokinin mRNAs are increased over post-natal development in humans, most strikingly in the first 5 post-natal years (**Figure 3**). Parvalbumin immunoreactivity first appears in the DLPFC around 3–6 months of age and mRNA is increased 20-fold from neonatal to adult years, indicating that arrival and/or robust maturation of these interneurons occurs in post-natal primate life (Reynolds and Beasley, 2001; Erickson and Lewis, 2002; Cruz et al., 2003; Grateron et al., 2003; Fung et al., 2010). The cell density or mRNA expression of other interneuron markers, such as calbindin and vasoactive intestinal peptide increase similarly in the early post-natal years reaching a peak in children (∼4–7 in human) prior to declining to adult levels in adolescence (Yan et al., 1995; Delalle et al., 1997; Fung et al., 2010; **Figure 3**). Post-natal expression of neuropeptide Y, somatostatin, and calretinin declines with age, indicating that these markers may contribute more to early brain development and become down-regulated with normal brain maturation (**Figure 3**). For example, density of dendrite-targeting somatostatin immunoreactive cells increases in the non-human primate cortex from E120 to E140, before declining through post-natal life and adulthood (Yamashita et al., 1989; Hayashi et al., 1990), with expression in human being dramatically reduced over the first 10 years of life (Fung et al., 2010). Taken together, these results suggest that recruitment, differentiation, growth, and refinement of cortical inhibitory interneurons is a major developmental event, if not the major developmental event, occurring as cognitive abilities like working memory and language are initially attained.

In addition to the developmental profiles of interneuron markers, high levels of neuronal migration markers such as DCX and poly-sialyated neuronal cell adhesion molecule (PSA-NCAM) in infants (Cox et al., 2009; Fung et al., 2011a; Kang et al., 2011; Xu et al., 2011) provide compelling evidence that cortical interneuron migration continues post-natally, particularly in the first few years of life (**Figure 2**). In the adult primate brain, new neurons are produced by two neurogenic regions, the sub-granular zone of the hippocampus (Eriksson et al., 1998) and the vSVZ (Curtis et al., 2003), producing granular cells in the dentate gyrus and interneurons thought to be destined for the olfactory bulb, respectively (Kornack and Rakic, 1999, 2001; Gould, 2007). In the rhesus macaque, we have found ∼50,000–80,000 dividing cells/12 h along the SVZ of juvenile and adult macaques, using both BrdU and Ki67 positive cell markers of cell proliferation (unpublished; Shalaev et al., 2002). Other studies have demonstrated BrdU and NeuN/GAD_{67} co-labeled cells in the principal sulcus of adult macaques (Gould et al., 1999; Koketsu et al., 2003; Runyan et al., 2006; Ashrafi et al., 2007) suggesting that some of these dividing cells may contribute to an adult generated population of interneurons in the cortex. The abundance of DCX positive cells around the ventricle, and the presence of PSA-NCAM positive neurons in the white matter of the principal sulcus in non-human primate brain, further support post-natal generation of neurons from the vSVZ (Fung et al., 2011a). Additionally, the presence of $GAD_{65/67}$+, somatostatin+ and neuropeptide Y+ neurons with morphology reminiscent of tangentially migrating cells (elongated cell body and leading and/or trailing processes parallel to the pial surface) in the post-natal white matter of primates may indicate the presence of post-natally migrating interneurons (Fung et al., 2011a; Yang et al., 2011; Joshi et al., 2012). A recent study by Alvarez-Buylla and colleagues (Sanai et al., 2011) provides further evidence of a major migratory pathway (DCX+, PSA-NCAM+ cells) in post-natal human brains originating from the SVZ and targeting prefrontal cortex. In primates, there are several reports of immature neurons in the amygdala, and in piriform, inferior temporal and prefrontal cortices in post-natal life (Gould et al., 1999, 2001; Bernier et al., 2002; Runyan et al., 2006; Ashrafi et al., 2007). The recent and emerging post-natal primate studies provide new insight into our still incomplete knowledge of the neurodevelopmental trajectory for tangential neuronal migration and we suggest that cortical interneurogenesis is protracted in humans and may continue at a low level throughout human life and we have incorporated this possibility on our graph (**Figure 2**).

Interestingly, most biochemical markers of interneurons are reduced in schizophrenia, supportive of an interneuron deficit in the disease. Specifically, there is strong evidence for reduced

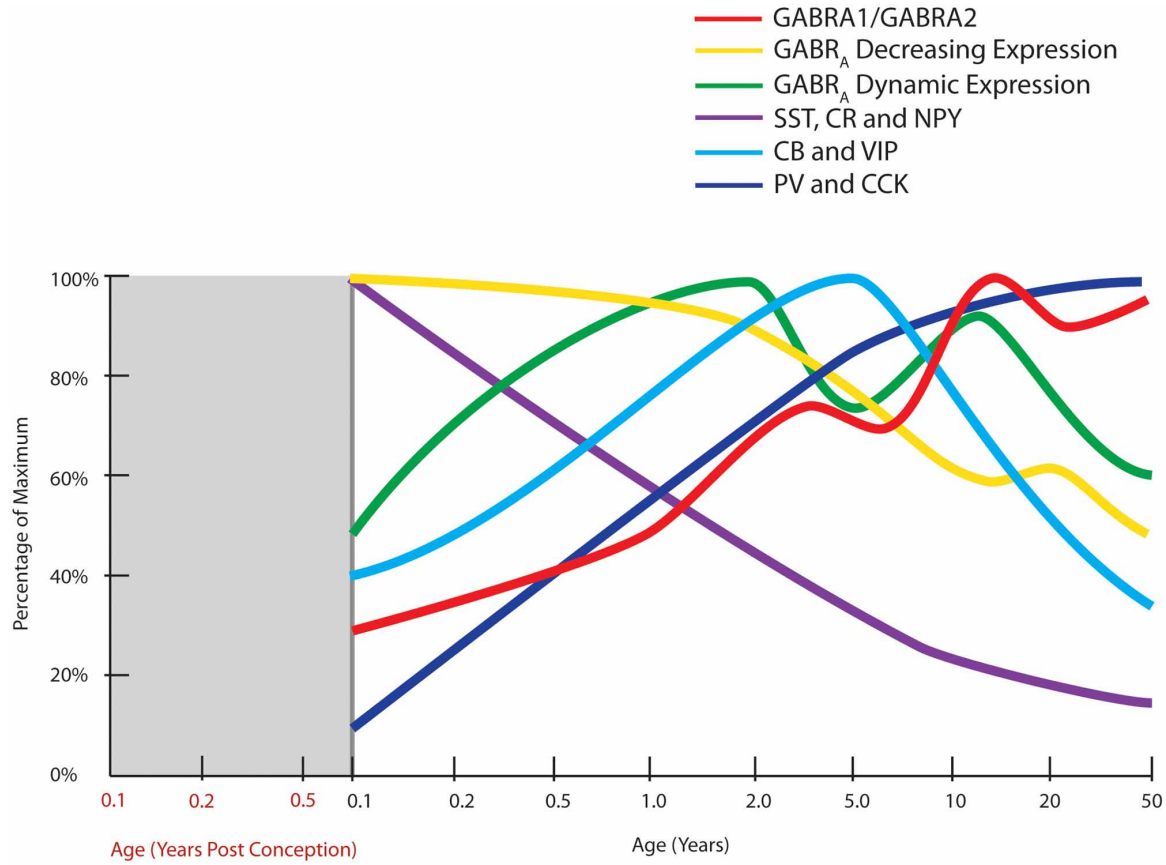

FIGURE 3 | Normal developmental trajectories of expression of inhibitory system components in the human prefrontal cortex. Normal development of GABA receptor components of the inhibitory system is dynamic until adolescence where the trajectories reach a steady or declining state. $GABA_A$ ($GABR_A$) subunits display two distinct patterns, one of decreasing expression following birth (α2, α5, β1, γ1, γ3 subunits) and a M pattern (dynamic expression) with peaks at toddler and teenage time periods (α1, α4, β2, β3, γ2 subunits; Fillman et al., 2010). The peak in the ratio of GABA α1 to α2 subunits (GABRA1/GABRA2) coincides with increased gamma band power in the prefrontal cortex (refer **Figure 1**). Expression of inhibitory neuron markers display either decline over post-natal life (SST, somatostatin; CR, calretinin; NPY, neuropeptide Y), initial up-regulation and then decline or plateau around school age (CB, calbindin; VIP, vasoactive intestinal peptide), or increased expression over post-natal life (PV, parvalbumin; CCK, cholecystokinin; Fung et al., 2010).

expression of the calcium binding protein parvalbumin (Beasley and Reynolds, 1997; Reynolds and Beasley, 2001; Reynolds et al., 2002; Hashimoto et al., 2008a,b; Morris et al., 2008; Sakai et al., 2008; Bitanihirwe et al., 2009; Mellios et al., 2009; Fung et al., 2010) and the neuromodulatory peptide, somatostatin (Hashimoto et al., 2008a,b; Morris et al., 2008; Fung et al., 2010). Given the developmental studies highlighting the protracted maturation of interneurons within the primate cortex over a decade or more (see **Figure 3**) the inhibitory neurons are implicated as a key substrate where brain development may be derailed in schizophrenia, with particular emphasis that a balance of cortical interneuron markers is normally achieved during adolescence (with stabilization of some markers, but down-regulation of others) and overlapping with timing of schizophrenia onset. This requires a new understanding of adolescence as a time of dynamic developmental change, not just as a time of regressive events, but one where the inhibitory system is still being rearranged and positioned for adult life.

There is new evidence that post-natal cortical interneuron migration is of relevance to the pathophysiology of schizophrenia, but this requires a re-interpretation of existing data. Ample evidence for increased interstitial white matter neuron (IWMN) densities in schizophrenia exists (Akbarian et al., 1993; Anderson et al., 1996; Eastwood and Harrison, 2003, 2005a; Kirkpatrick et al., 2003; Yang et al., 2011; Joshi et al., 2012). These observations were initially interpreted as increased remnants of early-generated cortical subplate neurons (a transient population of white matter neurons) derived from the dorsal pallium that failed to undergo programmed cell death (Kostovic and Rakic, 1980; Chun and Shatz, 1989). However, the observations of increased subcortical neurons found in schizophrenia can also be interpreted as being due to abnormal tangential migration of cortical inhibitory interneurons from the vSVZ (Yang et al., 2011; Joshi et al., 2012; Volk et al., 2012). The increase in IWMN density may be indicative of arrested migration of cortical interneurons during their journey to the cortex earlier in life or increased genesis and

migration of new interneurons toward the cortex in response to possible cortical trauma, such as increased inflammation (Fillman et al., 2013). In either case, the recognition that cortical interneurons are generated from a unique birth place (vSVZ), require particular neurodevelopmental signals, can take years to establish themselves, and may have the ability to continually turn over, even at low levels, suggests that a greater understanding of the timing, the control and extent of interneurogenesis and migration may provide important clues as to the developmental origins of interneuron pathology in schizophrenia.

MYELINATION

Another developmental event that extends well into post-natal life is myelination (**Figure 2**). Brain axonal tracts are myelinated progressively across the lifespan in a region- and function-specific manner. Early human post-mortem work first described regional differences in the degree of myelination across the lifespan and highlighted the protracted development of a number of white matter tracts. Comparison of qualitative myelin staining in 200 cases from mid-gestation to 1-year old, from 1 to 30-years old, and numerous older cases yielded findings of earlier myelination of the hippocampus, primary motor, and primary sensory areas, and later myelination of frontal cortical white matter, extending into the 4th decade of life (Yakovlev and Lecours, 1967). A follow-up study ($n = 12$ brains) corroborated these findings in the primary motor and sensory cortices, but failed to confirm protracted myelination of the prefrontal cortex (Benes, 1989). Both studies were limited by critical age gaps in the cohort or very small sample sizes at certain stages. A comprehensive larger study graded myelination in 62 white matter sites within 162 post-mortem brains from newborn to 3-years of age (Brody et al., 1987; Kinney et al., 1988). This study confirmed that the major white matter tracts, subcortical regions, primary cortical areas, and hippocampus become myelinated sooner than association cortical areas, but estimated that even in association areas the majority of infants achieve substantial myelination by the age of 2. A more recent study investigated a marker for myelination, myelin basic protein (MBP), in the parietal cortex and found MBP expression commenced at around 3.5 months of age, and reached adult-like levels from 13 months of age (Haynes et al., 2005). Investigation of hippocampal myelination has also suggested the importance of early myelin development, with appearance of oligodendrocytes from gw 20 and of myelinated fibers from post-natal week 2 (Abraham et al., 2010). By 11 years of age, myelinated fiber density in the hippocampus was well developed but had still not reached the highest adult levels (Abraham et al., 2010). These findings suggest that most myelination and axonal growth occur within the first 2 years of early life, but may be ongoing in many telencephalic areas including the frontal cortex and hippocampus. Post-mortem microarray data from our laboratory (Harris et al., 2009; Weickert et al., 2009), have demonstrated dramatic increases in mRNA transcripts of myelin proteins in the prefrontal cortex across human post-natal life. We observed increasing mRNA expression of MBP, myelin-associated oligodendrocyte basic protein (MOBP) and proteolipid protein 1 (PLP1) during the first decade of life (all $p < 0.001$). From birth to 2 years of age, there was an approximately 100 times increase in MBP expression, a 20 times increase in MOBP expression and a 10 times increase in PLP1 expression, followed by a much more gradual increase of all three transcripts from early childhood into their peak in adolescence. These findings are supported by other recent microarray data, in which expression of genes associated with myelination reached approximately 50% of adult levels by birth and 95% of adult levels by 2–3 years of age in the neocortex (Kang et al., 2011). These post-mortem gene expression findings suggest that the most active myelination of the human prefrontal cortex occurs early in life, predominantly in the first 2 years of life, but continues throughout childhood and adolescence before full myelin maturation levels are reached in adulthood.

The molecular findings of developmental myelination trajectories in normal humans have been confirmed with brain imaging studies of growth of white matter tracts across human post-natal development. Throughout the brain, white matter, especially myelin, develops rapidly in the first 12 months of life (with most dramatic changes from 0 to 3 months) followed by slower change from 1 to 2 years and thereafter (Schneider et al., 2004; Dubois et al., 2006; Hermoye et al., 2006; Provenzale et al., 2007; Gao et al., 2009). After the first year of life, and throughout childhood and adolescence, changes in white matter measures (fractional anisotropy) are more subtle and are predominantly due to altered perpendicular diffusivity, reflecting increasing myelination (Giorgio et al., 2008; Lebel et al., 2008; Gao et al., 2009). However, global age-related white matter changes can still be observed in adolescents (Barnea-Goraly et al., 2005; Giorgio et al., 2008, 2010; Bava et al., 2010), while region-specific changes can continue in young adulthood (Giorgio et al., 2008). White matter maturation occurs in a region specific manner, with late maturation associated with the prefrontal cortex throughout the first 2 decades of life reaching full maturation at 18 years (Barnea-Goraly et al., 2005) and fronto-temporal tracts approaching full maturation after 25 years (Lebel et al., 2008).

Overall, these studies provide strong evidence that myelination throughout the brain takes place most rapidly early in life. However, in numerous regions, including prefrontal regions, the process continues throughout the second decade of life with full maturation only reached in late adolescence or young adulthood. In brain regions implicated in the pathogenesis of schizophrenia, such as the prefrontal cortex, full white matter maturation is mostly achieved prior to the period of life when schizophrenia emerges. However, improvements in working memory (which employs the prefrontal cortex) are associated with increased white matter maturation during adolescence (Nagy et al., 2004; Bava et al., 2010), while poorer performance is associated with less white matter maturation (Bava et al., 2010). Deficits of working memory are also consistently seen in schizophrenia (Goldman-Rakic, 1994; Meyer-Lindenberg et al., 2005), and structural abnormalities in white matter underlying the DLPFC have also been reported (Kyriakopoulos et al., 2008). Microarray studies of post-mortem brains from patients with schizophrenia have implicated myelination deficits in the pathology of schizophrenia (Hakak et al., 2001; Matthews et al., 2012). These observations have been validated by targeted investigations of cortex, which have revealed a decrease in mRNA (Matthews et al., 2012) and protein levels (Honer et al., 1999; Parlapani et al., 2009) of MBP

in schizophrenia, though not consistently (Beasley et al., 2009). Thus, it seems likely that abnormal maturation of white matter could contribute to cognitive disturbances found in schizophrenia and that a better understanding of the molecular control of white matter maturation and how it could be disrupted in schizophrenia may reveal novel clues to neurodevelopmental origins of schizophrenia.

EXCITATORY SYNAPSES

Probably the most popular theory for neurodevelopmental origins of schizophrenia is the theory that dendritic spines are over-pruned in adolescence. The formation and elimination of dendritic spines, subcellular structures specialized for receipt and integration of excitatory input to pyramidal neurons is important as spine numbers are thought to correlate with cognitive and learning ability (Elston, 2000).

DENDRITIC ARBORIZATION AND SPINE DENSITIES

An early study (Huttenlocher, 1979) quantified synaptic density in the cerebral cortex across the human lifespan and suggested that synaptic regression occurred after the first two post-natal years; however, this study (Huttenlocher, 1979) had only one brain available in the age group between 7 and 25 years of age and one between 25 and 50 years of age, requiring a great deal of extrapolation from few data points. On the basis of the available data, it was concluded that synaptic regression occurs anytime between 2 and 16 years of age in human frontal cortex. It was a few years later that Feinberg interpreted these data as supporting the notion that a physical change, namely "synaptic pruning" could be occurring in the human brain during adolescence, and that emergence of schizophrenia symptomatology may be due to an overexuberance of synaptic elimination (Feinberg, 1983; Keshavan et al., 1994; Selemon and Goldman-Rakic, 1999). While this speculation has had enormous influence on neurodevelopmental models of schizophrenia and is found in most reviews on this topic, few investigators seem to carefully scrutinize the evidence upon which this theory is based, often interpreting a "dotted" line plotting supposed time of synaptic reduction as adolescent pruning, when in fact it is based on only 3 individuals and shows a putative reduction in synaptic density starting several years before adolescence. Further, as synapse densities change with brain volume changes and possible gain or loss of whole neurons, glia and blood vessels, it is not clear if changes in synapse densities across development are attributable to a net loss of number of synapses per neuron (pruning) or changes in these other factors (Huttenlocher, 1990). A subsequent report by Huttenlocher and Dabholkar (1997) with four adolescent cases could suggest in fact that there may indeed be no change or a small net increase in synapse density in the time period between infancy and adolescence in the human prefrontal cortex.

A more recent Golgi study of dendritic development in human cortex by Petanjek and colleagues (2008) spans a broad age range (newborn to age 91 years, $n = 25$) and includes 13 cases between the ages of 1 week and 10 years and studied the development of the layer III basilar dendritic tree from birth to the beginning of adolescence. There was an effect of age on all dendritic variables measured, except for the number of basal dendrites, which remained constant. The dendritic segment count reached adult levels by the age of 12 months. A 3-fold increase in the length of the dendritic tree of layer III pyramidal cells by 2.5 months of age with an additional 50% increase in the length of terminal dendritic segments between 16 and 30 months of age was observed. A plateau in intermediate dendritic segment length was observed during late adolescence, a period which was represented by three cases in this study (**Figure 4**). These data suggest that the major developmental event during early childhood is exuberant early growth of structures that are specialized to receive synapses and is not consistent with large-scale "pruning" of synapses or synaptic reductions that were believed to occur during post-natal life based on synaptic density counts (Huttenlocher, 1979).

In another study, basilar dendritic complexity appeared greater in younger individuals (14–40 years of age) than in older individuals (40–106 years of age; Jacobs et al., 1997). This was reflected in a statistically significant reduction of 9–12% in total dendritic length and a slight (4%), but statistically significant increase in the number of dendritic branches per cell in the older adult age group compared with the younger age group. The most substantial change observed was in dendritic spine measures, with a gradual 50% decrease in the number and density of spines from the mid-twenties to mid-adulthood (40 years: Jacobs et al., 1997). This suggests that the interpretation of earlier findings of changes in synaptic density reported by Huttenlocher (1979) could have been influenced by the many individuals in the 60–80 year range. However, Jacobs et al. (1997) found the number and density of spines between the ages of 40 to 106 years appeared stable in this study and the variability due to aging was relatively constricted to early adulthood. A subsequent study details changes in spine density in the DLPFC across the lifespan (Petanjek et al., 2011) using a post-mortem brain collection overlapping with that used in Petanjek's study described above. A peak in layer III pyramidal neuron spine densities in early life with higher level in school age as compared to the first year of life and an apparent stability from childhood through adolescence (5–20 years of age), and a change that begins after the second decade of life where spine density appears to gradually decline over the next 30 years (to age 50, **Figure 4**) and where this loss extends into aging (not graphed).

The studies reviewed above (Jacobs et al., 1997; Petanjek et al., 2008, 2011) had substantial inter-individual variability attributable to differences in the populations studied, and relatively small collection sizes in the context of broad age-ranges of the samples used. Taken together, these studies suggest that the timing of synaptic density change likely involves very early post-natal increases and that the timing and extent of synaptic density decreases and reductions in dendritic spines may be gradual and protracted in humans. The studies available suggest that synaptic pruning may not actually be prominent in human adolescence but may have its onset in toddlerhood until school age and/or begin after adolescence and extend into the 5th decade of life. Thus, the reviewed data suggest that the onset of synaptic regression is unlikely to be the trigger for the onset of schizophrenia. Another way to gather support for the timing of synaptic regression would be to examine quantitative measures of molecular makers of synapses during normal human cortical development as we and others have done and elaborate on next.

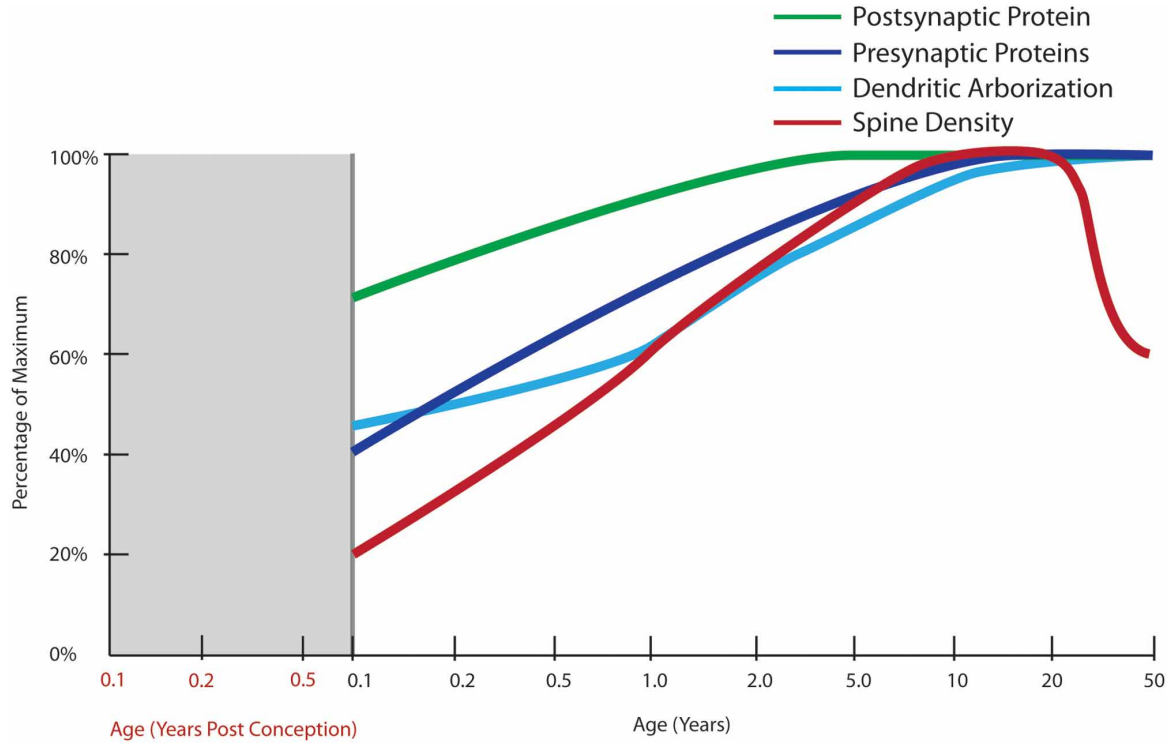

FIGURE 4 | Normal developmental trajectories of excitatory system components in the human prefrontal cortex. Normal development of the excitatory system involves increasing expression of presynaptic SNARE complex proteins (SNAP-25, syntaxin 1A, VAMP1) into early adulthood (Webster et al., 2011) and a similar increase in protein expressions of the post-synaptic markers, PSD95 and spinophilin, though starting at a higher baseline at birth (Kang et al., 2011; Webster et al., 2011). Dendritic arborization indexed by average length of the total dendritic tree of layer III pyramidal neurons does not reach its maximum until adulthood with a slight decline after the age of 30 years (Petanjek et al., 2008). Quantification of Golgi-impregnated tissue suggests an increase in spine density from birth until early school age, followed by a period of gradually decreasing density starting in early adulthood which lasts until middle age (Petanjek et al., 2011).

MOLECULAR MARKERS OF EXCITATORY SYNAPSES

While development of the glutamatergic synapses begins prenatally with the majority of synapses formed during the neonatal period (Feldmeyer and Radnikow, 2009) other work suggests the levels of one protein often used as marker of synapse density, synaptophysin, peaks much later in life during school age years (6–10 years of age) while levels of a protein thought to be marker of dendritic spines, PSD-95, peaks even later, in early adolescence and then marginally declines (Glantz et al., 2007). Consistent with this, Salimi et al. (2008) found a similar pattern of a late peak in the protein expression of complexin 2, a marker of excitatory synapses. Importantly, the ratio of excitatory to inhibitory synapse markers (complexin 2/complexin 1) decreased in adolescence (Salimi et al., 2008), again pointing to the maturation of the inhibitory system as an important adolescent event. Our recent data from multiple molecular markers suggest that developmental increases in synaptic proteins are not followed by a significant decline either in childhood or in adolescence as would be expected with major synaptic regression (Webster et al., 2011). We have found that a variety of synaptic mRNAs and proteins increase steadily after birth during childhood years (Webster et al., 2011). This pattern of increasing expression was found for many presynaptic proteins and was particularly true of the SNARE complex proteins (syntaxin 1A, SNAP-25, and VAMP1) where syntaxin-1A levels peaked in young adulthood or even in adult age (Fung et al., 2011b; Webster et al., 2011; **Figure 5**). In terms of molecules localized to the postsynaptic elements, PSD-95 and spinophillin both had highest mRNA levels during infancy with protein levels increasing until school age and with no evidence of an adolescent decline in protein (Webster et al., 2011; **Figure 5**). Overall there is a major developmental increase in synaptic components, especially at the protein level, from neonates through school age and adolescence that remains relatively stable through adolescence and into adulthood. These observations are contradictory to the model proposed by earlier studies that suggested the major developmental event in adolescence is regression or loss of synaptic elements (Huttenlocher, 1979; Feinberg, 1983; Glantz et al., 2007). The work reviewed here provides evidence that the theory stating that exuberant excitatory synapse loss could be the basis for the aberrant adolescent brain development in schizophrenia may need to be reconsidered. This position gains additional support from the lack of consistent molecular changes in presynaptic mRNAs and proteins in schizophrenia brains (see below).

Studies of markers of excitatory synapses in neocortex of patients with schizophrenia primarily find no change (Eastwood

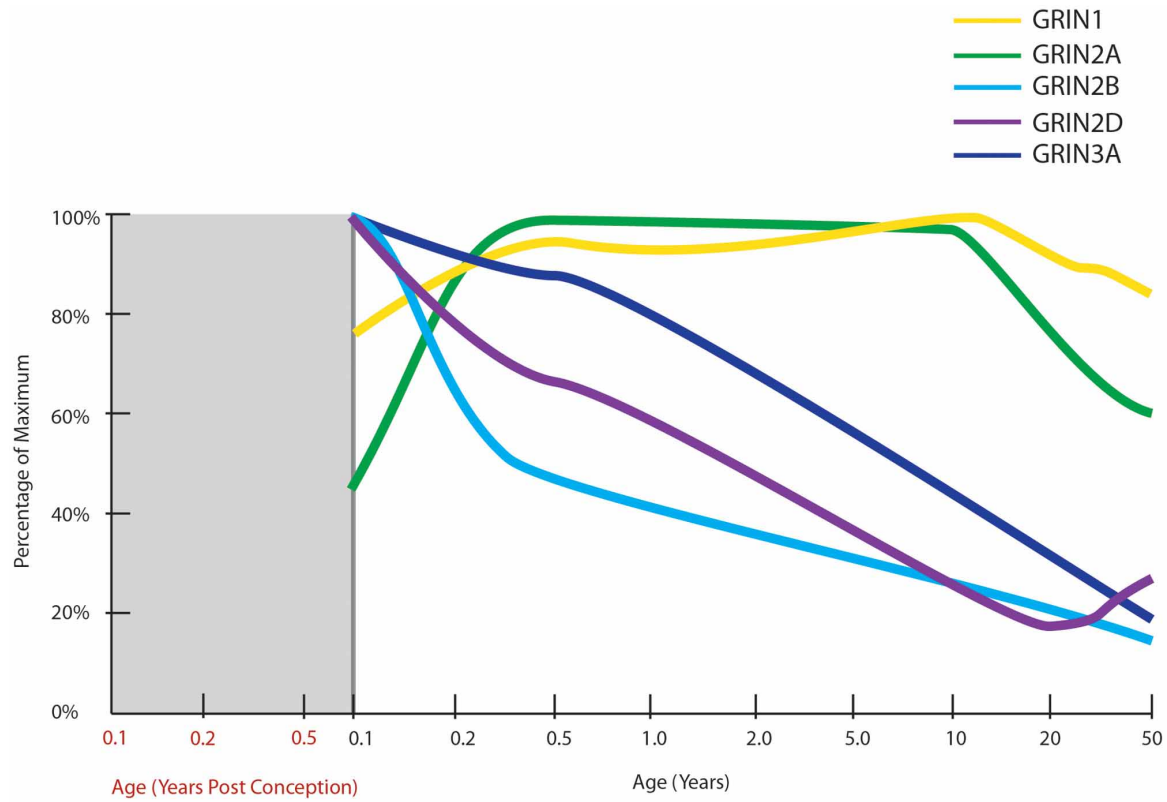

FIGURE 5 | Normal developmental trajectories of NMDA receptor subunits in the human prefrontal cortex. Normal development of NMDA receptor subunits (Choi et al., 2009; Colantuoni et al., 2011; Kang et al., 2011). mRNA of the obligatory NMDA receptor subunit, GRIN1 (NR1), is expressed at fairly steady levels post-natally, at least until middle age. GRIN2A expression increases in infancy to attain fairly steady levels throughout childhood and then decreases through adolescence and into adulthood. GRIN2B, GRIN2D, and GRIN3A all have their highest expressions at birth with decreasing trajectories thereafter.

and Harrison, 2001, 2005b; Sawada et al., 2002; Halim et al., 2003; Fung et al., 2011b) or slightly decreased levels (Eastwood and Harrison, 2005b). Similarly, measurement of excitatory spine markers has led to divergent results (Law et al., 2004; Weickert et al., 2004; Toro and Deakin, 2005; Kristiansen et al., 2006; Catts and Weickert, 2012), mainly suggesting there is no change or only slight decreased expression of spinophilin and PSD-95 in cortex of patients with schizophrenia. A molecular marker of dendrites, microtubule-associated protein 2, is also typically unaltered in level, but sometimes decreased in the cortex (Jones et al., 2002; Mukaetova-Ladinska et al., 2002; Somenarain and Jones, 2010). Even when slight decreases in molecular markers of excitatory synapses are found, they are typically identified in only a subset of cases (Faludi and Mirnics, 2011), not found in all anatomical regions (Webster et al., 2001; Law et al., 2004; Toro and Deakin, 2005), restricted to certain layers (layer III basilar dendrites only: Glantz and Lewis, 2000; Kolluri et al., 2005) and not consistent across cohorts (Halim et al., 2003; Eastwood and Harrison, 2005b; Castillo et al., 2010; Gray et al., 2010; Fung et al., 2011b). Taken together, schizophrenia, in general, does not appear to be characterized by a pervasive and consistent loss of excitatory synapse markers.

This raises the question of what physical elements could be responsible for cortical gray matter volume reductions found in schizophrenia? A recent and quite comprehensive consideration of the cellular components of cortical gray matter has yielded the following estimates for percentage of space occupied by each: neurons = ∼64%, glial = ∼12.5%, synapses = ∼6%, capillaries = ∼0.5%, and extracellular spaces = ∼17% (Bennett, 2011). Based on available histological evidence from studies on people with schizophrenia, Bennett suggests that a deficit in spines (Glantz and Lewis, 2000; Kolluri et al., 2005) alone would be insufficient to explain the gray matter loss found in imaging studies which is typically on the order of a 8–10% reduction. The fact that the loss of spines is quite anatomically restricted, i.e., occurs in deep layer III, but not in superficial layer III, and is not found in layers V or layers VI in frontal cortex also supports that spine loss is likely only a partial explanation for cortical thinning (Glantz and Lewis, 2000; Kolluri et al., 2005). Bennett concludes that other elements must be involved and he suggests that loss of dendrites is the likely suspect (Bennett, 2011). However, the 30% loss of dendrites used to form this argument appears to only apply to basilar dendrites as apical dendrites and segment length are actually non-significantly increased in the cortex of people with schizophrenia

(Kalus et al., 2000). Considering that basilar dendrites occupy an estimated 60% of the total dendritic length (Soloway et al., 2002), this reported loss of dendrites still may be only a partial explanation for cortical volume loss. What the anatomical substrate of the consistent reductions in gray matter volume found in patients with schizophrenia actually is, if it is not synaptic loss, remains an unanswered question. Clearly more quantitative anatomical studies of the brains of patients with schizophrenia are needed to determine the answer. We suggest that it is premature to consider all gray matter reduction in schizophrenia to be due solely to loss of synapses or to over-exuberant synaptic pruning.

RECEPTOR SYSTEMS

Schizophrenia pathophysiology likely involves disruptions to three neurotransmitter systems, namely; excitatory glutamatergic, inhibitory GABAergic, and modulating dopaminergic, input [reviewed in Seshadri et al. (2013)], the developmental trajectories of some of the main receptors for each are reviewed below.

NMDA RECEPTORS

The majority of excitatory glutamate synapses, important for learning, are primarily found on postsynaptic dendritic spines (Sheng and Hoogenraad, 2007) with synapses equipped with both ionotropic and metabotropic glutamate receptors (Hollmann and Heinemann, 1994). The expression trajectories of some ionotropic glutamate receptor subunits, those of AMPA and kainite, do not change during post-natal life. The expression profiles are in fact quite similar to one another, with increasing expression during gestation reaching 100% of maximum around the time of birth and remaining steady thereafter (Kang et al., 2011). Another ionotropic glutamate receptor, N-methyl-D-aspartate receptor (NMDAR), is critically important for the calcium flow in and out of cells that is responsible for both long-term potentiation (LTP) and long-term depression (LTD: Li and Tsien, 2009) while metabotropic glutamate receptors can increase or decrease the excitability of the postsynaptic neuron and regulate postsynaptic protein synthesis through second messenger systems, in effect modulating synaptic plasticity (Hollmann and Heinemann, 1994). Given the interest in NMDARs in schizophrenia, due to the psychotomimetic effect if blocked (Catts and Catts, 2010) and for their role in cognitive ability and synaptic plasticity across development, we will focus on developmental change in NMDARs.

NMDARs are heterotetrameric receptors, comprised of two obligatory NR1 subunits together with two NR2 (A-D) or NR3 (A-B) subunits, with the different assembly of subunits determining the functional differences in receptor properties (Cull-Candy et al., 2001). One of the first studies to examine NR1 mRNA and protein levels across human development in the PFC (Henson et al., 2008) found expression significantly changed across age in a sample from 18 gw to 25-years old. NR1 protein levels were low prenatally, rose to a peak at 11–15 years then reduced slightly in young adults. The mRNA expression followed a similar pattern but with a less marked reduction in adulthood. Two recent transcriptome analyses have also charted NMDAR subunit mRNAs in human prefrontal cortex from early gestation to old age (Colantuoni et al., 2011; Kang et al., 2011; **Figure 5**). NR1 mRNA expression was generally consistent across post-natal life, confirming our unpublished findings, and not consistent with the putative peak in NMDAR1 early adolescent peak reported earlier (Henson et al., 2008).

In general, in rodents NR2B, 2D, and 3A predominate in early life and 2A, 2C, and 3B are expressed to a greater extent in adults as compared to neonates. Thus, in the developing rodent brain, there is a shift in the ratio of NR2A/NR2B containing receptors during post-natal development in several brain regions, with a higher proportion of NR2A-rich receptors in later life (Ritter et al., 2002; Turman et al., 2002). This ratio switch is also present in the human hippocampus (Law et al., 2003) and the visual cortex (Murphy et al., 2005). NR2B-rich receptors exhibit longer excitatory postsynaptic currents (EPSCs; Monyer et al., 1994; Flint et al., 1997), enabling the coincidence of pre- and postsynaptic events and thus facilitating experience dependent synaptic plasticity in the critical early post-natal period (Philpot et al., 2001). Thus, the decreasing NR2A/NR2B ratio across life may be responsible for the decrease in plasticity seen during maturation and ageing (Crair and Malenka, 1995). In the rat hippocampus, the NR2A/NR2B ratio is believed to mediate the process of synaptic remodeling (Gambrill and Barria, 2011). Higher NR2B expression increases the rate of addition and subtraction of spines, and higher NR2A expression reduces the number of synapses and their volume.

In two recent transcriptome analyses, the prenatal expression of all NMDAR subunit mRNAs were found to be low in both studies, with exception of NR2B mRNA which is high prenatally and peaks shortly before birth (Colantuoni et al., 2011; Kang et al., 2011). While NR2C mRNA expression was steady across most of the life span, it displayed a slight increase after 70 years of age (Colantuoni et al., 2011; Kang et al., 2011). In Henson and colleagues' study of the PFC, NR3A mRNA and protein were very low during prenatal development, at maximum levels in the first year after birth and then declined gradually from ages 1 to 25, to be at 30% of the maximum expression (Henson et al., 2008). We also studied NMDAR mRNA levels in individuals aged from 1 month to 49 years (Choi et al., 2009) and found NR3A ($r = -0.933$) and NR2D ($r = -0.785$) mRNA levels were high within the first few years of human life and were significantly and gradually down-regulated with advancing age. Adult NR3A mRNA expression decreased to 35% of neonatal levels, consistent with the findings of Henson and colleagues (2008). Adult NR2D mRNA decreased to 50% of neonatal levels in our microarray study (Choi et al., 2009). Unlike other NMDAR subunits, NR2D expression is thought to be absent from cell bodies (Thompson et al., 2002) and little is known of how this specific subunit influences the physiology of the receptor, however, there is evidence that recruitment of extrasynaptic NMDARs containing NR2D are important for the expression of LTP in the hippocampus in adult rodents (Harney et al., 2008). Similar to what we find in human cortical development, rodent NR3A expression peaks early in life during the period of synaptogenesis and is down-regulated prior to the critical period of plasticity. This suggests that the critical period for NMDAR mediated plasticity in humans may start around toddlerhood in the prefrontal cortex (Wong et al., 2002; Perez-Otano et al., 2006) at a time where language acquisition and

utilization grows at a rapid pace. In rodents, deletion of NR3A increases spine density and leads to failure to maintain juvenile-type synapses (Das et al., 1998; Roberts et al., 2009). Taken together, these results suggest that the major post-natal change in human cortical NMDARs may not be the NR2B to NR2A switch, but rather may feature the down-regulation of NR2D and NR3A more prominently.

The extent to which NMDAR subunit mRNAs are altered in the brains of people with schizophrenia varies with subunit and brain region, however, mRNA changes remain controversial, with decreases (Humphries et al., 1996; Sokolov, 1998; Law and Deakin, 2001; Beneyto and Meador-Woodruff, 2008; Weickert et al., 2012), increases (Akbarian et al., 1996; Dracheva et al., 2001; Schmitt et al., 2010) and no change (Akbarian et al., 1996) observed in NR1. Direct studies of NMDAR protein expression in schizophrenia patients are rare. One study found no significant changes in NR1 or NR2A-D protein levels in schizophrenia patients, however, an alternatively spliced isoform of NR1, $NR1^{C2}$, was increased in the anterior cingulate cortex (Kristiansen et al., 2006). Binding studies also show mixed results depending on brain region and binding site. In the frontal cortex, studies have shown no alteration in binding at the PCP (Dean et al., 1999, 2001; Scarr et al., 2005; Beneyto and Meador-Woodruff, 2008), glycine (Nudmamud and Reynolds, 2001, polyamine or glutamate sites (Beneyto and Meador-Woodruff, 2008) in schizophrenia, while one study found an increase in glycine site binding in the schizophrenia orbital frontal cortex (Simpson et al., 1991) and increases in glycine site binding have also been demonstrated in various other cortical areas including superior temporal cortex (Nudmamud and Reynolds, 2001), somesthetic, visual, and premotor areas (Ishimaru et al., 1992). One study found increased [^{3} H]MK-801 binding in the putamen in schizophrenia (Kornhuber et al., 1989), however, a subsequent study showed no change in binding in the major striatal structures (Noga et al., 1997). We recently demonstrated for the first time a robust decrease in NR1 protein in schizophrenia patients in prefrontal cortex (Weickert et al., 2012). Our study also described decreased expressions of the NR1 and NR2C mRNA subunits in schizophrenia patients (Weickert et al., 2012), which are consistent with an earlier study (Beneyto and Meador-Woodruff, 2008). The Meador–Woodruff group also reported a significant increase in NR3A mRNA (Mueller and Meador-Woodruff, 2004) which could indicate that the NMDARs are in a more immature state and that higher NR3A could interfere with age-appropriate cortical plasticity in schizophrenia. Elimination of interneuronal expression of mature NMDARs post-natally, but prior to onset of adolescence, leads to a schizophrenia-like phenotype in mice (Belforte et al., 2010), suggesting that either the lower levels of the obligatory subunit (NR1) or higher levels of the NR3A subunit could interfere with the maturational changes in NMDARs and contribute to schizophrenia.

INHIBITORY NEUROTRANSMITTER RECEPTORS

The major inhibitory neurotransmitter we will consider here is gamma-aminobutyric acid (GABA) which binds ligand gated ion channel ($GABA_A$ receptor) and G-protein coupled receptors ($GABA_B$ receptor; Aguayo et al., 2004; Olsen and Sieghart, 2008). GABA is synthesized by two enzymes, GAD_{65} (GAD2) and GAD_{67} (GAD1). GAD_{67} is responsible for 90% of the basal GABA synthesis and is produced at limiting levels in the brain (Asada et al., 1997; Kash et al., 1997). In the human, strong basal expression of GAD_{67} was shown from neonate through adult, with GAD_{65} increasing and peaking in the teenage years (Pinto et al., 2010). However, in the DLPFC, our unpublished microarray data as well as that from Kang et al. (2011) show the mRNA for GAD_{65} peaks around one year of age and stays consistently expressed from infants to adulthood with little or no drop-off. Our microarray data shows a similar pattern for GAD_{67} implying that mRNA for both constitutive and synaptic synthesis of GABA is fairly stable throughout post-natal life. This is interesting as the enzymes responsible for GABA production are expressed at a steady rate despite large changes in distribution, maturational state, and phenotype of the interneuron populations that occur in post-natal human life (see earlier section).

GABA receptors act to hyperpolarize adult neurons, but act to depolarize younger neurons (Flint et al., 1998; Ben-Ari et al., 2007). This reversal is due to the high chloride concentrations found in immature neurons due to high developmental expression of Na-K-Cl co-transporter 1 (NKCC1, also known as SLC12A2: Di Cristo, 2007). NKCC1 is highly expressed during early fetal human development and increases until reaching a plateau in early childhood in the DLPFC (Hyde et al., 2011). The other major potassium-chloride transporter member, KCC2 (SLC12A5), is also expressed in early fetal development, but at much lower levels and also increases before reaching its steady state during early childhood (Hyde et al., 2011). There is uncertainty in establishing an exact crossover point from which GABA switches from excitatory to inhibitory, but it could be as late as 10 years of age (Hyde et al., 2011). The uncertainty can be attributed to two main causes; firstly, the individual variability of the developmental expression of NKCC1 and KCC2, both in Hyde's study and our own unpublished microarray findings, is substantial and thus the point of crossover may occur anywhere from prenatal life to school age when examining the overall profiles. Secondly, it has been shown that individual cells may have differing chloride gradients due to variable subcellular distribution of the two chloride transporters even within the same neuron, particularly at the axon initial segment (Khirug et al., 2008). This would result in GABA having an inhibitory effect on the dendritic portions of a cell but perhaps an excitatory effect at the axon initial segment. In homogenate tissues of the DLPFC, this type of individual cell variation in chloride transporter expression would be diluted but may help to explain NKCC1's continued presence throughout life when GABA action is predominately inhibitory.

Since more comprehensive and validated developmental data are available for the $GABA_A$ receptor as compared to the $GABA_B$ receptor, we will consider the developmental changes for the $GABA_A$ receptor mRNAs here. The $GABA_A$ receptor is a pentameric ion channel that is made up from a number of possible subunits, namely 6α, 3β, 3γ, δ, ε, and π subunits (Szabadics et al., 2006; Olsen and Sieghart, 2008). In the DLPFC, the human post-natal developmental change in the $GABA_A$ receptor α/β/γ subunits can be divided into two groups (**Figure 3**). The first comprises the subunits with decreasing mRNA expression over

the course of development (α2, α5, β1, γ1, γ3) and the second comprises those with a more dynamic expression pattern (α1, α4, β2, β3, γ2) with an M-shape peaking at the toddler and teenage time periods (Duncan et al., 2010; Fillman et al., 2010). The mature forms (predominant in adulthood) of the receptor subunits such as α1, β2, and γ2 typically have rapid channel opening/closing kinetics and greater sensitivity to GABA leading to greater temporal specificity of inhibitory action (Hevers and Lüddens, 1998; McClellan and Twyman, 1999).

The postsynaptic $GABA_A$ receptor α1 and α2 subunits show consistent change in post-natal life with α1 increasing in life with greatest rate of change soon after birth with more gradual increase until young adulthood and with quite dramatic down-regulation of α2 across post-natal life (Hashimoto et al., 2009; Duncan et al., 2010). This leads to an overall increase in the α1/α2 ratio in the prefrontal cortex, and it is interesting that the later peak in this ratio (in teenagers) aligns with the increase in gamma band power found in the prefrontal cortex in humans (Uhlhaas et al., 2009). However, changes in individual receptor subunit mRNAs for the β2, γ2 subunits do not achieve their peak of expression until the teenage/young adult years (Fillman et al., 2010) implying changes in the ratio of other $GABA_A$ receptor subunits also may contribute to increasing cognitive capacity of humans through development.

One of the most robust findings in schizophrenia neuropathology is deficits in cortical inhibitory interneurons across several cortical regions (Hashimoto et al., 2008a; Thompson et al., 2009), including DLPFC. Findings include consistent reductions in expression of GAD_{67}, which have been recently reviewed (Gonzalez-Burgos et al., 2010). Given the reproducible changes in GAD_{67}, one may expect to see correspondingly reproducible changes in the $GABA_A$ receptor. While there appears to be a replicable change toward an increase in GABA binding in the cortex of people with schizophrenia (Benes et al., 1992; Dean et al., 1999; Newell et al., 2007; Verdurand et al., 2013), there is less consensus as to changes occurring in the $GABA_A$ receptor mRNAs in schizophrenia. In DLPFC from schizophrenia patients, Volk et al. (2002) found an increase in $GABA_A$ α2 subunit labeled axon initial segments. However, as the axon initial segment lacks mature KCC2 transporters, the effect of this putative loss of GABA and increase in $GABA_A$ α2 is debated (Volk et al., 2002; Szabadics et al., 2006). It is noteworthy that the increased expression of $GABA_A$ α2 subunit has been independently replicated (Beneyto et al., 2011), but may not be found in all studies (Duncan et al., 2010). In contrast, decreased expression of the $GABA_A$ subunits α1 and α5 in the DLPFC from patients with schizophrenia has been more consistently observed (Hashimoto et al., 2008a; Duncan et al., 2010; Beneyto et al., 2011). The changes occurring in $GABA_A$ subunits in schizophrenia (an increase in α2 and a decrease α1) could be interpreted to reflect a cortex that may be held in a state of immaturity into adulthood. This concept is interesting as it fits with a number of other changes that would also indicate that the schizophrenia cortex could resemble a more immature cortical state, like increased NR3A, decreased parvalbumin and more calbindin with less cholecystokinin expression as just a few other notable examples consistent with this model.

DOPAMINE RECEPTORS

Functions of the DLPFC, like working memory, cognitive control, and reasoning (Goldman-Rakic, 1996; Nieoullon, 2002; Glickstein et al., 2005; Mizoguchi et al., 2009) are impacted in schizophrenia and are sensitive to changes in cortical dopamine (Williams and Goldman-Rakic, 1995; Lewis, 1997; Romanides et al., 1999) As these cognitive functions change across development, it is of interest to consider how dopamine receptors may change as humans grow and mature. Cortical dopamine receptors fall into two distinct categories. DRD2-like receptors [DRD2 (S = short and L = long), DRD4] and DRD1-like receptors (DRD1, DRD5: Monsma et al., 1989; Missale et al., 1998). The expression of DRD2 (both forms), DRD4, DRD5 mRNAs all display a predominate pattern of developmental change with highest levels of expression in infants that then declines with age (Weickert et al., 2007; Rothmond et al., 2012; **Figure 6**). Interestingly, the opposite pattern of expression for both mRNA and protein is observed for DRD1 making DRD1 unique among the cortical dopamine receptors in terms of its developmental expression profile and suggests that there is an increased role of DRD1 as the human cortex matures (Weickert et al., 2007; Rothmond et al., 2012; **Figure 6**). By early adulthood DRD1 appears to be the most prevalent receptor in the human PFC (Lidow and Rakic, 1992; Meador-Woodruff et al., 1997) and DRD1 is critical to PFC cognitive functioning and in particular working memory (Arnsten et al., 1994). Thus, the gradual increase in DRD1 protein levels from pre-adolescence into young adulthood happens during a time in development when the cortex functionally matures. It coincides with improved performance on a cognitive test thought to tap into a core cognitive deficit in schizophrenia, the Wisconsin Card Sorting Test, which measures behavioral flexibility and working memory (see earlier section), suggesting that DRD1 function may have a particularly salient role in working memory. Indeed, DRD1 receptors in adolescent rodents were shown to interact with NMDARs on pyramidal neurons to increase the likelihood that they would be driven into "up-states" thought to be necessary for working memory function (Tseng et al., 2007).

Electrophysiological studies show that in cortical pyramidal neurons, DRD1-like receptor agonism is excitatory (enhancing NMDA effects) and DRD2-like receptor agonism is inhibitory (attenuating AMPA and NMDA response: Wang and O'Donnell, 2001; Tseng and O'Donnell, 2004). In interneurons, DRD1-like receptors are excitatory throughout life (Tseng and O'Donnell, 2007), whereas DRD2-like receptors are weakly inhibitory in juvenile rodents but switch to being strongly excitatory in adult rodents, i.e., strongly increasing inhibitory firing of interneurons onto pyramidal neurons (Tseng and O'Donnell, 2004, 2007). In conditions of high dopamine, this leads to a greater contrast between pyramidal neurons firing due to strong excitatory input and the suppression of background activity of surrounding pyramidal cells (see O'Donnell, 2010 for a review). The cellular mechanisms underlying the switch from inhibitory to excitatory effect of DRD2-like receptors in interneurons are unclear (O'Donnell, 2010), but it yet again implicates peri-adolescent changes in cortical inhibitory circuits as critical foci of research for those seeking to understand the neurodevelopmental neuropathology of schizophrenia.

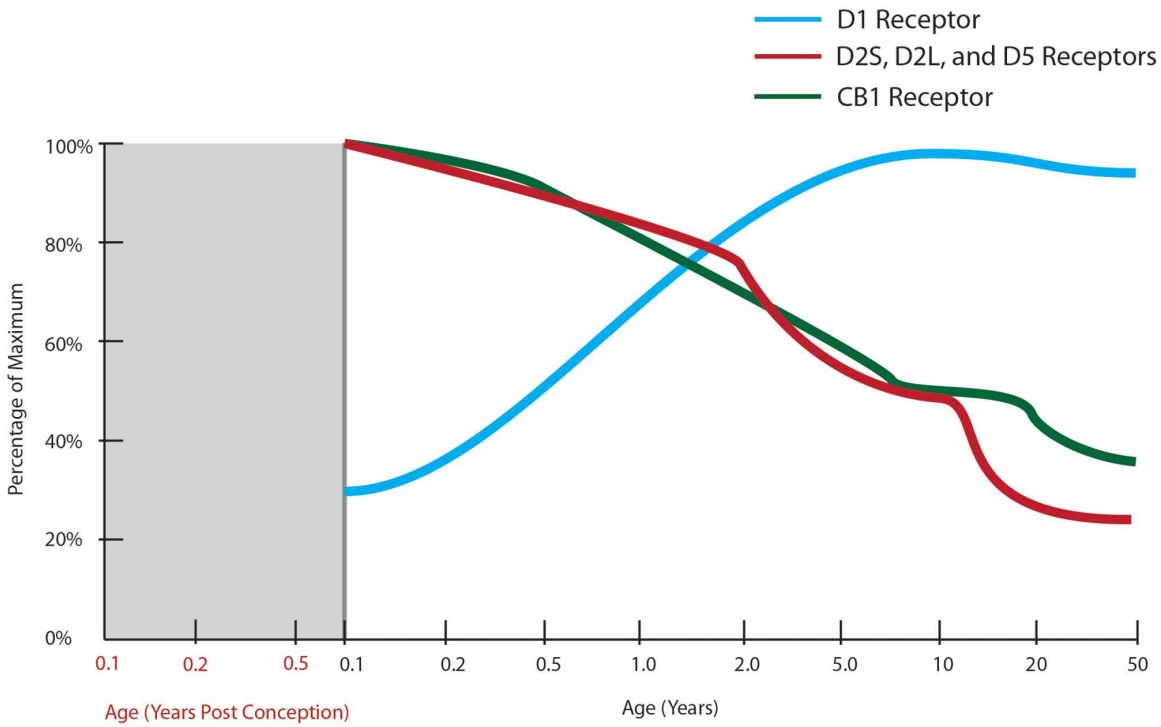

FIGURE 6 | Normal developmental trajectories of dopamine and cannabinoid expressions in the human prefrontal cortex. Expression of dopamine receptor, DRD1 suggests an inverted U-shape with increasing expression of receptors until the mid-twenties after which there is a slow decline (Rothmond et al., 2012). The DRD2 isoforms and DRD5 show an inverse pattern, peaking prenatally with a continuous decline in expression throughout life, excepting a small increase in the early twenties (Rothmond et al., 2012). The expression of DRD4 as determined by *in situ* hybridization is stable across the lifespan (Weickert et al., 2007). Measurement of this receptor by quantitative PCR has been quite variable and no statistical differences in expression across age groups have been detected (Rothmond et al., 2012). Cannabinoid receptor mRNA has its highest measured expression at the time of birth and shows a stepwise decline with age, reaching 30% of maximal expression in middle age (Long et al., 2012).

It is interesting to note that the other dopamine receptor linked to neuronal excitation, DRD5, has a developmental expression pattern opposite to that of DRD1 expression with the highest levels of expression within the first year of life, given that both receptors are nearly indistinguishable pharmacologically. DRD5 is consistently co-localized with DRD1 in the PFC (Bordelon-Glausier et al., 2008) and both are found on pyramidal neurons that are the neural substrates of working memory (Goldman-Rakic et al., 1989). Dopamine has a 10-fold higher affinity to DRD5 compared to DRD1 (Sunahara et al., 1991) suggesting DRD5 even when expressed at lower levels than DRD1 may be functionally important to cognitive processing of working memory (Amico et al., 2007). These results suggest that DRD5 may be the dominant receptor working in opposition to DRD2 early in life in infancy and toddlerhood, and that there may be a developmental switch that occurs such that DRD1 plays a more salient role in dopamine mediated excitation in neural processing during childhood and adolescence.

Schizophrenia studies investigating mRNA expression of all five dopamine receptors have found no change in DRD3, DRD4, and DRD5 in the DLPFC (Meador-Woodruff et al., 1997; Zhan et al., 2011). However, given the timing of late developmental up-regulation and the putative working model of an immature cortex found in schizophrenia, one may predict that DRD1 should be decreased in schizophrenia. While several *in vivo* binding studies support this (Okubo et al., 1997; Kosaka et al., 2010), only one post-mortem study has found a decrease in cortical DRD1 binding (Hess et al., 1987), which appears to be reflected in DRD1 mRNA expression levels (Meador-Woodruff et al., 1997; Zhan et al., 2011). There is, however, more support for the idea that DRD2 can be elevated in schizophrenia DLPFC (Hess et al., 1987; Kestler et al., 2001; Tallerico et al., 2001). Both DRD1 and DRD2 continue to be expressed throughout life and the interplay of the DRD1 and DRD2 receptors are integral to excitation and inhibition of adult cortical neurons (Onn et al., 2005; So et al., 2005; Cropley et al., 2006; Floresco and Magyar, 2006; Winter et al., 2009). Identifying the cellular localization of this up-regulation of DRD2 would be important for determining whether the increased DRD2 is perhaps counteracting DRD1 effects on pyramidal neurons or bolstering the inhibitory drive of interneurons in schizophrenia prefrontal cortex.

PSYCHOPHARMACOLOGICAL RESPONSES TO DRUGS OF ABUSE

When considering schizophrenia from a developmental standpoint, it is of interest to consider that the time of onset of schizophrenia is also when humans are, for the first time, more likely to come in contact with mind-altering drugs. The

adolescent brain seems to have unique responses to psychoactive drugs compared with children and adults. Adolescent mammals are less sensitive to the locomotor enhancing and cortisol-releasing effects of stimulants such as amphetamine and cocaine compared with adults (Laviola et al., 2002; Zombeck et al., 2010). Furthermore, the behavioral sensitization to stimulant drugs observed in adults can be absent in adolescent rodents (Laviola et al., 1995; Adriani et al., 1998). In contrast, effects of dopamine D2 receptor antagonism such as catalepsy, or prolonged fixed body posture, are more prominent in adolescents than adults (Spear et al., 1980; Campbell et al., 1988) perhaps due to the late up-regulation of D1 receptor and the changing D2R/D1R ratio, although these behaviors are thought to be more related to subcortical dopamine action than cortical dopamine action. Also, the ability of dopamine auto-receptors to suppress subcortical dopamine release matures during adolescence (Hedner and Lundborg, 1985), which might alter dopamine release following stimulant administration. Many people with schizophrenia show increased susceptibility to behavioral and dopamine-releasing effects of amphetamine (Lieberman et al., 1987; Laruelle et al., 1996), and hypersensitivity to typical psychostimulant effects such as locomotor hyperactivity is a benchmark of face validity of animal models of schizophrenia (Powell and Miyakawa, 2006).

Non-competitive NMDAR antagonists such as ketamine and phencyclidine produce psychotic and cognitive symptoms in healthy volunteers, exacerbate schizophrenia symptoms in patients and exert greater cognitive impairment in patients with schizophrenia as compared to healthy controls (Yago et al., 1981; Lahti et al., 1995, 2001; Malhotra et al., 1997). Ketamine and phencyclidine do not readily produce hallucinations in children, despite doing so in normal adults (Hirsch et al., 1997) perhaps due to post-natal difference in NMDAR subunit composition in children compared to adults (see earlier). Perinatal NMDAR antagonist administration induces impairments in attention, working memory, executive function, and social cognition in adulthood (Du Bois and Huang, 2007). In addition, these changes may be more pronounced in rodent pups treated perinatally than in animals exposed to NMDAR antagonists at later developmental stages (Mouri et al., 2007). This suggests that age of exposure to drugs may interact with normal developmental changes in NMDARs to bring about differential cortical effects and can result in distinct behavioral effects.

Compared with adults, adolescent rats find repeated administration of the active ingredient in cannabis, delta-9-tetrahydrocannabinol (THC), less aversive but are more likely to show residual deficits in social interaction and working memory after single or chronic THC exposure (Quinn et al., 2008). Similarly, adolescent rats show higher tolerance to the effects of alcohol (Swartzwelder et al., 1998) and are more likely to "binge-drink" than adults (Hargreaves et al., 2009a), but may also be more vulnerable to neurobiological change following alcohol exposure (Hargreaves et al., 2009b). Thus, while some drugs are more rewarding in younger individuals, this can lead to an increased potential for drug dependence (Quinn et al., 2008; Hargreaves et al., 2009a) and the likelihood of other detrimental effects of drugs of abuse. In humans, cannabinoid CB_1 receptor mRNA is highly expressed in the DLPFC from birth until toddlerhood, when it begins to fall rapidly until adolescence and is reduced to a trough by adulthood (Long et al., 2012; **Figure 6**). Synthetic capacity for the major cortical endogenous cannabinoid neurotransmitter peaks at adolescence, but is followed by a reduction in adulthood (Long et al., 2012). This identifies significant turning points in the prominence of normative endogenous cannabinoid signaling and the subsequent developmental regulation of inhibitory neurotransmission, since the majority of CB_1 receptors are localized to GABAergic terminals (Eggan et al., 2010a). Thus, the increased susceptibility to psychotomimetic effects of THC in people with schizophrenia (D'Souza et al., 2005) may be due to aberrant development of the endocannabinoid system during a critical period of change, and indeed numerous reports show that CB_1 receptors are altered in schizophrenia (Zavitsanou et al., 2004; Newell et al., 2006; Eggan et al., 2008, 2010b; Dalton et al., 2011). While both increases and decreases in CB_1 receptors are reported depending on brain region and if receptor binding, mRNA or protein is measured, it is possible that subtypes of schizophrenia may present with different alterations in the endogenous cannabinoid system (Dalton et al., 2011). In general, observations of the changes in the response to psychoactive drugs during development can be used together with developmental processes to understand not only detrimental effects of these drugs, such as psychotic reactions, but also the way in which development may have gone awry in schizophrenia.

CONCLUSION

The schizophrenia prodrome can occur during the adolescent years when the human prefrontal cortex is undergoing molecular and functional change (Cornblatt et al., 2003). Deficits in higher order cognitive abilities remain among the most important causes of persistent functional disability in schizophrenia. Therefore, closer examination of the normal developmental trajectories of these cognitive functions and their underlying neural substrates should be considered in the context of schizophrenia in order to adequately identify the key risk factors and determinants of abnormal cortical development in schizophrenia. To date, the predominant neurodevelopmental model used to underpin the emergence of schizophrenia during adolescence is one that posits synaptic regression as the dominant feature of adolescence, a theory based largely on work done over 25 years ago. We suggest that this view is an overly simplistic and a temporally inaccurate way to frame adolescent development of the human cortex. When considering schizophrenia in the context of neurodevelopment and giving appropriate emphasis on the more recently described post-natal developmental changes, a more modern, dynamic, and complex picture emerges. While many developmental changes are yet to be confirmed, many others have been replicated and these provide ample scope for changes in risk genes and environments to alter post-natal developmental trajectories. We also suggest that while some processes may be attenuated with human post-natal brain growth, many processes are accelerated and others are more accurately viewed as in an active growth phase and more dynamic state of change. Thus, it may be inaccurate to consider the major developmental event of the human cortex to be one of

synaptic regression. Certainly, there is an overwhelming increase in brain size, in interneuron differentiation, in synaptic molecule expression, and in GABA and dopamine receptor mRNA levels that would indicate that gains in synaptic strength predominate in early life and continue during childhood. What appears to be the major developmental switch at adolescence is the slowing of the exuberant growth period typical of infants, toddlers, and children. We suggest that, when theorizing about the neurodevelopmental basis of schizophrenia, schizophrenia could be considered to result from a failure to reach the final state of cortical maturation resulting in retainment of an immature cortex (at least transiently) rather than resulting from excess of adolescent synaptic pruning. Further, it is clear that adolescence is a time of dynamic brain change and that schizophrenia could be viewed as resulting from a destabilization of a normal adolescence process. Since the adolescent brain is in a state of flux it may be possible to stabilize the adolescent brain of those at risk so that any disruption is only transitory and so that chronic schizophrenia does not emerge (McGorry, 2011).

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REFERENCES

Abraham, H., Vincze, A., Jewgenow, I., Veszpremi, B., Kravjak, A., Gomori, E., et al. (2010). Myelination in the human hippocampal formation from midgestation to adulthood. *Int. J. Dev. Neurosci.* 28, 401–410.

Adriani, W., Chiarotti, F., and Laviola, G. (1998). Elevated novelty seeking and peculiar d-amphetamine sensitization in periadolescent mice compared with adult mice. *Behav. Neurosci.* 112, 1152–1166.

Aguayo, L. G., van Zundert, B., Tapia, J. C., Carrasco, M. A., and Alvarez, F. J. (2004). Changes on the properties of glycine receptors during neuronal development. *Brain Res. Brain Res. Rev.* 47, 33–45.

Akbarian, S., Bunney, W. E., Potkin, S. G., Wigal, S. B., Hagman, J. O., Sandman, C. A., et al. (1993). Altered distribution of nicotinamide-adenine dinucleotide phosphate diaphorase cells in frontal lobe of schizophrenics implies disturbances of cortical development. *Arch. Gen. Psychiatry* 50, 169–177.

Akbarian, S., Sucher, N. J., Bradley, D., Tafazzoli, A., Trinh, D., Hetrick, W. P., et al. (1996). Selective alterations in gene expression for NMDA receptor subunits in prefrontal cortex of schizophrenics. *J. Neurosci.* 16, 19–30.

Amico, F., Spowart-Manning, L., Anwyl, R., and Rowan, M. J. (2007). Performance- and task-dependent effects of the dopamine D-1/D-5 receptor agonist SKF 38393 on learning and memory in the rat. *Eur. J. Pharmacol.* 577, 71–77.

Anderson, S., Eisenstat, D., Shi, L., and Rubenstein, J. (1997). Interneuron migration from basal forebrain to neocortex: dependence on Dlx genes. *Science* 278, 474–476.

Anderson, S. A., Volk, D. W., and Lewis, D. A. (1996). Increased density of microtubule associated protein 2-immunoreactive neurons in the prefrontal white matter of schizophrenic subjects. *Schizophr. Res.* 19, 111–119.

Anderson, V. A., Anderson, P., Northam, E., Jacobs, R., and Catroppa, C. (2001). Development of executive functions through late childhood and adolescence in an Australian sample. *Dev. Neuropsychol.* 20, 385–406.

Andreasen, N. C., O'Leary, D. S., Flaum, M., Nopoulos, P., Watkins, G. L., Boles Ponto, L. L., et al. (1997). Hypofrontality in schizophrenia: distributed dysfunctional circuits in neuroleptic-naive patients. *Lancet* 349, 1730–1734.

Arnold, S. E., Hyman, B. T., Van Hoesen, G. W., and Damasio, A. R. (1991). Some cytoarchitectural abnormalities of the entorhinal cortex in schizophrenia. *Arch. Gen. Psychiatry* 48, 625–632.

Arnold, S. E., Ruscheinsky, D. D., and Han, L. Y. (1997). Further evidence of abnormal cytoarchitecture of the entorhinal cortex in schizophrenia using spatial point pattern analyses. *Biol. Psychiatry* 42, 639–647.

Arnsten, A. F. T., Cai, J. X., Murphy, B. L., and Goldmanrakic, P. S. (1994). Dopamine D1 receptor mechanisms in the cognitive performance of young adult and aged monkeys. *Psychopharmacology (Berl.)* 116, 143–151.

Asada, H., Kawamura, Y., Maruyama, K., Kume, H., Ding, R. G., Kanbara, N., et al. (1997). Cleft palate and decreased brain gamma-aminobutyric acid in mice lacking the 67-kDa isoform of glutamic acid decarboxylase. *Proc. Natl. Acad. Sci. U.S.A.* 94, 6496–6499.

Ashrafi, S., Runyan, C. A., Weickert, C. S., and Saunders, R. C. (2007). "Newly generated GABAergic cells in the adult primate prefrontal cortex," in *Society for Neuroscience* (San Diego, CA, USA: Society for Neuroscience).

Ayala, R., Shu, T., and Tsai, L.-H. (2007). Trekking across the brain: the journey of neuronal migration. *Cell* 128, 29–43.

Barbeau, D., Laing, J., Robitalille, Y., Quirion, R., and Srivastava, L. (1995). Decreased expression of the embryonic form of the neural cell adhesion molecule in schizophrenic brains. *Proc. Natl. Acad. Sci. U.S.A.* 92, 2785–2789.

Barch, D. M., Carter, C. S., Braver, T. S., Sabb, F. W., MacDonald, A. 3rd, Noll, D. C., et al. (2001). Selective deficits in prefrontal cortex function in medication-naive patients with schizophrenia. *Arch. Gen. Psychiatry* 58, 280–288.

Barnea-Goraly, N., Menon, V., Eckert, M., Tamm, L., Bammer, R., Karchemskiy, A., et al. (2005). White matter development during childhood and adolescence: a cross-sectional diffusion tensor imaging study. *Cereb. Cortex* 15, 1848–1854.

Bava, S., Thayer, R., Jacobus, J., Ward, M., Jernigan, T. L., and Tapert, S. F. (2010). Longitudinal characterization of white matter maturation during adolescence. *Brain Res.* 1327, 38–46.

Bayatti, N., Moss, J. A., Sun, L., Ambrose, P., Ward, J. F., Lindsay, S., et al. (2008). A molecular neuroanatomical study of the developing human neocortex from 8 to 17 postconceptional weeks revealing the early differentiation of the subplate and subventricular zone. *Cereb. Cortex* 18, 1536–1548.

Beasley, C. L., Dwork, A. J., Rosoklija, G., Mann, J. J., Mancevski, B., Jakovski, Z., et al. (2009). Metabolic abnormalities in fronto-striatal-thalamic white matter tracts in schizophrenia. *Schizophr. Res.* 109, 159–166.

Beasley, C. L., and Reynolds, G. P. (1997). Parvalbumin-immunoreactive neurons are reduced in the prefrontal cortex of schizophrenics. *Schizophr. Res.* 24, 349–355.

Belforte, J. E., Zsiros, V., Sklar, E. R., Jiang, Z. H., Yu, G., Li, Y. Q., et al. (2010). Postnatal NMDA receptor ablation in corticolimbic interneurons confers schizophrenia-like phenotypes. *Nat. Neurosci.* 13, 76–83.

Beltaifa, S., Webster, M. J., Ligons, D. L., Fatula, R. J., Herman, M. M., Kleinman, J. E., et al. (2005). Discordant changes in cortical TrkC mRNA and protein during the human lifespan. *Eur. J. Neurosci.* 21, 2433–2444.

Ben-Ari, Y., Gaiarsa, J.-L., Tyzio, R., and Khazipov, R. (2007). GABA: a pioneer transmitter that excites immature neurons and generates primitive oscillations. *Physiol. Rev.* 87, 1215–1284.

Benes, F. M. (1989). Myelination of cortical-hippocampal relays during late adolescence. *Schizophr. Bull.* 15, 585–593.

Benes, F. M., Vincent, S. L., Alsterberg, G., Bird, E. D., and Sangiovanni, J. P. (1992). Increased GABA-A receptor binding in superficial layers of cingulate cortex in schizophrenics. *J. Neurosci.* 12, 924–929.

Beneyto, M., Abbott, A., Hashimoto, T., and Lewis, D. A. (2011). Lamina-specific alterations in cortical GABA(A) receptor subunit expression in schizophrenia. *Cereb. Cortex* 21, 999–1011.

Beneyto, M., and Meador-Woodruff, J. H. (2008). Lamina-specific abnormalities of NMDA receptor-associated postsynaptic protein transcripts in the prefrontal cortex in schizophrenia and bipolar disorder. *Neuropsychopharmacology* 33, 2175–2186.

Bennett, M. R. (2011). Schizophrenia: susceptibility genes, dendritic-spine pathology and gray matter loss. *Prog. Neurobiol.* 95, 275–300.

Bernier, P. J., Bedard, A., Vinet, J., Levesque, M., and Parent, A. (2002). Newly generated neurons in the amygdala and adjoining cortex of adult primates. *Proc. Natl. Acad. Sci. U.S.A.* 99, 11464–11469.

Bernstein, H. G., Krell, D., Baumann, B., Danos, P., Falkai, P., Diekmann, S., et al. (1998). Morphometric studies of the entorhinal cortex in neuropsychiatric patients and controls: clusters of heterotopically displaced lamina II neurons are not indicative of schizophrenia. *Schizophr. Res.* 33, 125–132.

Bhardwaj, R. D., Curtis, M. A., Spalding, K. L., Buchholz, B. A., Fink, D., Bjork-Eriksson, T., et al. (2006). Neocortical neurogenesis in humans is restricted to development. *Proc. Natl. Acad. Sci. U.S.A.* 103, 12564–12568.

Bhojraj, T. S., Diwadkar, V. A., Sweeney, J. A., Prasad, K. M., Eack, S. M., Montrose, D. M., et al. (2010). Longitudinal alterations of executive function in non-psychotic adolescents at familial risk for schizophrenia. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 34, 469–474.

Bitanihirwe, B. K. Y., Lim, M. P., Kelley, J. F., Kaneko, T., and Woo, T. U. W. (2009). Glutamatergic deficits and parvalbumin-containing inhibitory neurons in the prefrontal cortex in schizophrenia. *BMC Psychiatry* 9:71. doi: 10.1186/1471-244X-9-71

Blakemore, S. J., and Choudhury, S. (2006). Development of the adolescent brain: implications for executive function and social cognition. *J. Child Psychol. Psychiatry* 47, 296–312.

Bordelon-Glausier, J. R., Khan, Z. U., and Muly, E. C. (2008). Quantification of D-1 and D-5 dopamine receptor localization in layers I, III, and V of *Macaca mulatta* prefrontal cortical area 9: coexpression in dendritic spines and axon terminals. *J. Comp. Neurol.* 508, 893–905.

Borgwardt, S. J., Dickey, C., Pol, H. H., Whitford, T. J., and Delisi, L. E. (2009). Workshop on defining the significance of progressive brain change in schizophrenia: December 12, 2008 American College of Neuropsychopharmacology (ACNP) all-day satellite, Scottsdale, Arizona The rapporteurs' report. *Schizophr. Res.* 112, 32–45.

Brocki, K. C., and Bohlin, G. (2004). Executive functions in children aged 6 to 13: a dimensional and developmental study. *Dev. Neuropsychol.* 26, 571–593.

Brody, B. A., Kinney, H. C., Kloman, A. S., and Gilles, F. H. (1987). Sequence of central nervous system myelination in human infancy. I. An autopsy study of myelination. *J. Neuropathol. Exp. Neurol.* 46, 283–301.

Bunge, S. A., Dudukovic, N. M., Thomason, M. E., Vaidya, C. J., and Gabrieli, J. D. E. (2002). Immature frontal lobe contributions to cognitive control in children: evidence from fMRI. *Neuron* 33, 301–311.

Bunge, S. A., Helskog, E. H., and Wendelken, C. (2009). Left, but not right, rostrolateral prefrontal cortex meets a stringent test of the relational integration hypothesis. *Neuroimage* 46, 338–342.

Bunge, S. A., Wendelken, C., Badre, D., and Wagner, A. D. (2005). Analogical reasoning and prefrontal cortex: evidence for separable retrieval and integration mechanisms. *Cereb. Cortex* 15, 239–249.

Callicott, J. H., Mattay, V. S., Verchinski, B. A., Marenco, S., Egan, M. F., and Weinberger, D. R. (2003). Complexity of prefrontal cortical dysfunction in schizophrenia: more than up or down. *Am. J. Psychiatry* 160, 2209–2215.

Campbell, A., Baldessarini, R. J., and Teicher, M. H. (1988). Decreasing sensitivity to neuroleptic agents in developing rats; evidence for a pharmacodynamic factor. *Psychopharmacology (Berl.)* 94, 46–51.

Cannon, M., Caspi, A., Moffitt, T. E., Harrington, H., Taylor, A., Murray, R. M., et al. (2002a). Evidence for early-childhood, pan-developmental impairment specific to schizophreniform disorder – results from a longitudinal birth cohort. *Arch. Gen. Psychiatry* 59, 449–456.

Cannon, T. D., Thompson, P. M., van Erp, T. G. M., Toga, A. W., Poutanen, V. P., Huttunen, M., et al. (2002b). Cortex mapping reveals regionally specific patterns of genetic and disease-specific gray-matter deficits in twins discordant for schizophrenia. *Proc. Natl. Acad. Sci. U.S.A.* 99, 3228–3233.

Casey, B. J., Giedd, J. N., and Thomas, K. M. (2000). Structural and functional brain development and its relation to cognitive development. *Biol. Psychol.* 54, 241–257.

Casey, B. J., Trainor, R. J., Orendi, J. L., Schubert, A. B., Nystrom, L. E., Giedd, J. N., et al. (1997). A developmental functional MRI study of prefrontal activation during performance of a Go-No-Go task. *J. Cogn. Neurosci.* 9, 835–847.

Castillo, M. A., Ghose, S., Tamminga, C. A., and Ulery-Reynolds, P. G. (2010). Deficits in syntaxin 1 phosphorylation in schizophrenia prefrontal cortex. *Biol. Psychiatry* 67, 208–216.

Cattell, R. B. (1987). *Intelligence: Its Structure, Growth, and Action*. Amsterdam: North Holland: Elsevier.

Catts, V. S., and Catts, S. V. (2010). "The psychotomimetic effects of PCP, LSD and MDMA: Pharmacological models of schizophrenia?," in *Secondary Schizophrenia,* eds. P. S. Sachdev and M. S. Keshavan (Cambridge: Cambridge Press), 141–168.

Catts, V. S., and Weickert, C. S. (2012). Gene expression analysis implicates a death receptor pathway in schizophrenia pathology. *PLoS ONE* 7:e35511. doi: 10.1371/journal.pone.0035511

Caviness, V. S. Jr. (1976). Patterns of cell and fiber distribution in the neocortex of the reeler mutant mouse. *J. Comp. Neurol.* 170, 435–447.

Chai, X., Forster, E., Zhao, S., Bock, H. H., and Frotscher, M. (2009). Reelin acts as a stop signal for radially migrating neurons by inducing phosphorylation of n-cofilin at the leading edge. *Commun. Integr. Biol.* 2, 375–377.

Choi, K. H., Zepp, M. E., Higgs, B. W., Weickert, C. S., and Webster, M. J. (2009). Expression profiles of schizophrenia susceptibility genes during human prefrontal cortical development. *J. Psychiatry Neurosci.* 34, 450–458.

Chong, V. Z., Webster, M. J., Rothmond, D., and Weickert, C. S. (2008). Specific developmental reductions in subventricular zone ErbB1 and ErbB4 mRNA in the human brain. *Int. J. Dev. Neurosci.* 26, 791–803.

Christoff, K., Prabhakaran, V., Dorfman, J., Zhao, Z., Kroger, J. K., Holyoak, K. J., et al. (2001). Rostrolateral prefrontal cortex involvement in relational integration during reasoning. *Neuroimage* 14, 1136–1149.

Chun, J. J. M., and Shatz, C. J. (1989). Interstitial cells of the adult neocortical white matter are the remnant of the early generated subplate neuron population. *J. Comp. Neurol.* 282, 555–569.

Colantuoni, C., Lipska, B. K., Ye, T., Hyde, T. M., Tao, R., Leek, J. T., et al. (2011). Temporal dynamics and genetic control of transcription in the human prefrontal cortex. *Nature* 478, 519–523.

Corbin, J., and Butt, S. (2011). Developmental mechanisms for the generation of telencephalic interneurons. *Dev. Neurobiol.* 71, 710–732.

Corbin, J. G., Nery, S., and Fishell, G. (2001). Telencephalic cells take a tangent: non-radial migration in the mammalian forebrain. *Nat. Neurosci.* 4, 1177–1182.

Cornblatt, B. A., Lencz, T., Smith, C. W., Correll, C. U., Auther, A. M., and Nakayama, E. (2003). The schizophrenia prodrome revisited: a neurodevelopmental perspective. *Schizophr. Bull.* 29, 633–651.

Costa, E., Chen, Y., Davis, J., Dong, E., Noh, J. S., Tremolizzo, L., et al. (2002). REELIN and schizophrenia: a disease at the interface of the genome and the epigenome. *Mol. Interv.* 2, 47–57.

Cox, E. T., Brennaman, L. H., Gable, K. L., Hamer, R. M., Glantz, L. A., Lamantia, A. S., et al. (2009). Developmental regulation of neural cell adhesion molecule in human prefrontal cortex. *Neuroscience* 162, 96–105.

Crair, M. C., and Malenka, R. C. (1995). A critical period for long-term potentiation at thalamocortical synapses. *Nature* 375, 325–328.

Crone, E. A., Wendelken, C., Donohue, S., van Leijenhorst, L., and Bunge, S. A. (2006). Neurocognitive development of the ability to manipulate information in working memory. *Proc. Natl. Acad. Sci. U.S.A.* 103, 9315–9320.

Crone, E. A., Wendelken, C., van Leijenhorst, L., Honomichl, R. D., Christoff, K., and Bunge, S. A. (2009). Neurocognitive development of relational reasoning. *Dev. Sci.* 12, 55–66.

Cropley, V. L., Fujita, M., Innis, R. B., and Nathan, P. J. (2006). Molecular imaging of the dopaminergic system and its association with human cognitive function. *Biol. Psychiatry* 59, 898–907.

Cruz, D. A., Eggan, S. M., and Lewis, D. A. (2003). Postnatal development of pre- and postsynaptic GABA markers at chandelier cell connections with pyramidal neurons in monkey prefrontal cortex. *J. Comp. Neurol.* 465, 385–400.

Cull-Candy, S., Brickley, S., and Farrant, M. (2001). NMDA receptor subunits: diversity, development and disease. *Curr. Opin. Neurobiol.* 11, 327–335.

Curtis, M. A., Penney, E. B., Pearson, A. G., van Roon-Mom, W. M., Butterworth, N. J., Dragunow, M., et al. (2003). Increased cell proliferation and neurogenesis in the adult human Huntington's disease brain. *Proc. Natl. Acad. Sci. U.S.A.* 100, 9023–9027.

D'Arcangelo, G., Miao, G. G., Chen, S. C., Soares, H. D., Morgan, J. I., and Curran, T. (1995). A protein related to extracellular matrix proteins deleted in the mouse mutant reeler. *Nature* 374, 719–723.

D'Souza, D. C., Abi-Saab, W. M., Madonick, S., Forselius-Bielen, K., Doersch, A., Braley, G., et al. (2005). Delta-9-tetrahydrocannabinol effects in schizophrenia: implications for cognition, psychosis, and addiction. *Biol. Psychiatry* 57, 594–608.

Dalton, V. S., Long, L. E., Weickert, C. S., and Zavitsanou, K. (2011). Paranoid schizophrenia is characterized by increased CB1 receptor binding in the dorsolateral prefrontal cortex. *Neuropsychopharmacology* 36, 1620–1630.

Das, S., Sasaki, Y. F., Rothe, T., Premkumar, L. S., Takasu, M., Crandall, J. E., et al. (1998). Increased NMDA current and spine density in mice lacking the NMDA receptor subunit NR3A. *Nature* 393, 377–381.

David, A. S., Malmberg, A., Brandt, L., Allebeck, P., and Lewis, G. (1997). IQ and risk for schizophrenia: a population-based cohort study. *Psychol. Med.* 27, 1311–1323.

Dean, B., Hussain, T., Hayes, W., Scarr, E., Kitsoulis, S., Hill, C., et al. (1999). Changes in serotonin(2A) and GABA(A) receptors in schizophrenia: studies on the human dorsolateral prefrontal cortex. *J. Neurochem.* 72, 1593–1599.

Dean, B., Pavey, G., McLeod, M., Opeskin, K., Keks, N., and Copolov, D. (2001). A change in the density of [(3)H]flumazenil, but not [(3)H]muscimol binding, in Brodmann's Area 9 from subjects with bipolar disorder. *J. Affect. Disord.* 66, 147–158.

Dekaban, A. S., and Sadowsky, D. (1978). Changes in brain weights during span of human life – relation of brain weights to body heights and body weights. *Ann. Neurol.* 4, 345–356.

Del Rio, J. A., Heimrich, B., Borrell, V., Forster, E., Drakew, A., Alcantara, S., et al. (1997). A role for Cajal-Retzius cells and reelin in the development of hippocampal connections. *Nature* 385, 70–74.

Delalle, I., Evers, P., Kostovic, I., and Uylings, H. B. (1997). Laminar distribution of neuropeptide Y-immunoreactive neurons in human prefrontal cortex during development. *J. Comp. Neurol.* 379, 515–522.

Di Cristo, G. (2007). Development of cortical GABAergic circuits and its implications for neurodevelopmental disorders. *Clin. Genet.* 72, 1–8.

Dickson, H., Laurens, K. R., Cullen, A. E., and Hodgins, S. (2012). Meta-analyses of cognitive and motor function in youth aged 16 years and younger who subsequently develop schizophrenia. *Psychol. Med.* 42, 743–755.

Dracheva, S., Marras, S. A., Kramer, F. R., Davis, K. L., and Haroutunian, V. (2001). N-methyl-D-aspartic acid receptor expression in the dorsolateral prefrontal cortex of elderly patients with schizophrenia. *Am. J. Psychiatry* 158, 2107.

Du Bois, T. M., and Huang, X. F. (2007). Early brain development disruption from NMDA receptor hypofunction: relevance to schizophrenia. *Brain Res. Rev.* 53, 260–270.

Duan, X., Chang, J. H., Ge, S., Faulkner, R. L., Kim, J. Y., Kitabatake, Y., et al. (2007). Disrupted-In-Schizophrenia 1 regulates integration of newly generated neurons in the adult brain. *Cell* 130, 1146–1158.

Dubois, J., Hertz-Pannier, L., Dehaene-Lambertz, G., Cointepas, Y., and Le Bihan, D. (2006). Assessment of the early organization and maturation of infants' cerebral white matter fiber bundles: a feasibility study using quantitative diffusion tensor imaging and tractography. *Neuroimage* 30, 1121–1132.

Duncan, C., Webster, M., Rothmond, D. A., Bahn, S., Elashoff, M., and Shannon Weickert, C. (2010). Prefrontal GABA-A receptor α subunit expression in normal postnatal human development and schizophrenia. *J. Psychiatr. Res.* 44, 673–681.

Durston, S., Davidson, M. C., Tottenham, N., Galvan, A., Spicer, J., Fossella, J. A., et al. (2006). A shift from diffuse to focal cortical activity with development. *Dev. Sci.* 9, 1–8.

Durston, S., Pol, H. E. H., Casey, B. J., Giedd, J. N., Buitelaar, J. K., and van Engeland, H. (2001). Anatomical MRI of the developing human brain: what have we learned? *J. Am. Acad. Child Adolesc. Psychiatry* 40, 1012–1020.

Eastwood, S. L., and Harrison, P. J. (2001). Synaptic pathology in the anterior cingulate cortex in schizophrenia and mood disorders. A review and a Western blot study of synaptophysin, GAP-43 and the complexins. *Brain Res. Bull.* 55, 569–578.

Eastwood, S. L., and Harrison, P. J. (2003). Interstitial white matter neurons express less reelin and are abnormally distributed in schizophrenia: towards an integration of molecular and morphologic aspects of the neurodevelopmental hypothesis. *Mol. Psychiatry* 8, 821–831.

Eastwood, S. L., and Harrison, P. J. (2005a). Interstitial white matter neuron density in the dorsolateral prefrontal cortex and parahippocampal gyrus in schizophrenia. *Schizophr. Res.* 79, 181–188.

Eastwood, S. L., and Harrison, P. J. (2005b). Decreased expression of vesicular glutamate transporter 1 and complexin II mRNAs in schizophrenia: further evidence for a synaptic pathology affecting glutamate neurons. *Schizophr. Res.* 73, 159–172.

Edin, F., MacOveanu, J., Olesen, P., Tegner, J., and Klingberg, T. (2007). Stronger synaptic connectivity as a mechanism behind development of working memory-related brain activity during childhood. *J. Cogn. Neurosci.* 19, 750–760.

Eggan, S. M., Hashimoto, T., and Lewis, D. A. (2008). Reduced cortical cannabinoid 1 receptor messenger RNA and protein expression in schizophrenia. *Arch. Gen. Psychiatry* 65, 772–784.

Eggan, S. M., Melchitzky, D. S., Sesack, S. R., Fish, K. N., and Lewis, D. A. (2010a). Relationship of cannabinoid CB1 receptor and cholecystokinin immunoreactivity in monkey dorsolateral prefrontal cortex. *Neuroscience* 169, 1651–1661.

Eggan, S. M., Stoyak, S. R., Verrico, C. D., and Lewis, D. A. (2010b). Cannabinoid CB1 receptor immunoreactivity in the prefrontal cortex: comparison of schizophrenia and major depressive disorder. *Neuropsychopharmacology* 35, 2060–2071.

Elston, G. N. (2000). Pyramidal cells of the frontal lobe: all the more spinous to think with. *J. Neurosci.* 20, RC95.

Eluvathingal, T. J., Hasan, K. M., Kramer, L., Fletcher, J. M., and Ewing-Cobbs, L. (2007). Quantitative diffusion tensor tractography of association and projection fibers in normally developing children and adolescents. *Cereb. Cortex* 17, 2760–2768.

Erickson, S. L., and Lewis, D. A. (2002). Postnatal development of parvalbumin- and GABA transporter-immunoreactive axon terminals in monkey prefrontal cortex. *J. Comp. Neurol.* 448, 186–202.

Eriksson, P. S., Perfilieva, E., Bjork-Eriksson, T., Alborn, A. M., Nordborg, C., Peterson, D. A., et al. (1998). Neurogenesis in the adult human hippocampus. *Nat. Med.* 4, 1313–1317.

Fair, D. A., Dosenbach, N. U. F., Church, J. A., Cohen, A. L., Brahmbhatt, S., Miezin, F. M., et al. (2007). Development of distinct control networks through segregation and integration. *Proc. Natl. Acad. Sci. U.S.A.* 104, 13507–13512.

Falkai, P., Schneider-Axmann, T., and Honer, W. G. (2000). Entorhinal cortex pre-alpha cell clusters in schizophrenia: quantitative evidence of a developmental abnormality. *Biol. Psychiatry* 47, 937–943.

Faludi, G., and Mirnics, K. (2011). Synaptic changes in the brain of subjects with schizophrenia. *Int. J. Dev. Neurosci.* 29, 305–309.

Feinberg, I. (1983). Schizophrenia – caused by a fault in programmed synaptic elimination during adolescence. *J. Psychiatr. Res.* 17, 319–334.

Feldmeyer, D., and Radnikow, G. (2009). Developmental alterations in the functional properties of excitatory neocortical synapses. *J. Physiol.* 587, 1889–1896.

Fertuzinhos, S., Krsnik, Z., Kawasawa, Y., Rasin, M., Kwan, K., Chen, J., et al. (2009). Selective depletion of molecularly defined cortical interneurons in human holoprosencephaly with severe striatal hypoplasia. *Cereb. Cortex* 19, 2196–2207.

Fillman, S. G., Cloonan, N., Catts, V. S., Miller, L. C., Wong, J., McCrossin, T., et al. (2013). Increased inflammatory markers identified in the dorsolateral prefrontal cortex of individuals with schizophrenia. *Mol. Psychiatry* 18, 206–214.

Fillman, S. G., Duncan, C. E., Webster, M. J., Elashoff, M., and Weickert,

C. S. (2010). Developmental co-regulation of the beta and gamma GABAA receptor subunits with distinct alpha subunits in the human dorsolateral prefrontal cortex. *Int. J. Dev. Neurosci.* 28, 513–519.

Flint, A. C., Liu, X., and Kriegstein, A. R. (1998). Nonsynaptic glycine receptor activation during early neocortical development. *Neuron* 20, 43–53.

Flint, A. C., Maisch, U. S., Weishaupt, J. H., Kriegstein, A. R., and Monyer, H. (1997). NR2A subunit expression shortens NMDA receptor synaptic currents in developing neocortex. *J. Neurosci.* 17, 2469–2476.

Floresco, S. B., and Magyar, O. (2006). Mesocortical dopamine modulation of executive functions: beyond working memory. *Psychopharmacology (Berl.)* 188, 567–585.

Fung, S., Joshi, D., Allen, K., Sivagnanasundaram, S., Rothmond, D., Saunders, R., et al. (2011a). Developmental patterns of doublecortin expression and white matter neuron density in the postnatal primate prefrontal cortex and schizophrenia. *PLoS ONE* 6:e25194. doi: 10.1371/journal.pone.0025194

Fung, S. J., Sivagnanasundaram, S., and Weickert, C. S. (2011b). Lack of change in markers of presynaptic terminal abundance alongside subtle reductions in markers of presynaptic terminal plasticity in prefrontal cortex of schizophrenia patients. *Biol. Psychiatry* 69, 71–79.

Fung, S. J., Webster, M. J., Sivagnanasundaram, S., Duncan, C., Elashoff, M., and Weickert, C. S. (2010). Expression of interneuron markers in the dorsolateral prefrontal cortex of the developing human and in schizophrenia. *Am. J. Psychiatry* 167, 1479–1488.

Gambrill, A. C., and Barria, A. (2011). NMDA receptor subunit composition controls synaptogenesis and synapse stabilization. *Proc. Natl. Acad. Sci. U.S.A.* 108, 5855–5860.

Gao, W., Lin, W., Chen, Y., Gerig, G., Smith, J. K., Jewells, V., et al. (2009). Temporal and spatial development of axonal maturation and myelination of white matter in the developing brain. *Am. J. Neuroradiol.* 30, 290–296.

Ghashghaei, H. T., Weber, J., Pevny, L., Schmid, R., Schwab, M. H., Lloyd, K. C., et al. (2006). The role of neuregulin-ErbB4 interactions on the proliferation and organization of cells in the subventricular zone. *Proc. Natl. Acad. Sci. U.S.A.* 103, 1930–1935.

Giedd, J. N., Blumenthal, J., Jeffries, N. O., Castellanos, F. X., Liu, H., Zijdenbos, A., et al. (1999). Brain development during childhood and adolescence: a longitudinal MRI study. *Nat. Neurosci.* 2, 861–863.

Giorgio, A., Watkins, K. E., Chadwick, M., James, S., Winmill, L., Douaud, G., et al. (2010). Longitudinal changes in grey and white matter during adolescence. *Neuroimage* 49, 94–103.

Giorgio, A., Watkins, K. E., Douaud, G., James, A. C., James, S., De Stefano, N., et al. (2008). Changes in white matter microstructure during adolescence. *Neuroimage* 39, 52–61.

Glantz, L. A., Gilmore, J. H., Hamer, R. M., Lieberman, J. A., and Jarskog, L. F. (2007). Synaptophysin and postsynaptic density protein 95 in the human prefrontal cortex from mid-gestation into early adulthood. *Neuroscience* 149, 582–591.

Glantz, L. A., and Lewis, D. A. (2000). Decreased dendritic spine density on prefrontal cortical pyramidal neurons in schizophrenia. *Arch. Gen. Psychiatry* 57, 65–73.

Glickstein, S. B., Desteno, D. A., Hof, P. R., and Schmauss, C. (2005). Mice lacking dopamine D2 and D3 receptors exhibit differential activation of prefrontal cortical neurons during tasks requiring attention. *Cereb. Cortex* 15, 1016–1024.

Goffinet, A. M. (1984). Events governing organization of postmigratory neurons: studies on brain development in normal and reeler mice. *Brain Res.* 319, 261–296.

Gogtay, N., Giedd, J. N., Lusk, L., Hayashi, K. M., Greenstein, D., Vaituzis, A. C., et al. (2004). Dynamic mapping of human cortical development during childhood through early adulthood. *Proc. Natl. Acad. Sci. U.S.A.* 101, 8174–8179.

Goldberg, T. E., Goldman, R. S., Burdick, K. E., Malhotra, A. K., Lencz, T., Patel, R. C., et al. (2007). Cognitive improvement after treatment with second-generation antipsychotic medications in first-episode schizophrenia: is it a practice effect? *Arch. Gen. Psychiatry* 64, 1115–1122.

Goldman-Rakic, P. S. (1994). Working memory dysfunction in schizophrenia. *J. Neuropsychiatry Clin. Neurosci.* 6, 348–357.

Goldman-Rakic, P. S. (1996). Regional and cellular fractionation of working memory. *Proc. Natl. Acad. Sci. U.S.A.* 93, 13473–13480.

Goldman-Rakic, P. S., Leranth, C., Williams, S. M., Mons, N., and Geffard, M. (1989). Dopamine synaptic complex with pyramidal neurons in primate cerebral cortex. *Proc. Natl. Acad. Sci. U.S.A.* 86, 9015–9019.

Gonzalez-Burgos, G., Hashimoto, T., and Lewis, D. A. (2010). Alterations of cortical GABA neurons and network oscillations in schizophrenia. *Curr. Psychiatry Rep.* 12, 335–344.

Goswami, U. (1989). Relational complexity and the development of analogical reasoning. *Cogn. Dev.* 4, 251–268.

Gould, E. (2007). How widespread is adult neurogenesis in mammals? *Nat. Rev. Neurosci.* 8, 481–488.

Gould, E., Reeves, A. J., Graziano, M. S., and Gross, C. G. (1999). Neurogenesis in the neocortex of adult primates. *Science* 286, 548–552.

Gould, E., Vail, N., Wagers, M., and Gross, C. G. (2001). Adult-generated hippocampal and neocortical neurons in macaques have a transient existence. *Proc. Natl. Acad. Sci. U.S.A.* 98, 10910–10917.

Grateron, L., Cebada-Sanchez, S., Marcos, P., Mohedano-Moriano, A., Insausti, A. M., Munoz, M., et al. (2003). Postnatal development of calcium-binding proteins immunoreactivity (parvalbumin, calbindin, calretinin) in the human entorhinal cortex. *J. Chem. Neuroanat.* 26, 311–316.

Gray, L. J., Dean, B., Kronsbein, H. C., Robinson, P. J., and Scarr, E. (2010). Region and diagnosis-specific changes in synaptic proteins in schizophrenia and bipolar I disorder. *Psychiatry Res.* 178, 374–380.

Grayson, D. R., Jia, X., Chen, Y., Sharma, R. P., Mitchell, C. P., Guidotti, A., et al. (2005). Reelin promoter hypermethylation in schizophrenia. *Proc. Natl. Acad. Sci. U.S.A.* 102, 9341–9346.

Green, M. F. (1996). What are the functional consequences of neurocognitive deficits in schizophrenia? *Am. J. Psychiatry* 153, 321–330.

Hakak, Y., Walker, J. R., Li, C., Wong, W. H., Davis, K. L., Buxbaum, J. D., et al. (2001). Genome-wide expression analysis reveals dysregulation of myelination-related genes in chronic schizophrenia. *Proc. Natl. Acad. Sci. U.S.A.* 98, 4746–4751.

Halim, N. D., Weickert, C. S., McClintock, B. W., Hyde, T. M., Weinberger, D. R., Kleinman, J. E., et al. (2003). Presynaptic proteins in the prefrontal cortex of patients with schizophrenia and rats with abnormal prefrontal development. *Mol. Psychiatry* 8, 797–810.

Hargreaves, G. A., Monds, L., Gunasekaran, N., Dawson, B., and McGregor, I. S. (2009a). Intermittent access to beer promotes binge-like drinking in adolescent but not adult Wistar rats. *Alcohol* 43, 305–314.

Hargreaves, G. A., Quinn, H., Kashem, M. A., Matsumoto, I., and McGregor, I. S. (2009b). Proteomic analysis demonstrates adolescent vulnerability to lasting hippocampal changes following chronic alcohol consumption. *Alcohol. Clin. Exp. Res.* 33, 86–94.

Harney, S. C., Jane, D. E., and Anwyl, R. (2008). Extrasynaptic NR2D-containing NMDARs are recruited to the synapse during LTP of NMDAR-EPSCs. *J. Neurosci.* 28, 11685–11694.

Harris, L. W., Lockstone, H. E., Khaitovich, P., Weickert, C. S., Webster, M. J., and Bahn, S. (2009). Gene expression in the prefrontal cortex during adolescence: implications for the onset of schizophrenia. *BMC Med. Genomics* 2:28. doi: 10.1186/1755-8794-2-28

Hashimoto, T., Arion, D., Unger, T., Maldonado-Aviles, J. G., Morris, H. M., Volk, D. W., et al. (2008a). Alterations in GABA-related transcriptome in the dorsolateral prefrontal cortex of subjects with schizophrenia. *Mol. Psychiatry* 13, 147–161.

Hashimoto, T., Bazmi, H. H., Mirnics, K., Wu, Q., Sampson, A. R., and Lewis, D. A. (2008b). Conserved regional patterns of GABA-related transcript expression in the neocortex of subjects with schizophrenia. *Am. J. Psychiatry* 165, 479–489.

Hashimoto, T., Nguyen, Q. L., Rotaru, D., Keenan, T., Arion, D., Beneyto, M., et al. (2009). Protracted developmental trajectories of GABA(A) receptor alpha 1 and alpha 2 subunit expression in primate prefrontal cortex. *Biol. Psychiatry* 65, 1015–1023.

Hatten, M. E. (2002). New directions in neuronal migration. *Science* 297, 1660–1663.

Haubensak, W., Attardo, A., Denk, W., and Huttner, W. B. (2004). Neurons arise in the basal neuroepithelium of the early mammalian telencephalon: a major site of neurogenesis. *Proc. Natl. Acad. Sci. U.S.A.* 101, 3196–3201.

Hayashi, M., Yamashita, A., Shimizu, K., Sogawa, K., and Fujii, Y. (1990). Somatostatin gene expression in the developing monkey frontal and cerebellar cortices. *Brain Res. Dev. Brain Res.* 57, 37–41.

Haynes, R. L., Borenstein, N. S., Desilva, T. M., Folkerth, R. D., Liu, L. G., Volpe, J. J., et al. (2005). Axonal development in the cerebral white matter of the human fetus and infant. *J. Comp. Neurol.* 484, 156–167.

Heaton, R. K., Chelune, G. J., Talley, T. L., Kay, G. G., and Curtiss, G. (1993). *Wisconsin Card Sorting Test Manual*. Odessa, FL: Psychological Assessment Resources.

Hedner, T., and Lundborg, P. (1985). Development of dopamine autoreceptors in the postnatal rat brain. *J. Neural Transm.* 62, 53–63.

Heinrichs, R. W., and Zakzanis, K. K. (1998). Neurocognitive deficit in schizophrenia: a quantitative review of the evidence. *Neuropsychology* 12, 426–445.

Henson, M. A., Roberts, A. C., Salimi, K., Vadlamudi, S., Hamer, R. M., Gilmore, J. H., et al. (2008). Developmental regulation of the NMDA receptor subunits, NR3A and NR1, in human prefrontal cortex. *Cereb. Cortex* 18, 2560–2573.

Hermoye, L., Saint-Martin, C., Cosnard, G., Lee, S.-K., Kim, J., Nassogne, M.-C., et al. (2006). Pediatric diffusion tensor imaging: normal database and observation of the white matter maturation in early childhood. *Neuroimage* 29, 493–504.

Hess, E. J., Bracha, H. S., Kleinman, J. E., and Creese, I. (1987). Dopamine receptor subtype imbalance in schizophrenia. *Life Sci.* 40, 1487–1497.

Hevers, W., and Lüddens, H. (1998). The diversity of GABAA receptors. Pharmacological and electrophysiological properties of GABAA channel subtypes. *Mol. Neurobiol.* 18, 35–86.

Hirsch, S. R., Das, I., Garey, L. J., and De Belleroche, J. (1997). A pivotal role for glutamate in the pathogenesis of schizophrenia, and its cognitive dysfunction. *Pharmacol. Biochem. Behav.* 56, 797–802.

Hollmann, M., and Heinemann, S. (1994). Cloned glutamate receptors. *Annu. Rev. Neurosci.* 17, 31–108.

Honer, W. G., Falkal, P., Chen, C., Arango, V., Mann, J. J., and Dwork, A. J. (1999). Synaptic and plasticity-associated proteins in anterior frontal cortex in severe mental illness. *Neuroscience* 91, 1247–1255.

Hudspeth, W. J., and Pribram, K. H. (1990). Stages of brain and cognitive maturation. *J. Educ. Psychol.* 82, 881–884.

Hudspeth, W. J., and Pribram, K. H. (1992). Psychophysiological indexes of cerebral maturation. *Int. J. Psychophysiol.* 12, 19–29.

Huizinga, M., Dolan, C. V., and van der Molen, M. W. (2006). Age-related change in executive function: developmental trends and a latent variable analysis. *Neuropsychologia* 44, 2017–2036.

Humphries, C., Mortimer, A., Hirsch, S., and Debelleroche, J. (1996). NMDA receptor mRNA correlation with antemortem cognitive impairment in schizophrenia. *Neuroreport* 7, 2051–2055.

Huttenlocher, P. (1979). Synaptic density in human frontal cortex – developmental changes and effects of aging. *Brain Res.* 163, 195–205.

Huttenlocher, P. R. (1990). Morphometric study of human cerebral cortex development. *Neuropsychologia* 28, 517–527.

Huttenlocher, P. R., and Dabholkar, A. S. (1997). Regional differences in synaptogenesis in human cerebral cortex. *J. Comp. Neurol.* 387, 167–178.

Hyde, T. M., Lipska, B. K., Ali, T., Mathew, S. V., Law, A. J., Metitiri, O. E., et al. (2011). Expression of GABA signaling molecules KCC2, NKCC1, and GAD1 in cortical development and schizophrenia. *J. Neurosci.* 31, 11088–11095.

Impagnatiello, F., Guidotti, A. R., Pesold, C., Dwivedi, Y., Caruncho, H., Pisu, M. G., et al. (1998). A decrease of reelin expression as a putative vulnerability factor in schizophrenia. *Proc. Natl. Acad. Sci. U.S.A.* 95, 15718–15723.

Insel, T. R. (2010). Rethinking schizophrenia. *Nature* 468, 187–193.

Ishimaru, M., Kurumaji, A., and Toru, M. (1992). NMDA-associated glycine binding site increases in schizophrenic brains. *Biol. Psychiatry* 32, 379–381.

Iwasaki, N., Hamano, K., Okada, Y., Horigome, Y., Nakayama, J., Takeya, T., et al. (1997). Volumetric quantification of brain development using MRI. *Neuroradiology* 39, 841–846.

Jacobs, B., Driscoll, L., and Schall, M. (1997). Life-span dendritic and spine changes in areas 10 and 18 of human cortex: a quantitative Golgi study. *J. Comp. Neurol.* 386, 661–680.

Jakob, H., and Beckmann, H. (1986). Prenatal developmental disturbances in the limbic allocortex in schizophrenics. *J. Neural Transm.* 65, 303–326.

Jones, L. B., Johnson, N., and Byne, W. (2002). Alterations in MAP2 immunocytochemistry in areas 9 and 32 of schizophrenic prefrontal cortex. *Psychiatry Res.* 114, 137–148.

Jones, P., Rodgers, B., Murray, R., and Marmot, M. (1994). Child developmental risk-factors for adult schizophrenia in the British 1946 birth cohort. *Lancet* 344, 1398–1402.

Joshi, D., Fung, S. J., Rothwell, A., and Weickert, C. S. (2012). Higher gamma-aminobutyric acid neuron density in the white matter of orbital frontal cortex in schizophrenia. *Biol. Psychiatry* 72, 725–733.

Kalus, P., Muller, T. J., Zuschratter, W., and Senitz, D. (2000). The dendritic architecture of prefrontal pyramidal neurons in schizophrenic patients. *Neuroreport* 11, 3621–3625.

Kandel, E., Schwartz, J., and Jessell, T. (2000). *Principles of Neural Science*. New York, NY: McGraw-Hill.

Kang, H. J., Kawasawa, Y. I., Cheng, F., Zhu, Y., Xu, X., Li, M., et al. (2011). Spatio-temporal transcriptome of the human brain. *Nature* 478, 483–489.

Kash, S. F., Johnson, R. S., Tecott, L. H., Noebels, J. L., Mayfield, R. D., Hanahan, D., et al. (1997). Epilepsy in mice deficient in the 65-kDa isoform of glutamic acid decarboxylase. *Proc. Natl. Acad. Sci. U.S.A.* 94, 14060–14065.

Keshavan, M. S., Anderson, S., and Pettegrew, J. W. (1994). Is schizophrenia due to excessive synaptic pruning in the prefrontal cortex – the Feinberg hypothesis revisisted. *J. Psychiatr. Res.* 28, 239–265.

Kestler, L. P., Walker, E., and Vega, E. M. (2001). Dopamine receptors in the brains of schizophrenia patients: a meta-analysis of the findings. *Behav. Pharmacol.* 12, 355–371.

Khandaker, G. M., Barnett, J. H., White, I. R., and Jones, P. B. (2011). A quantitative meta-analysis of population-based studies of premorbid intelligence and schizophrenia. *Schizophr. Res.* 132, 220–227.

Khirug, S., Yamada, J., Afzalov, R., Voipio, J., Khiroug, L., and Kaila, K. (2008). GABAergic depolarization of the axon initial segment in cortical principal neurons is caused by the Na-K-2Cl cotransporter NKCC1. *J. Neurosci.* 28, 4635–4639.

Kinney, H. C., Brody, B. A., Kloman, A. S., and Gilles, F. H. (1988). Sequence of central nervous system myelination in human infancy. II. Patterns of myelination in autopsied infants. *J. Neuropathol. Exp. Neurol.* 47, 217–234.

Kirkpatrick, B., Messias, N. C., Conley, R. R., and Roberts, R. C. (2003). Interstitial cells of the white matter in the dorsolateral prefrontal cortex in deficit and nondeficit schizophrenia. *J. Nerv. Ment. Dis.* 191, 563–567.

Klingberg, T., Forssberg, H., and Westerberg, H. (2002). Increased brain activity in frontal and parietal cortex underlies the development of visuospatial working memory capacity during childhood. *J. Cogn. Neurosci.* 14, 1–10.

Koketsu, D., Mikami, A., Miyamoto, Y., and Hisatsune, T. (2003). Nonrenewal of neurons in the cerebral neocortex of adult macaque monkeys. *J. Neurosci.* 23, 937–942.

Kolluri, N., Sun, Z. X., Sampson, A. R., and Lewis, D. A. (2005). Lamina-specific reductions in dendritic spine density in the prefrontal cortex of subjects with schizophrenia. *Am. J. Psychiatry* 162, 1200–1202.

Kornack, D. R., and Rakic, P. (1999). Continuation of neurogenesis in the hippocampus of the adult macaque monkey. *Proc. Natl. Acad. Sci. U.S.A.* 96, 5768–5773.

Kornack, D. R., and Rakic, P. (2001). The generation, migration, and differentiation of olfactory neurons in the adult primate brain. *Proc. Natl. Acad. Sci. U.S.A.* 98, 4752–4757.

Kornhuber, J., Mack-Burkhardt, F., Riederer, P., Hebenstreit, G. F., Reynolds, G. P., Andrews, H. B., et al. (1989). [3H]MK-801 binding sites in postmortem brain regions of schizophrenic patients. *J. Neural Transm.* 77, 231–236.

Korr, H., and Schmitz, C. (1999). Facts and fictions regarding postnatal neurogenesis in the developing human cerebral cortex. *J. Theor. Biol.* 200, 291–297.

Kosaka, J., Takahashi, H., Ito, H., Takano, A., Fujimura, Y., Matsumoto, R., et al. (2010). Decreased binding of C-11 NNC112 and C-11 SCH23390 in patients with chronic schizophrenia. *Life Sci.* 86, 814–818.

Kosodo, Y., and Huttner, W. B. (2009). Basal process and cell divisions of neural progenitors in the developing brain. *Dev. Growth Differ.* 51, 251–261.

Kosodo, Y., Roper, K., Haubensak, W., Marzesco, A. M., Corbeil, D., and Huttner, W. B. (2004). Asymmetric distribution of the apical plasma membrane during neurogenic divisions of mammalian neuroepithelial cells. *EMBO J.* 23, 2314–2324.

Kostovic, I., and Rakic, P. (1980). Cytology and time of origin of interstitial neurons in the white matter in infant and adult human and monkey telencephalon. *J. Neurocytol.* 9, 219–242.

Krimer, L. S., Herman, M. M., Saunders, R. C., Boyd, J. C., Hyde, T. M., Carter, J. M., et al. (1997). A qualitative and quantitative analysis of the entorhinal cortex in schizophrenia. *Cereb. Cortex* 7, 732–739.

Kristiansen, L. V., Beneyto, M., Haroutunian, V., and Meador-Woodruff, J. H. (2006). Changes in NMDA receptor subunits and interacting PSD proteins in dorsolateral prefrontal and anterior cingulate cortex indicate abnormal regional expression in schizophrenia. *Mol. Psychiatry* 11, 737–747.

Kroger, J. K., Sabb, F. W., Fales, C. L., Bookheimer, S. Y., Cohen, M. S., and Holyoak, K. J. (2002). Recruitment of anterior dorsolateral prefrontal cortex in human reasoning: a parametric study of relational complexity. *Cereb. Cortex* 12, 477–485.

Kwon, H., Reiss, A. L., and Menon, V. (2002). Neural basis of protracted developmental changes in visuo-spatial working memory. *Proc. Natl. Acad. Sci. U.S.A.* 99, 13336–13341.

Kyriakopoulos, M., Bargiotas, T., Barker, G. J., and Frangou, S. (2008). Diffusion tensor imaging in schizophrenia. *Biol. Psychiatry* 23, 255–273.

Lahti, A. C., Koffel, B., Laporte, D., and Tamminga, C. A. (1995). Subanesthetic doses of ketamine stimulate psychosis in schizophrenia. *Neuropsychopharmacology* 13, 9–19.

Lahti, A. C., Weiler, M. A., Tamara Michaelidis, B. A., Parwani, A., and Tamminga, C. A. (2001). Effects of ketamine in normal and schizophrenic volunteers. *Neuropsychopharmacology* 25, 455–467.

Laruelle, M., Abi-Dargham, A., van Dyck, C. H., Gil, R., D'Souza, C. D., Erdos, J., et al. (1996). Single photon emission computerized tomography imaging of amphetamine-induced dopamine release in drug-free schizophrenic subjects. *Proc. Natl. Acad. Sci. U.S.A.* 93, 9235–9240.

Laviola, G., Adriani, W., Morley-Fletcher, S., and Terranova, M. L. (2002). Peculiar response of adolescent mice to acute and chronic stress and to amphetamine: evidence of sex differences. *Behav. Brain Res.* 130, 117–125.

Laviola, G., Wood, R. D., Kuhn, C., Francis, R., and Spear, L. P. (1995). Cocaine sensitization in periadolescent and adult rats. *J. Pharmacol. Exp. Ther.* 275, 345–357.

Law, A. J., and Deakin, J. F. W. (2001). Asymmetrical reductions of hippocampal NMDAR1 glutamate receptor mRNA in the psychoses. *Neuroreport* 12, 2971–2974.

Law, A. J., Weickert, C. S., Hyde, T. M., Kleinman, J. E., and Harrison, P. J. (2004). Reduced spinophilin but not microtubule-associated protein 2 expression in the hippocampal formation in schizophrenia and mood disorders: molecular evidence for a pathology of dendritic spines. *Am. J. Psychiatry* 161, 1848–1855.

Law, A. J., Weickert, C. S., Webster, M. J., Herman, M. M., Kleinman, J. E., and Harrison, P. J. (2003). Changes in NMDA receptor subunit mRNAs and cyclophilin mRNA during development of the human hippocampus. *Ann. N.Y. Acad. Sci.* 1003, 426–430.

Lebel, C., Walker, L., Leemans, A., Phillips, L., and Beaulieu, C. (2008). Microstructural maturation of the human brain from childhood to adulthood. *Neuroimage* 40, 1044–1055.

Lenroot, R. K., Gogtay, N., Greenstein, D. K., Wells, E. M., Wallace, G. L., Clasen, L. S., et al. (2007). Sexual dimorphism of brain developmental trajectories during childhood and adolescence. *Neuroimage* 36, 1065–1073.

Lequin, M. H., and Barkovich, A. J. (1999). Current concepts of cerebral malformation syndromes. *Curr. Opin. Pediatr.* 11, 492–496.

Letinic, K., Zoncu, R., and Rakic, P. (2002). Origin of GABAergic neurons in the human neocortex. *Nature* 417, 645–649.

Levin, H. S., Culhane, K. A., Hartmann, J., Evankovich, K., Mattson, A. J., Harward, H., et al. (1991). Developmental changes in performance on tests of purported frontal lobe functioning. *Dev. Neuropsychol.* 7, 377–395.

Lewis, D. A. (1997). Development of the prefrontal cortex during adolescence: insights into vulnerable neural circuits in schizophrenia. *Neuropsychopharmacology* 16, 385–398.

Li, F., and Tsien, J. Z. (2009). Memory and the NMDA receptors. *N. Engl. J. Med.* 361, 302–303.

Lidow, M. S., and Rakic, P. (1992). Scheduling of monoaminergic neurotransmitter receptor expression in the primate neocortex during postnatal development. *Cereb. Cortex* 2, 401–416.

Lieberman, J. A., Kane, J. M., and Alvir, J. (1987). Provocative tests with psychostimulant drugs in schizophrenia. *Psychopharmacology (Berl.)* 91, 415–433.

Liston, C., Watts, R., Tottenham, N., Davidson, M. C., Niogi, S., Ulug, A. M., et al. (2006). Frontostriatal microstructure modulates efficient recruitment of cognitive control. *Cereb. Cortex* 16, 553–560.

Long, L. E., Lind, J., Webster, M., and Weickert, C. S. (2012). Developmental trajectory of the endocannabinoid system in human dorsolateral prefrontal cortex. *BMC Neurosci.* 13:87. doi: 10.1186/1471-2202-13-87

Maccabe, J. H., Wicks, S., Lofving, S., David, A. S., Berndtsson, A., Gustafsson, J. E., et al. (2013). Decline in cognitive performance between ages 13 and 18 years and the risk for psychosis in adulthood. A Swedish longitudinal cohort study in males. *JAMA Psychiatry.* 70, 261–270.

Malhotra, A. K., Pinals, D. A., Adler, C. M., Elman, I., Clifton, A., Pickar, D., et al. (1997). Ketamine-induced exacerbation of psychotic symptoms and cognitive impairment in neuroleptic-free schizophrenics. *Neuropsychopharmacology* 17, 141–150.

Manoach, D. S., Gollub, R. L., Benson, E. S., Searl, M. M., Goff, D. C., Halpern, E., et al. (2000). Schizophrenic subjects show aberrant fMRI activation of dorsolateral prefrontal cortex and basal ganglia during working memory performance. *Biol. Psychiatry* 48, 99–109.

Manoach, D. S., Press, D. Z., Thangaraj, V., Searl, M. M., Goff, D. C., Halpern, E., et al. (1999). Schizophrenic subjects activate dorsolateral prefrontal cortex during a working memory task, as measured by fMRI. *Biol. Psychiatry* 45, 1128–1137.

Mao, Y., Ge, X., Frank, C. L., Madison, J. M., Koehler, A. N., Doud, M. K., et al. (2009). Disrupted in schizophrenia 1 regulates neuronal progenitor proliferation via modulation of GSK3beta/beta-catenin signaling. *Cell* 136, 1017–1031.

Marin-Padilla, M. (1990). Three-dimensional structural organization of layer I of the human cerebral cortex: a Golgi study. *J. Comp. Neurol.* 299, 89–105.

Marin, O., and Rubenstein, J. L. (2003). Cell migration in the forebrain. *Annu. Rev. Neurosci.* 26, 441–483.

Matthews, P. R., Eastwood, S. L., and Harrison, P. J. (2012). Reduced myelin basic protein and actin-related gene expression in visual cortex in schizophrenia. *PLoS ONE* 7:e38211. doi: 10.1371/journal.pone.0038211

McArdle, J. J., Ferrer-Caja, E., Hamagami, F., and Woodcock, R. W. (2002). Comparative longitudinal structural analyses of the growth and decline of multiple intellectual abilities over the life span. *Dev. Psychol.* 38, 115–142.

McClellan, A. M., and Twyman, R. E. (1999). Receptor system response kinetics reveal functional subtypes of native murine and recombinant human GABAA receptors. *J. Physiol.* 515, 711–727.

McGorry, P. (2011). Transition to adulthood: the critical period for pre-emptive, disease-modifying care for schizophrenia and related disorders. *Schizophr. Bull.* 37, 524–530.

Meador-Woodruff, J. H., Haroutunian, V., Powchik, P., Davidson, M., Davis, K. L., and Watson, S. J. (1997). Dopamine receptor transcript expression in striatum and prefrontal and occipital cortex – focal abnormalities in orbitofrontal cortex in schizophrenia. *Arch. Gen. Psychiatry* 54, 1089–1095.

Mellios, N., Huang, H. S., Baker, S. P., Gaidzicka, M., Ginns, E., and Akbarian, S. (2009). Molecular determinants of dysregulated GABAergic gene expression in the prefrontal cortex of subjects with schizophrenia. *Biol. Psychiatry* 65, 1006–1014.

Meyer-Lindenberg, A., Poline, J. B., Kohn, P. D., Holt, J. L., Egan, M. F., Weinberger, D. R., et al. (2001). Evidence for abnormal cortical functional connectivity during working memory in schizophrenia. *Am. J. Psychiatry* 158, 1809–1817.

Meyer-Lindenberg, A. S., Olsen, R. K., Kohn, P. D., Brown, T., Egan, M. F., Weinberger, D. R., et al. (2005). Regionally specific disturbance of dorsolateral prefrontal-hippocampal functional connectivity in schizophrenia. *Arch. Gen. Psychiatry* 62, 379–386.

Meyer, G. (2001). Human neocortical development: the importance of embryonic and early fetal events. *Neuroscientist* 7, 303–314.

Millar, J. K., Wilson-Annan, J. C., Anderson, S., Christie, S., Taylor, M. S., Semple, C. A., et al. (2000). Disruption of two novel genes by a translocation co-segregating with schizophrenia. *Hum. Mol. Genet.* 9, 1415–1423.

Missale, C., Nash, S. R., Robinson, S. W., Jaber, M., and Caron, M. G. (1998). Dopamine receptors: from structure to function. *Physiol. Rev.* 78, 189–225.

Mizoguchi, K., Shoji, H., Tanaka, Y., Maruyama, W., and Tabira, T.

(2009). Age-related spatial working memory impairment is caused by prefrontal cortical dopaminergic dysfunction in rats. *Neuroscience* 162, 1192–1201.

Monsma, F. J., McVittie, L. D., Gerfen, C. R., Mahan, L. C., and Sibley, D. R. (1989). Multiple D2 dopamine receptors produced by alternative RNA splicing. *Nature* 342, 926–929.

Monyer, H., Burnashev, N., Laurie, D. J., Sakmann, B., and Seeburg, P. H. (1994). Developmental and regional expression in the rat brain and functional properties of four NMDA receptors. *Neuron* 12, 529–540.

Morris, H. M., Hashimoto, T., and Lewis, D. A. (2008). Alterations in somatostatin mRNA expression in the dorsolateral prefrontal cortex of subjects with schizophrenia or schizoaffective disorder. *Cereb. Cortex* 18, 1575–1587.

Mouri, A., Noda, Y., Enomoto, T., and Nabeshima, T. (2007). Phencyclidine animal models of schizophrenia: approaches from abnormality of glutamatergic neurotransmission and neurodevelopment. *Neurochem. Int.* 51, 173–184.

Mueller, H. T., and Meador-Woodruff, J. H. (2004). NR3A NMDA receptor subunit mRNA expression in schizophrenia, depression and bipolar disorder. *Schizophr. Res.* 71, 361–370.

Mukaetova-Ladinska, E. B., Hurt, J., Honer, W. G., Harrington, C. R., and Wischik, C. M. (2002). Loss of synaptic but not cytoskeletal proteins in the cerebellum of chronic schizophrenics. *Neurosci. Lett.* 317, 161–165.

Murphy, K. M., Beston, B. R., Boley, P. M., and Jones, D. G. (2005). Development of human visual cortex: a balance between excitatory and inhibitory plasticity mechanisms. *Dev. Psychobiol.* 46, 209–221.

Nadarajah, B. (2003). Radial glia and somal translocation of radial neurons in the developing cerebral cortex. *Glia* 43, 33–36.

Nadarajah, B., Brunstrom, J. E., Grutzendler, J., Wong, R. O., and Pearlman, A. L. (2001). Two modes of radial migration in early development of the cerebral cortex. *Nat. Neurosci.* 4, 143–150.

Nadarajah, B., and Parnavelas, J. G. (2002). Modes of neuronal migration in the developing cerebral cortex. *Nat. Rev. Neurosci.* 3, 423–432.

Nagy, Z., Westerberg, H., and Klingberg, T. (2004). Maturation of white matter is associated with the development of cognitive functions during childhood. *J. Cogn. Neurosci.* 16, 1227–1233.

Newell, K. A., Deng, C., and Huang, X. F. (2006). Increased cannabinoid receptor density in the posterior cingulate cortex in schizophrenia. *Exp. Brain Res.* 172, 556–560.

Newell, K. A., Zavitsanou, K., Jew, S. K., and Huang, X. F. (2007). Alterations of muscarinic and GABA receptor binding in the posterior cingulate cortex in schizophrenia. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 31, 225–233.

Nieoullon, A. (2002). Dopamine and the regulation of cognition and attention. *Prog. Neurobiol.* 67, 53–83.

Noga, J. T., Hyde, T. M., Herman, M. M., Spurney, C. F., Bigelow, L. B., Weinberger, D. R., et al. (1997). Glutamate receptors in the postmortem striatum of schizophrenic, suicide, and control brains. *Synapse* 27, 168–176.

Nudmamud, S., and Reynolds, G. P. (2001). Increased density of glutamate/N-methyl-D-aspartate receptors in superior temporal cortex in schizophrenia. *Neurosci. Lett.* 304, 9–12.

O'Donnell, P. (2010). Adolescent maturation of cortical dopamine. *Neurotox. Res.* 18, 306–312.

O'Rahilly, R., and Muller, F. (1994). "Neurulation in the normal human embryo," in *Neural Tube Effects*, eds G. Bock and J. Marsh (Chichester: Wiley), 70–82.

Okubo, Y., Suhara, T., Suzuki, K., Kobayashi, K., Inoue, O., Terasaki, O., et al. (1997). Decreased prefrontal dopamine D1 receptors in schizophrenia revealed by PET. *Nature* 385, 634–636.

Olesen, P. J., Nagy, Z., Westerberg, H., and Klingberg, T. (2003). Combined analysis of DTI and fMRI data reveals a joint maturation of white and grey matter in a fronto-parietal network. *Cogn. Brain Res.* 18, 48–57.

Olsen, R. W., and Sieghart, W. (2008). International Union of Pharmacology. LXX. Subtypes of gamma-aminobutyric acid(A) receptors: classification on the basis of subunit composition, pharmacology, and function. Update. *Pharmacol. Rev.* 60, 243–260.

Onn, S. P., Wang, X. B., Lin, M., and Grace, A. A. (2005). Dopamine D1 and D4 receptor subtypes differentially modulate recurrent excitatory synapses in prefrontal cortical pyramidal neurons. *Neuropsychopharmacology* 31, 318–338.

Pantelis, C., Yucel, M., Wood, S. J., McGorry, P. D., and Velakoulis, D. (2003). Early and late neurodevelopmental disturbances in schizophrenia and their functional consequences. *Aust. N.Z. J. Psychiatry* 37, 399–406.

Parlapani, E., Schmitt, A., Erdmann, A., Bernstein, H. G., Breunig, B., Gruber, O., et al. (2009). Association between myelin basic protein expression and left entorhinal cortex pre-alpha cell layer disorganization in schizophrenia. *Brain Res.* 1301, 126–134.

Perez-Otano, I., Lujan, R., Tavalin, S. J., Plomann, M., Modregger, J., Liu, X. B., et al. (2006). Endocytosis and synaptic removal of NR3A-containing NMDA receptors by PACSIN1/syndapin1. *Nat. Neurosci.* 9, 611–621.

Perlstein, W. M., Carter, C. S., Noll, D. C., and Cohen, J. D. (2001). Relation of prefrontal cortex dysfunction to working memory and symptoms in schizophrenia. *Am. J. Psychiatry* 158, 1105–1113.

Perlstein, W. M., Dixit, N. K., Carter, C. S., Noll, D. C., and Cohen, J. D. (2003). Prefrontal cortex dysfunction mediates deficits in working memory and prepotent responding in schizophrenia. *Biol. Psychiatry* 53, 25–38.

Petanjek, Z., Berger, B., and Esclapez, M. (2009a). Origins of cortical GABAergic neurons in the cynomolgus monkey. *Cereb. Cortex* 19, 249–262.

Petanjek, Z., Kostovic, I., and Esclapez, M. (2009b). Primate-specific origins and migration of cortical GABAergic neurons. *Front. Neuroanat.* 3:26. doi: 10.3389/neuro.05.026.2009

Petanjek, Z., Judas, M., Kostovic, I., and Uylings, H. B. M. (2008). Lifespan alterations of basal dendritic trees of pyramidal neurons in the human prefrontal cortex: a layer-specific pattern. *Cereb. Cortex* 18, 915–929.

Petanjek, Z., Judas, M., Simic, G., Rasin, M. R., Uylings, H. B. M., Rakic, P., et al. (2011). Extraordinary neoteny of synaptic spines in the human prefrontal cortex. *Proc. Natl. Acad. Sci. U.S.A.* 108, 13281–13286.

Philpot, B. D., Sekhar, A. K., Shouval, H. Z., and Bear, M. F. (2001). Visual experience and deprivation bidirectionally modify the composition and function of NMDA receptors in visual cortex. *Neuron* 29, 157–169.

Pinto, J. G. A., Hornby, K. R., Jones, D. G., and Murphy, K. M. (2010). Developmental changes in GABAergic mechanisms in human visual cortex across the lifespan. *Front. Cell. Neurosci.* 4:16. doi: 10.3389/fncel.2010.00016

Potkin, S. G., Turner, J. A., Brown, G. G., McCarthy, G., Greve, D. N., Glover, G. H., et al. (2009). Working memory and DLPFC inefficiency in schizophrenia: the FBIRN study. *Schizophr. Bull.* 35, 19–31.

Powell, C. M., and Miyakawa, T. (2006). Schizophrenia-relevant behavioral testing in rodent models: a uniquely human disorder? *Biol. Psychiatry* 59, 1198–1207.

Prabhakaran, V., Smith, J. A. L., Desmond, J. E., Glover, G. H., and Gabrieli, J. D. E. (1997). Neural substrates of fluid reasoning: an fMRI study of neocortical activation during performance of the Raven's Progressive Matrices Test. *Cogn. Psychol.* 33, 43–63.

Provenzale, J. M., Liang, L., Delong, D., and White, L. E. (2007). Diffusion tensor imaging assessment of brain white matter maturation during the first postnatal year. *Am. J. Roentgenol.* 189, 476–486.

Quinn, H. R., Matsumoto, I., Callaghan, P. D., Long, L. E., Arnold, J. C., Gunasekaran, N., et al. (2008). Adolescent rats find repeated Delta(9)-THC less aversive than adult rats but display greater residual cognitive deficits and changes in hippocampal protein expression following exposure. *Neuropsychopharmacology* 33, 1113–1126.

Rakic, P. (1972). Mode of cell migration to the superficial layers of fetal monkey neocortex. *J. Comp. Neurol.* 145, 61–83.

Rakic, S., and Zecevic, N. (2003). Emerging complexity of layer I in human cerebral cortex. *Cereb. Cortex* 13, 1072–1083.

Ravizza, S. M., Moua, K. C. K., Long, D., and Carter, C. S. (2010). The impact of context processing deficits on task-switching performance in schizophrenia. *Schizophr. Res.* 116, 274–279.

Reichenberg, A., Weiser, M., Rapp, M. A., Rabinowitz, J., Caspi, A., Schmeidler, J., et al. (2005). Elaboration on premorbid intellectual performance in schizophrenia – premorbid intellectual decline and risk for schizophrenia. *Arch. Gen. Psychiatry* 62, 1297–1304.

Reif, A., Fritzen, S., Finger, M., Strobel, A., Lauer, M., Schmitt, A., et al. (2006). Neural stem cell proliferation is decreased in schizophrenia, but not in depression. *Mol. Psychiatry* 11, 514–522.

Reynolds, G. P., and Beasley, C. L. (2001). GABAergic neuronal subtypes in the human frontal cortex – development and deficits in

schizophrenia. *J. Chem. Neuroanat.* 22, 95–100.

Reynolds, G. P., Beasley, C. L., and Zhang, Z. J. (2002). Understanding the neurotransmitter pathology of schizophrenia: selective deficits of subtypes of cortical GABAergic neurons. *J. Neural Transm.* 109, 881–889.

Richland, L. E., Morrison, R. G., and Holyoak, K. J. (2006). Children's development of analogical reasoning: insights from scene analogy problems. *J. Exp. Child Psychol.* 94, 249–273.

Ritter, L. M., Vazquez, D. M., and Meador-Woodruff, J. H. (2002). Ontogeny of ionotropic glutamate receptor subunit expression in the rat hippocampus. *Brain Res. Dev. Brain Res.* 139, 227–236.

Roberts, A. C., Diez-Garcia, J., Rodriguiz, R. M., Lopez, I. P., Lujan, R., Martinez-Turrillas, R., et al. (2009). Downregulation of NR3A-containing NMDARs is required for synapse maturation and memory consolidation. *Neuron* 63, 342–356.

Romanides, A. J., Duffy, P., and Kalivas, P. W. (1999). Glutamatergic and dopaminergic afferents to the prefrontal cortex regulate spatial working memory in rats. *Neuroscience* 92, 97–106.

Rothmond, D. A., Weickert, C. S., and Webster, M. J. (2012). Developmental changes in human dopamine neurotransmission: cortical receptors and terminators. *BMC Neurosci.* 13:18. doi: 10.1186/1471-2202-13-18

Runyan, C. A., Weickert, C. S., and Saunders, R. C. (2006). "Adult neurogenesis and immediate early gene response to working memory stimulation in the primate prefrontal cortex," in *Society for Neuroscience* (Atlanta, GA, USA: Society for Neuroscience).

Sakai, T., Oshima, A., Nozaki, Y., Ida, I., Haga, C., Akiyama, H., et al. (2008). Changes in density of calcium-binding-protein-immunoreactive GABAergic neurons in prefrontal cortex in schizophrenia and bipolar disorder. *Neuropathology* 28, 143–150.

Salimi, K., Glantz, L. A., Hamer, R. M., German, T. T., Gilmore, J. H., and Jarskog, L. F. (2008). Regulation of complexin 1 and complexin 2 in the developing human prefrontal cortex. *Synapse* 62, 273–282.

Sanai, N., Nguyen, T., Ihrie, R. A., Mirzadeh, Z., Tsai, H. H., Wong, M., et al. (2011). Corridors of migrating neurons in the human brain and their decline during infancy. *Nature* 478, 382–386.

Sawada, K., Young, C. E., Barr, A. M., Longworth, K., Takahashi, S., Arango, V., et al. (2002). Altered immunoreactivity of complexin protein in prefrontal cortex in severe mental illness. *Mol. Psychiatry* 7, 484–492.

Scarr, E., Beneyto, M., Meador-Woodruff, J. H., and Dean, B. (2005). Cortical glutamatergic markers in schizophrenia. *Neuropsychopharmacology* 30, 1521–1531.

Scherf, K. S., Sweeney, J. A., and Luna, B. (2006). Brain basis of developmental change in visuospatial working memory. *J. Cogn. Neurosci.* 18, 1045–1058.

Schmitt, A., Koschel, J., Zink, M., Bauer, M., Sommer, C., Frank, J., et al. (2010). Gene expression of NMDA receptor subunits in the cerebellum of elderly patients with schizophrenia. *Eur. Arch. Psychiatry Clin. Neurosci.* 260, 101–111.

Schneider, J. F., Il'yasov, K. A., Hennig, J., and Martin, E. (2004). Fast quantitative diffusion-tensor imaging of cerebral white matter from the neonatal period to adolescence. *Neuroradiology* 46, 258–266.

Schulz, J., Sundin, J., Leask, S., and Done, D. J. (2012). Risk of adult schizophrenia and its relationship to childhood IQ in the 1958 British Birth Cohort. *Schizophr. Bull.* doi: 10.1093/schbul/sbs157. [Epub ahead of print].

Seidman, L. J., Cherkerzian, S., Goldstein, J. M., Agnew-Blais, J., Tsuang, M. T., and Buka, S. L. (2013). Neuropsychological performance and family history in children at age 7 who develop adult schizophrenia or bipolar psychosis in the New England Family Studies. *Psychol. Med.* 43, 119–131.

Selemon, L. D., and Goldman-Rakic, P. S. (1999). The reduced neuropil hypothesis: a circuit based model of schizophrenia. *Biol. Psychiatry* 45, 17–25.

Seshadri, S., Zeledon, M., and Sawa, A. (2013). Synapse-specific contributions in the cortical pathology of schizophrenia. *Neurobiol. Dis.* 53, 26–35.

Shalaev, I., Weickert, C. S., Webster, M. J., and Saunders, R. C. (2002). "Neurogenesis in the dentate gurys and subventricular zone of the macaque," in *Society for Neuroscience* (Orlando, FL, USA: Society for Neuroscience).

Shankle, W. R., Landing, B. H., Rafii, M. S., Schiano, A., Chen, J. M., and Hara, J. (1998a). Evidence for a postnatal doubling of neuron number in the developing human cerebral cortex between 15 months and 6 years. *J. Theor. Biol.* 191, 115–140.

Shankle, W. R., Romney, A. K., Landing, B. H., and Hara, J. (1998b). Developmental patterns in the cytoarchitecture of the human cerebral cortex from birth to 6 years examined by correspondence analysis. *Proc. Natl. Acad. Sci. U.S.A.* 95, 4023–4028.

Sheng, M., and Hoogenraad, C. C. (2007). The postsynaptic architecture of excitatory synapses: a more quantitative view. *Annu. Rev. Biochem.* 76, 823–847.

Sidman, R. L., and Rakic, P. (1973). Neuronal migration, with special reference to developing human brain – review. *Brain Res.* 62, 1–35.

Silver, H., Feldman, P., Bilker, W., and Gur, R. C. (2003). Working memory deficit as a core neuropsychological dysfunction in schizophrenia. *Am. J. Psychiatry* 160, 1809–1816.

Simpson, M. D., Slater, P., Royston, M. C., and Deakin, J. F. (1991). Alterations in phencyclidine and sigma binding sites in schizophrenic brains. Effects of disease process and neuroleptic medication. *Schizophr. Res.* 6, 41–48.

Snook, L., Paulson, L. A., Roy, D., Phillips, L., and Beaulieu, C. (2005). Diffusion tensor imaging of neuro development in children and young adults. *Neuroimage* 26, 1164–1173.

So, C. H., Varghese, G., Curley, K. J., Kong, M. M. C., Alijaniaram, M., Ji, X. D., et al. (2005). D1 and D2 dopamine receptors form heterooligomers and cointernalize after selective activation of either receptor. *Mol. Pharmacol.* 68, 568–578.

Sokolov, B. P. (1998). Expression of NMDAR1, GluR1, GluR7, and KA1 glutamate receptor mRNAs is decreased in frontal cortex of "neuroleptic-free" schizophrenics: evidence on reversible up-regulation by typical neuroleptics. *J. Neurochem.* 71, 2454–2464.

Soloway, A. S., Pucak, M. L., Melchitzky, D. S., and Lewis, D. A. (2002). Dendritic morphology of callosal and ipsilateral projection neurons in monkey prefrontal cortex. *Neuroscience* 109, 461–471.

Somenarain, L., and Jones, L. B. (2010). A comparative study of MAP2 immunostaining in areas 9 and 17 in schizophrenia and Huntington chorea. *J. Psychiatr. Res.* 44, 694–699.

Spalding, K. L., Bhardwaj, R. D., Buchholz, B. A., Druid, H., and Frisen, J. (2005). Retrospective birth dating of cells in humans. *Cell* 122, 133–143.

Spear, L. P. (2007). *Adolescent Psychopathology and the Developing Brain: Integrating Brain and Prevention Science*. New York, NY: Oxford University Press.

Spear, L. P., Shalaby, I. A., and Brick, J. (1980). Chronic administration of haloperidol during development: behavioral and psychopharmacological effects. *Psychopharmacology (Berl.)* 70, 47–58.

Sternberg, R. J., and Rifkin, B. (1979). Development of analogical reasoning processes. *J. Exp. Child Psychol.* 27, 195–232.

Sunahara, R. K., Guan, H. C., Odowd, B. F., Seeman, P., Laurier, L. G., Ng, G., et al. (1991). Cloning of the gene for a human dopamine D5 receptor with higher affinity for dopamine than D1. *Nature* 350, 614–619.

Swartzwelder, H. S., Richardson, R. C., Markwiese-Foerch, B., Wilson, W. A., and Little, P. J. (1998). Developmental differences in the acquisition of tolerance to ethanol. *Alcohol* 15, 311–314.

Szabadics, J., Varga, C., Molnar, G., Olah, S., Barzo, P., and Tamas, G. (2006). Excitatory effect of GABAergic axo-axonic cells in cortical microcircuits. *Science* 311, 233–235.

Takemura, N. U. (2005). Evidence for neurogenesis within the white matter beneath the temporal neocortex of the adult rat brain. *Neuroscience* 134, 121–132.

Tallerico, T., Novak, G., Liu, I. S. C., Ulpian, C., and Seeman, P. (2001). Schizophrenia: elevated mRNA for dopamine D2(Longer) receptors in frontal cortex. *Mol. Brain Res.* 87, 160–165.

Tamamaki, N., Fujimori, K., and Takauji, R. (1997). Origin and route of tangentially migrating neurons in the developing neocortical intermediate zone. *J. Neurosci.* 17, 8313–8323.

Tamm, L., Menon, V., and Reiss, A. L. (2002). Maturation of brain function associated with response inhibition. *J. Am. Acad. Child Adolesc. Psychiatry* 41, 1231–1238.

Tan, H. Y., Sust, S., Buckholtz, J. W., Mattay, V. S., Meyer-Lindenberg, A., Egan, M. F., et al. (2006). Dysfunctional prefrontal regional specialization and compensation in schizophrenia. *Am. J. Psychiatry* 163, 1969–1977.

Thatcher, R. W. (1991). Maturation of the human frontal lobes – physiological evidence for staging. *Dev. Neuropsychol.* 7, 397–419.

Thatcher, R. W. (1992). Cyclic cortical reorganization during early childhood. *Brain Cogn.* 20, 24–50.

Thatcher, R. W. (1994). Psychopathology for early frontal lobe damage – dependence on cycles of development. *Dev. Psychopathol.* 6, 565–596.

Thompson, C. L., Drewery, D. L., Atkins, H. D., Stephenson, F. A., and Chazot, P. L. (2002). Immunohistochemical localization of N-methyl-D-aspartate receptor subunits in the adult murine hippocampal formation: evidence for a unique role of the NR2D subunit. *Brain Res. Mol. Brain Res.* 102, 55–61.

Thompson, M., Weickert, C. S., Wyatt, E., and Webster, M. J. (2009). Decreased glutamic acid decarboxylase(67) mRNA expression in multiple brain areas of patients with schizophrenia and mood disorders. *J. Psychiatr. Res.* 43, 970–977.

Toro, C., and Deakin, J. F. W. (2005). NMDA receptor subunit NRI and postsynaptic protein PSD-95 in hippocampus and orbitofrontal cortex in schizophrenia and mood disorder. *Schizophr. Res.* 80, 323–330.

Tseng, K. Y., Lewis, B. L., Lipska, B. K., and O'Donnell, P. (2007). Post-pubertal disruption of medial prefrontal cortical dopamine-glutamate interactions in a developmental animal model of schizophrenia. *Biol. Psychiatry* 62, 730–738.

Tseng, K. Y., and O'Donnell, P. (2004). Dopamine-glutamate interactions controlling prefrontal cortical pyramidal cell excitability involve multiple signaling mechanisms. *J. Neurosci.* 24, 5131–5139.

Tseng, K. Y., and O'Donnell, P. (2007). D-2 dopamine receptors recruit a GABA component for their attenuation of excitatory synaptic transmission in the adult rat prefrontal cortex. *Synapse* 61, 843–850.

Turman, J. E. Jr., Lee, O. K., and Chandler, S. H. (2002). Differential NR2A and NR2B expression between trigeminal neurons during early postnatal development. *Synapse* 44, 76–85.

Uhlhaas, P. J., Haenschel, C., Nikolic, D., and Singer, W. (2008). The role of oscillations and synchrony in cortical networks and their putative relevance for the pathophysiology of schizophrenia. *Schizophr. Bull.* 34, 927–943.

Uhlhaas, P. J., Roux, F., Singer, W., Haenschel, C., Sireteanu, R., and Rodriguez, E. (2009). The development of neural synchrony reflects late maturation and restructuring of functional networks in humans. *Proc. Natl. Acad. Sci. U.S.A.* 106, 9866–9871.

Velanova, K., Wheeler, M. E., and Luna, B. (2008). Maturational changes in anterior cingulate and frontoparietal recruitment support the development of error processing and inhibitory control. *Cereb. Cortex* 18, 2505–2522.

Verdurand, M., Fillman, S. G., Weickert, C. S., and Zavitsanou, K. (2013). Increases in [3H]muscimol and [3H]flumazenil binding in the dorsolateral prefrontal cortex in schizophrenia are linked to alpha4 and gamma2s mRNA levels respectively. *PLoS ONE* 8:e52724. doi: 10.1371/journal.pone.0052724

Volk, D. W., Matsubara, T., Li, S. Y., Sengupta, E. J., Georgiev, D., Minabe, Y., et al. (2012). Deficits in transcriptional regulators of cortical parvalbumin neurons in schizophrenia. *Am. J. Psychiatry* 169, 1082–1091.

Volk, D. W., Pierri, J. N., Fritschy, J. M., Auh, S., Sampson, A. R., and Lewis, D. A. (2002). Reciprocal alterations in pre- and postsynaptic inhibitory markers at chandelier cell inputs to pyramidal neurons in schizophrenia. *Cereb. Cortex* 12, 1063–1070.

Wang, C., Liu, F., Liu, Y. Y., Zhao, C. H., You, Y., Wang, L., et al. (2011). Identification and characterization of neuroblasts in the subventricular zone and rostral migratory stream of the adult human brain. *Cell Res.* 21, 1534–1550.

Wang, J., and O'Donnell, P. (2001). D-1 dopamine receptors potentiate NMDA-mediated excitability increase in layer V prefrontal cortical pyramidal neurons. *Cereb. Cortex* 11, 452–462.

Webster, M. J., Elashoff, M., and Weickert, C. S. (2011). Molecular evidence that cortical synaptic growth predominates during the first decade of life in humans. *Int. J. Dev. Neurosci.* 29, 225–236.

Webster, M. J., Weickert, C. S., Herman, M. M., Hyde, T. M., and Kleinman, J. E. (2001). Synaptophysin and GAP-43 mRNA levels in the hippocampus of subjects with schizophrenia. *Schizophr. Res.* 49, 89–98.

Weickert, C. S., Elashoff, M., Richards, A. B., Sinclair, D., Bahn, S., Paabo, S., et al. (2009). Transcriptome analysis of male-female differences in prefrontal cortical development. *Mol. Psychiatry* 14, 558–561.

Weickert, C. S., Fung, S. J., Catts, V. S., Schofield, P. R., Allen, K. M., Moore, L. T., et al. (2012). Molecular evidence of N-methyl-D-aspartate receptor hypofunction in schizophrenia. *Mol. Psychiatry*. doi: 10.1038/mp.2012.137. [Epub ahead of print].

Weickert, C. S., Straub, R. E., McClintock, B. W., Matsumoto, M., Hashimoto, R., Hyde, T. M., et al. (2004). Human dysbindin (DTNBP1) gene expression in normal brain and in schizophrenic prefrontal cortex and midbrain. *Arch. Gen. Psychiatry* 61, 544–555.

Weickert, T. W., Goldberg, T. E., Gold, J. M., Bigelow, L. B., Egan, M. F., and Weinberger, D. R. (2000a). Cognitive impairments in patients with schizophrenia displaying preserved and compromised intellect. *Arch. Gen. Psychiatry* 57, 907–913.

Weickert, C. S., Webster, M. J., Colvin, S. M., Herman, M. M., Hyde, T. M., Weinberger, D. R., et al. (2000b). Localization of epidermal growth factor receptors and putative neuroblasts in human subependymal zone. *J. Comp. Neurol.* 423, 359–372.

Weickert, C. S., Webster, M. J., Gondipalli, P., Rothmond, D., Fatula, R. J., Herman, M. M., et al. (2007). Postnatal alterations in dopaminergic markers in the human prefrontal cortex. *Neuroscience* 144, 1109–1119.

Weickert, T. W., and Goldberg, T. E. (2000). "The course of cognitive impairments in patients with schizophrenia," in *Cognition in Schizophrenia: Impairments, Importance and Treatment Strategies,* eds T. Sharma and P. Harvey (New York, NY: Oxford University Press), 3–15.

Weinberger, D. R. (1987). Implications of normal brain development for the pathogenesis of schizophrenia. *Arch. Gen. Psychiatry* 44, 660–669.

Weinberger, D. R., Berman, K. F., and Zec, R. F. (1986). Physiological dysfunction of dorsolateral prefrontal cortex in schizophrenia. 1. Regional cerebral blood flow evidence. *Arch. Gen. Psychiatry* 43, 114–124.

Williams, G. V., and Goldman-Rakic, P. S. (1995). Modulation of memory fields by dopamine D1 receptors in prefrontal cortex. *Nature* 376, 572–575.

Winter, S., Dieckmann, M., and Schwabe, K. (2009). Dopamine in the prefrontal cortex regulates rats behavioral flexibility to changing reward value. *Behav. Brain Res.* 198, 206–213.

Wong, H. K., Liu, X. B., Matos, M. F., Chan, S. F., Perez-Otano, I., Boysen, M., et al. (2002). Temporal and regional expression of NMDA receptor subunit NR3A in the mammalian brain. *J. Comp. Neurol.* 450, 303–317.

Wood, S. J., Pantelis, C., Velakoulis, D., Yucel, M., Fornito, A., and McGorry, P. D. (2008). Progressive changes in the development toward schizophrenia: studies in subjects at increased symptomatic risk. *Schizophr. Bull.* 34, 322–329.

Woodberry, K. A., Giuliano, A. J., and Seidman, L. J. (2008). Premorbid IQ in schizophrenia: a meta-analytic review. *Am. J. Psychiatry* 165, 579–587.

Wright, S. B., Matlen, B. J., Baym, C. L., Ferrer, E., and Bunge, S. A. (2008). Neural correlates of fluid reasoning in children and adults. *Front. Hum. Neurosci.* 1:8. doi: 10.3389/neuro.09.008.2007

Xu, G., Broadbelt, K. G., Haynes, R. L., Folkerth, R. D., Borenstein, N. S., Belliveau, R. A., et al. (2011). Late development of the GABAergic system in the human cerebral cortex and white matter. *J. Neuropathol. Exp. Neurol.* 70, 841–858.

Yago, K. B., Pitts, F. N. Jr., Burgoyne, R. W., Aniline, O., Yago, L. S., and Pitts, A. F. (1981). The urban epidemic of phencyclidine (PCP) use: clinical and laboratory evidence from a public psychiatric hospital emergency service. *J. Clin. Psychiatry* 42, 193–196.

Yakovlev, P. I., and Lecours, A. (1967). "The myelogenetic cycles of regional maturation of the brain," in *Regional Development of the Brain Early in Life,* ed A. Minkowski (Oxford: Blackwell Scientific Publications Inc.).

Yamashita, A., Hayashi, M., Shimizu, K., and Oshima, K. (1989). Ontogeny of somatostatin in cerebral cortex of macaque monkey: an immunohistochemical study. *Brain Res. Dev. Brain Res.* 45, 103–111.

Yan, Y. H., Van Brederode, J. F., and Hendrickson, A. E. (1995). Transient co-localization of calretinin, parvalbumin, and calbindin-D28K in developing visual cortex of monkey. *J. Neurocytol.* 24, 825–837.

Yang, Y., Fung, S. J., Rothwell, A., Tianmei, S., and Weickert, C. S. (2011). Increased interstitial white matter neuron density in the dorsolateral prefrontal cortex of people with schizophrenia. *Biol. Psychiatry* 69, 63–70.

Zavitsanou, K., Garrick, T., and Huang, X. F. (2004). Selective antagonist [3H]SR141716A binding to cannabinoid CB1 receptors

is increased in the anterior cingulate cortex in schizophrenia. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 28, 355–360.

Zecevic, N. (2004). Specific characteristic of radial glia in the human fetal telencephalon. *Glia* 48, 27–35.

Zecevic, N., Chen, Y., and Filipovic, R. (2005). Contributions of cortical subventricular zone to the development of the human cerebral cortex. *J. Comp. Neurol.* 491, 109–122.

Zecevic, N., Hu, F., and Jakovcevski, I. (2011). Interneurons in the developing human neocortex. *Dev. Neurobiol.* 71, 18–33.

Zhan, L., Kerr, J. R., Lafuente, M. J., Maclean, A., Chibalina, M. V., Liu, B., et al. (2011). Altered expression and coregulation of dopamine signalling genes in schizophrenia and bipolar disorder. *Neuropathol. Appl. Neurobiol.* 37, 206–219.

Zombeck, J. A., Swearingen, S. P., and Rhodes, J. S. (2010). Acute locomotor responses to cocaine in adolescents vs. adults from four divergent inbred mouse strains. *Genes Brain Behav.* 9, 892–898.

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