Review

The involvement of the NMDA receptor ϖ-serine/glycine site in the pathophysiology and treatment of schizophrenia

Viviane Labrie, John C. Roder

Abstract

Hypofunction of the N-methyl-D-aspartate receptor (NMDAR) has been implicated in the pathophysiology of schizophrenia. The NMDAR contains a ϖ-serine/glycine site on the NR1 subunit that may be a promising therapeutic target for psychiatric illness. This review outlines the complex regulation of endogenous NMDAR ϖ-serine/glycine site agonists and explores their contribution to schizophrenia pathogenesis and their potential clinical utility. Genetic studies have associated genes influencing NMDAR ϖ-serine/glycine site activation with an increased susceptibility to schizophrenia. Postmortem studies have identified abnormalities in several transcripts affecting ϖ-serine/glycine site activity, consistent with in vivo reports of alterations in levels of endogenous ϖ-serine/glycine site agonists and antagonists. Genetically modified mice with aberrant NMDAR ϖ-serine/glycine site function model certain features of the negative and cognitive symptoms of schizophrenia, and similar behavioral abnormalities have been observed in other candidate genes models. Compounds that directly activate the NMDAR ϖ-serine/glycine site or inhibit glycine transport have demonstrated beneficial effects in preclinical models and clinical trials. Future pharmacological approaches for schizophrenia treatment may involve targeting enzymes that affect ϖ-serine synthesis and metabolism.

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1. Introduction

Schizophrenia is a chronic and severely debilitating psychiatric disorder affecting nearly 1% of the population worldwide. It is characterized by positive symptoms that include hallucinations, delusions, and thought disorder, by negative symptoms comprised of affective flattening and social isolation, and by profound cognitive deficits in attention, learning, memory, and behavioral flexibility (Ross et al., 2006; Lewis and Gonzalez-Burgos, 2006). Symptoms of schizophrenia typically emerge during adolescence or early adulthood and while positive symptoms often fluctuate, negative and cognitive symptoms are more enduring, causing great disability and deterioration in the quality of life of patients (Yamauchi et al., 2008; Milne et al., 2005). Current antipsychotic treatments for schizophrenia show success in reducing the severity of positive symptoms, but have limited efficacy in ameliorating negative and cognitive deficits (Ross et al., 2006). Furthermore, antipsychotic regimens are often poorly tolerated, leading to poor compliance and symptomatic relapse (Lewis and Gonzalez-Burgos, 2006). In order to develop effective therapies, much effort has been made to further understand the molecular alterations involved in the pathophysiology of schizophrenia.

Abnormalities in several neurotransmitter systems have been implicated in the pathophysiological processes underlying schizophrenia. The dopamine hypothesis has been the dopaminergic theory, which postulates that schizophrenic symptoms arise from excessive dopaminergic transmission, particularly in the striatum, and the presence of dopaminergic deficits in prefrontal brain regions (Davis et al., 1991). This theory was based on the observation that blockade of D2 receptors is a mechanism of action for antipsychotics (Seeman et al., 1975; Creese et al., 1976) and the ability of dopamine-releasing stimulants, such as amphetamine, to induce psychosis (Janowsky and Risch, 1979). Amphetamine elicits only the positive symptoms of schizophrenia, consistent with the greater efficacy of antipsychotics in relieving the positive symptoms rather than the negative symptoms, cognitive impairments, and cortical atrophy seen in schizophrenia patients. In addition to the dopaminergic abnormalities, NMDA receptor (NMDAR) hypofunction has been proposed to be involved in schizophrenia. This theory originated from studies demonstrating that non-competitive NMDAR antagonists like phencyclidine (PCP) reliably and immediately induce a syndrome similar to schizophrenia in healthy individuals and exacerbate symptoms in schizophrenia patients (Javitt and Zukin, 1991; Krystal et al., 1994). Serum concentrations of PCP that are able to produce psychiatric symptoms correspond to the level that blocks NMDARs (Javitt and Zukin, 1991). Moreover, NMDAR inhibitors generate the negative and cognitive disturbances as well as the psychotic symptoms characteristic of the disorder (Javitt and Zukin, 1991; Krystal et al., 1994). Negative symptoms induced by NMDAR antagonists are not worsened by amphetamine administration in healthy humans and differences in the psychotic states produced by psychostimulants and NMDAR inhibitors have been observed (Krystal et al., 2005).

Since these initial observations, convergent evidence has supported a role for aberrant NMDAR-mediated neurotransmission in schizophrenia pathogenesis (Coyle, 2006; Milan, 2005) and the glutamatergic system in schizophrenia is considered to be part of a larger complex framework involving the interaction of multiple neurotransmitters and risk genes. More recently, studies have indicated that the NMDAR D-serine/glycine binding site and its modulatory enzymes may be crucially involved in the glutamatergic dysfunction thought to occur (Milan, 2005; Coyle, 2006). Here, we examine the significance of the NMDAR D-serine/glycine site and its related modulators to the pathophysiology of schizophrenia. Genetic, neurochemical, and postmortem studies in humans have begun to distinguish pathogenetic events that may alter glutamatergic transmission and plausibly lead to abnormal NMDAR D-serine/glycine site activity and the formation of schizophrenic symptoms. Evidence from pharmacological and genetic animal models have provided further support for aberrant NMDAR D-serine/glycine site function in endophenotypes relevant to schizophrenia. Finally, D-serine/glycine site agonists assessed in clinical studies have shown promising ameliorative effects. Overall, these findings present novel molecular alterations and therapeutic interventions for schizophrenia.

2. The NMDAR: structure and regulation

NMDARs in the central nervous system (CNS) are heteromeric protein complexes composed of at least one NR1 subunit together with different combinations of NR2 and/or NR3 subunits (Cull-Candy et al., 2001). Alternative splicing of the Grin1 gene produces eight NR1 isoforms and by associating with different constellations of NR2 (NR2A-D) and NR3 subunits (NR3A and NR3B) form a multitude of different NMDAR receptors with distinct biophysical properties (Monyer et al., 1994) and specific patterns of expression during development and in the mature mammalian CNS (Dumas, 2005). NMDAR complexity is further enhanced through post-translational modifications, such as phosphorylation, glycosylation, and ubiquitination, affecting cellular localization and function of the receptor (Dingledine et al., 1999).

At resting membrane potential, the pore of the NMDAR channel is blocked by magnesium, and this block can be removed via a-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate (AMPA) receptor-mediated membrane depolarization (Dingledine et al., 1999). The NMDAR also contains a glutamate recognition site on the NR2 subunit and a glycine or D-serine modulatory site on the NR1 subunit (Fig. 1) (Johnson and Ascher, 1987; Clements and Westbrook, 1991). The D-serine/glycine site on the NMDAR must be occupied for glutamate to activate the receptor (Clements and Westbrook, 1991). The unique property of being both voltage-dependent and ligand-gated gives the NMDAR the ability to act as a coincidence detector for presynaptic activity (glutamate release) and post-synaptic activity (adequate depolarization of the post-synaptic membrane). Once activated, the NMDAR channel permits the influx of calcium, which stimulates intracellular signaling cascades that can subsequently affect synaptic plasticity and gene transcription (Bading et al., 1993). Induction of NMDAR-dependent forms of synaptic plasticity, such as long-term potentiation (LTP), is thought to underlie many types of learning and memory formation (Nicoll, 2003).

NMDARs are present throughout the brain and are principally neuronal, though they can also be expressed on astrocytes. Beyond the glutamate and D-serine/glycine binding site, they contain several regulatory sites sensitive to polyamines, Zn2+, protons, and glutathione (Cull-Candy et al., 2001; Dingledine et al., 1999). The numerous influences that converge on the NMDAR highlight the importance of these receptors in diverse brain functions. Additionally, the NMDAR can also be modulated by several artificially derived inhibitory compounds, including PCP, MK-801, and ketamine, which are high affinity open channel blockers (Cull-Candy et al., 2001; Dingledine et al., 1999).

3. Endogenous modulators of the NMDAR D-serine/glycine site

In NMDAR complexes containing NR1 and NR2 subunits, D-serine and glycine both have excitatory effects, with D-serine being up to three times more potent than glycine (Matsui et al., 1995). NMDARs with NR2/NR1 subunits have been implicated in numerous physiological processes, including synaptic plasticity and development, as well as in several pathological conditions, such as neurodegenerative and psychiatric diseases (Cull-Candy et al., 2001).
et al., 2001). In receptors composed of NR1 and NR3 subunits, glycine continues to act as an activator, while D-serine exerts weak partial agonistic effects (Chatterton et al., 2002; Smothers and Woodward, 2007). To date, the role of NR3/NR1 heteromers in the adult brain is unclear and they display a peculiar resistance to psychotomimetic NMDAR antagonists (Smothers and Woodward, 2007; Chatterton et al., 2002), thus bringing their relevance to the induction of psychotic syndromes into question.

In addition to enabling NMDAR activation, endogenous D-serine/glycine site agonists have a role in neuromodulation. Binding to the D-serine/glycine site allosterically influences the NMDAR to enhance the affinity and efficacy of glutamate (Fadda et al., 1988), delays receptor desensitization to increase the duration and frequency of the open channel state (Vyklický et al., 1990), and promotes NMDAR turnover through priming of the receptor for internalization (Nong et al., 2003).

Examination of the crystal structure of the NR1 binding core provided further insight on the modes of interaction and selectivity of D-serine/glycine site agonists. D-Serine binds more tightly to the receptor than glycine, due to its ability to make 3 additional hydrogen bonds and displace a water molecule within the binding pocket (Furukawa and Gouaux, 2003). The binding of the D-enantiomer to NR1 is selective, as L-serine contains a hydroxyl group that interacts unfavorably with a phenyl ring in the binding site (Furukawa and Gouaux, 2003).

Whether the D-serine/glycine site on NMDARs is saturated by glycine or D-serine at physiological conditions remains somewhat controversial, though most evidence indicates that the D-serine/glycine site is not saturated in several brain areas. Elevations in glycine and/or D-serine concentrations evoke NMDAR-mediated responses in the prefrontal cortex, neocortex, hippocampus, and brainstem slices in vitro (Chen et al., 2003; Thomson et al., 1989; Wilcox et al., 1996; Depoortere et al., 2005; Berger and Isaacs, 1999) and in the prefrontal cortex, hippocampus, and thalamus in vivo (Chen et al., 2003; Panizzutti et al., 2005; Kinney et al., 2003; Thiels et al., 1992; Salt, 1989), signifying that the NMDAR glycine may not be fully saturated at synapses in these brain regions. Incomplete saturation of the D-serine/glycine site suggests that agonists of the D-serine/glycine site are capable of regulating NMDAR-mediated neurotransmission.

3.1. Glycine

Although glycine is an abundant amino acid found throughout the brain, its synaptic concentrations are tightly regulated by glycine transporters. At NMDAR-expressing synapses, extracellular glycine concentrations are primarily derived from astroglial cells and clearance is mediated by glycine transporter 1 (GlyT-1) (Kinney et al., 2003; Lim et al., 2004). GlyT-1s are closely associated to NMDARs (Cubelos et al., 2005) and there at least 6 glial and neuronal subtypes (GlyT-1a–f) (Chen et al., 2004b; Hanley et al., 2000). GlyT-1 effectively maintains low, subsaturating levels of glycine, as GlyT-1 blockers like ALX-5407 are capable of enhancing NMDAR activity (Chen et al., 2003; Bergeron et al., 1998; Martina et al., 2004). Spillover from glycinergic neurons also contributes a small amount of glycine to NMDARs (Ahmadi et al., 2003); although distant diffusion from glycinergic neurons is limited by the high affinity glycine transporter 2 (GlyT-2) that is responsible for glycine reuptake near strychnine-sensitive glycine A receptors (Poyatos et al., 1997). In addition, System A-family transporters (SNAT) on astrocytes and neurons transport a range of small neutral amino acids including glycine, and by modulating glycine uptake and release may also contribute to the dynamic regulation of extracellular glycine (Mackenzie and Erickson, 2004; Javitt et al., 2005).

Biosynthesis of glycine occurs through the conversion of L-serine to glycine by the enzyme serine hydroxymethyltransferase (Appaji Rao et al., 2003). This enzyme is also capable of functioning in a reverse direction, thereby eliminating glycine (Appaji Rao et al., 2003). Additionally, the glycine cleavage system in astrocytes efficiently degrades glycine and generates the concentration gradient between the cytosol and extracellular space that allows glycine transporters to remove glycine from the synaptic cleft (Sakata et al., 2001; Oda et al., 2007).

3.2. D-Serine

The discovery of D-serine in the brain revolutionized the long-standing belief that only L-isomers of amino acids existed in mammalian tissues. D-Serine was found to be a highly selective endogenous activator of the NMDAR D-serine/glycine site (Mothet
The brain, SRR protein has also been detected in the murine liver where D-serine released from Bergman glia mediates NMDAR-levels are relatively high in caudal regions of the developing brain, of D-amino acid oxidase (DAO), the D-serine catabolic enzyme concentrations in caudal brain areas coincide with the emergence (Wang and Zhu, 2003). Prior to the appearance of DAO, D-serine produce an lower affinity and possesses (D’Aniello et al., 1993; Pollegioni et al., 1992). The brain distribution of DAO is inversely related to that of endogenous D-serine (et al., 2005; De Miranda et al., 2002). Like D-serine, SRR is present in both astrocytes and neurons of the brain (Williams et al., 2006b; Wolosker et al., 1999; Yoshikawa et al., 2006; Miya et al., 2008; Kartvelishvily et al., 2006), with a regional distribution that closely resembles that of NMDARs (Schell et al., 1997). Though enriched in the brain, SRR protein has also been detected in the murine liver and the human liver, kidney, and heart (Wolosker et al., 1999; Xia et al., 2004). Similarly, D-serine levels are much higher in the CNS than in peripheral tissues (Hashimoto et al., 1995). In the adult human and rodent brain, D-serine and SRR are predominantly localized to the forebrain, with high levels in the cerebral cortex and hippocampus, and minimal levels in the cerebellum and brainstem (Xia et al., 2004; Schell et al., 1995). The low D-serine concentrations in caudal brain areas coincide with the emergence of D-amino acid oxidase (DAO), the D-serine catabolic enzyme (Wang et al., 2003). Prior to the appearance of DAO, D-serine levels are relatively high in caudal regions of the developing brain, where D-serine released from Bergman glia mediates NMDAR-dependent neuronal migration in the cerebellum (Kim et al., 2005).

DAO is highly selective for D-serine degradation at physiological pH, where it catalyzes the oxidative deamination of D-serine to produce an α-keto acid, ammonia, and hydrogen peroxide (D’Aniello et al., 1993; Pollegioni et al., 1992). The brain distribution of DAO is inversely related to that of endogenous D-serine concentrations, with the highest levels of DAO in astrocytes, Golgi-Bergmann glia, and tanyocytes of the hindbrain and cerebellum (Moreno et al., 1999). Lower levels of DAO have been detected in the neurons of the prefrontal cortex, hippocampus, and substantia nigra (Verrall et al., 2007; Moreno et al., 1999). In the periphery, DAO is most highly expressed in the kidneys and liver (Katagiri et al., 1991).

Modulating DAO function is G72 (also known as LG72 or DAO activator), a gene unique to primates (Chumakov et al., 2002). Initially, G72 was reported to be an activator of DAO (Chumakov et al., 2002); however a recent study indicates that G72 may instead repress DAO activity (Sacchi et al., 2008). The function of G72 remains controversial, as in mammalian cell lines and rat primary hippocampal neurons, G72 was described to have an alternate role, acting as a mitochondrial protein that promoted mitochondrial fragmentation and denticr protein kinase C (PKC) to SRR, leading SRR phosphorylation and reductions in D-serine production and SRR activity by inducing phospholipase C (PLC) inhibition of SRR (Mustafa et al., 2009). Additionally, protein-interacting with kinase C (PICK1) has been shown to bind to the C-terminus of SRR (Fuji et al., 2006), possibly to directly modulate its activity. Alternatively or in addition, PICK1 may escort protein kinase C (PKC) to SRR, leading SRR phosphorylation and altered SRR activity (Fuji et al., 2006). Following synthesis, D-serine undergoes vesicular storage and release from astrocytes (Martineau et al., 2008; Mothet et al., 2005; Williams et al., 2006b) or is released through a nonvesicular pathway from neurons (Kartvelishvily et al., 2006) and possibly astrocytes (Ribeiro et al., 2002). Synaptic D-serine facilitates NMDAR activation, although active NMDARs are in turn capable limiting D-serine availability through translocation of cytosolic SRR to the plasma membrane which reduces its capacity to form D-serine (Balan et al., 2009) and/or by promoting release of nitric oxide that enhances DAO function and suppresses SRR activity (Shoji et al., 2006a,b; Mustafa et al., 2007). NMDAR-mediated feedback regulation of D-serine availability may be a means of preventing receptor over-excitation.

Intracellular levels of D-serine are regulated by SRR and DAO; however clearance of D-serine from the synaptic space is assured by various sodium-dependent and sodium-independent transporters expressed on neurons and glia (Ribeiro et al., 2002; O’Brien et al., 2005; Helboe et al., 2003; Javitt et al., 2002). Among the transporters, the alanine-serine-cysteine transporter 1 (Asc-1) mediates the majority of D-serine reuptake in the brain (Rutter et al., 2007). Asc-1 is located on the presynaptic terminal, dendrites, and cell body of neurons (Helboe et al., 2003) and is the only transporter that exhibits a high D-serine affinity (Rutter et al., 2007).

4. The predominant physiological co-agonist of the NMDAR D-serine/glycine site

Growing evidence indicates that D-serine, rather than glycine, is the dominant endogenous ligand for the D-serine/glycine site of most NMDARs. Depletion of D-serine by treatment with DAO has been shown to attenuate NMDAR activity, as measured by biochemical and electrophysiological approaches in cerebellar slices, hippocampal slices, hippocampal cell cultures, and retina preparations (Mothet et al., 2000; Yang et al., 2003; Gustafson et al., 2007). Moreover, in hypothalamic slices, NMDAR currents are substantially reduced following elimination of D-serine by DAO, while a loss of glycine by a glycine oxidase enzyme does not produce an effect (Panatier et al., 2006). Similarly, removal of D-serine with a recombinant D-serine deaminase enzyme suppressed NMDAR-mediated light-evoked responses in retinal cells and NMDAR-induced neurotoxicity in organotypic hippocampal slices (Gustafson et al., 2007; Shleper et al., 2005). In a senescence-accelerated mouse strain, deficient NMDAR-dependent LTP in the hippocampus was associated with a diminished production of D-serine, but not lowered levels of glycine (Mothet et al., 2006). The effects of diminished NMDAR-mediated neurotransmission in these experiments could be fully reversed by the application of exogenous D-serine (Mothet et al., 2000, 2006; Yang et al., 2003; Panatier et al., 2006; Gustafson et al., 2007). Together, these experiments favor D-serine as the predominant physiological co-agonist for the NMDAR D-serine/glycine site

Increases in D-serine are also capable of further enhancing NMDAR signaling. This has been demonstrated several in vitro studies examining NMDAR-evoked excitatory responses in the prefrontal cortex, hippocampus, striatum, and in hippocampal
cultures (Chen et al., 2003; Martina et al., 2003; Yang et al., 2003; Chapman et al., 2003). In mice that lack DAO activity, the resulting elevation in D-serine potentiates NMDAR-mediated currents in spinal cord neurons (Wake et al., 2001). Mice that lack the neuronal transporter Asc-1 also display NMDAR-dependent hyperexcitability (Xie et al., 2005), presumably from the elevations in extracellular D-serine. Finally, exogenous administration of D-serine has been shown to elevate hippocampal responses in vivo, as measured by changes in relative cerebral blood volume in a functional magnetic resonance imaging (fMRI) study (Panizzutti et al., 2005).

Thus, these findings demonstrate a capacity for D-serine and its modulatory enzymes to dynamically regulate NMDAR activity, and disturbances in this pathway could conceivably contribute to psychopathologies associated with abnormal NMDAR-mediated neurotransmission.

5. Dysfunction of the NMDAR in schizophrenia

A large body of evidence supports a central role for aberrant NMDAR function in the pathophysiology of schizophrenia. In addition to the induction of psychotomimetic effects, subanesthetic doses of a non-competitive NMDAR antagonist increase amphetamine-induced dopamine release in the striatum to an extent that mimics the exaggerated responses seen in schizophrenic subjects (Kegeles et al., 2000). Chronic exposure to NMDAR inhibitors in rodents and primates also lowers dopamine levels in the prefrontal cortex and affects dopamine receptor binding (Tsukada et al., 2005; Jentsch et al., 1997), consistent with findings in patients with schizophrenia (Abi-Dargham et al., 2002; Davis et al., 1991; Narendran et al., 2005). This suggests that aberrant dopaminergic transmission in schizophrenia may be the consequence of a defect in the regulatory glutamatergic neuronal pathway. In addition, antagonists of the NMDAR disrupt activity in the prefrontal cortex, affecting the efficiency of neuronal firing and synchronization (Jackson et al., 2004; Kargieman et al., 2007), which may contribute to disturbances in cortical processing and cognitive function observed in schizophrenia. Furthermore, genetic association studies have identified a number of susceptibility genes that influence NMDAR function (Ross et al., 2006; Harrison and Weinberger, 2005). In drug-naive schizophrenia patients, decreased in vivo hippocampal NMDAR binding and reduced plasma levels of endogenous NMDAR agonists have been reported (Pilowsky et al., 2006; Hashimoto et al., 2003; Yamada et al., 2005). Postmortem studies have found numerous alterations in NMDAR receptor binding, transcript levels, and subunit protein expression in the cortex, hippocampus, and thalamus of schizophrenic individuals (Kristiansen et al., 2007). Reductions in parvalbumin-immunoreactive cells (GABAergic interneurons) and diminished expression of GAD67, the GABA synthesis enzyme, are also frequently observed in the postmortem hippocampus and prefrontal cortex (Reynolds et al., 2004; Torrey et al., 2005). Administration of NMDAR antagonists can replicate the loss of parvalbumin and GAD67 (Keilhoff et al., 2004; Kinney et al., 2006), alter GABA-mediated inhibitory control of cortical neurons (Homayoun and Moghaddam, 2007), and disrupt the development of GABAergic neurons (Abekawa et al., 2007). Thus, NMDA hypofunction could contribute to the abnormalities in several genes and neurotransmitter systems implicated in the biological mechanism underlying schizophrenia. The indication of aberrant NMDAR function in schizophrenia pathogenesis prompts a need to
further understand how NMDAR hypofunction may arise in this disease and predicts that this system could be useful for the development of novel therapeutics. Genetic, clinical, postmortem, and pharmacological studies indicate that the NMDAR D-serine/glycine site and its regulators may be involved in the pathophysiology and treatment of schizophrenia.

6. Genetic studies: G72, DAO, and SRR

Archival family, twin, and adoption studies indicate that schizophrenia is highly heritable, but no single gene exhibits a strong effect. Instead, accumulating evidence indicates that schizophrenia has a heterogeneous etiology involving a complex interplay of multiple genes, epigenetics, and environmental factors (Harrison and Weinberger, 2005; Ross et al., 2006). Several of the genes associated with schizophrenia risk are modulators of NMDAR D-serine/glycine site activation. In particular, genes involved in D-serine catabolism and synthesis have been identified.

G72 was initially identified by Chumakov et al. (2002), who examined markers within a 5-Mb segment from chromosome 13q33, a region that had previously been linked to schizophrenia in earlier linkage analyses. A significant association between G72 and schizophrenia was identified in French Canadian and Russian populations (Chumakov et al., 2002). In a yeast two-hybrid screen, G72 strongly associated with DAO and in vitro assays confirmed a regulatory effect of G72 on DAO activity (Chumakov et al., 2002; Sacchi et al., 2008). The positive association of G72 with schizophrenia susceptibility has since been replicated in numerous studies (Shinkai et al., 2007; Schuchmer et al., 2004; Fallin et al., 2005; Korostishevsky et al., 2004; Opden-Rhein et al., 2008) and continues to be significant following meta-analyses (Shi et al., 2008). Additionally, G72 is one of the best supported loci for bipolar disorder (Prata et al., 2008; Schuchmer et al., 2004; Fallin et al., 2005; Williams et al., 2006a). Furthermore, some evidence indicates an association with major depression and panic disorder (Rietschel et al., 2008; Schuchmer et al., 2005), with one large study of 2831 individuals suggesting that G72 may influence predisposition to episodes of mood disorder across traditional bipolar and schizophrenia categories (Williams et al., 2006a).

Despite the number of positive associations, these studies have demonstrated considerable allelic heterogeneity, with few studies reporting association with the same allele at a SNP marker. The limited allelic compatibility along with the difficulties in identifying endogenous G72 protein (Benzel et al., 2008) have made it difficult to understand how G72 is dysregulated in schizophrenia and other psychiatric illnesses. However, Korostishevsky et al. (2004) did amplify G72 mRNA showing that it is overexpressed in the dorsolateral prefrontal cortex of postmortem schizophrenia patients.

Interestingly, recent genetic classification and fMRI investigations in healthy and schizophrenic populations report that genetic variation in the G72 gene may influence cognitive function (Jansen et al., 2009; Hall et al., 2008; Opden-Rhein et al., 2008; Goldberg et al., 2006). Carriers of G72 risk variants differed in their performance during tests of working memory, verbal initiation, attention, and semantic fluency, and displayed differential recruitment of brain regions relevant to cognitive ability, including the hippocampal complex and prefrontal cortex (Jansen et al., 2009; Hall et al., 2008; Opden-Rhein et al., 2008; Goldberg et al., 2006). By modulating DAO activity, G72 could contribute to the regulation of NMDAR-mediated cognitive function in the healthy brain and impact an array of diseases characterized by cognitive impairment.

The Chumakov et al. (2002) study also demonstrated that SNP markers within the DAO gene may confer an increased vulnerability to schizophrenia, as surveyed in a French Canadian population. This has been independently replicated in a number of subsequent genetic investigations in German, Han Chinese, Irish, American schizophrenia samples (Schumacher et al., 2004; Liu et al., 2004; Corvin et al., 2007; Wood et al., 2007). Furthermore, some studies have indicated epistasis between DAO and G72, where the combined effect of polymorphisms in these genes results in a greater risk of schizophrenia (Chumakov et al., 2002; Corvin et al., 2007). However, negative studies examining DAO have been reported (Shinkai et al., 2007; Yamada et al., 2005), and the evidence for an association between DAO and schizophrenia susceptibility is not as prevalent as that for G72. Similar to G72, functional variants in DAO have yet to be identified.

Preliminary studies have indicated that genetic variation in the SRR gene could contribute to schizophrenia. Investigations examining variants in the promoter and 5′-terminus of SRR have demonstrated significant associations with schizophrenia (Goltsov et al., 2006; Morita et al., 2006; Labrie et al., 2009), contrary to polymorphisms in the central and 3′ region of SRR (Strohmaier et al., 2007; Labrie et al., 2009). Consequently, it is possible that the 5′ end of SRR is of importance in mediating abnormal SRR function in schizophrenia. Accordingly, a significantly associated variant was found to induce a 60% reduction in SRR promoter function (Morita et al., 2006). Furthermore, all SRR markers demonstrating a positive association are in close proximity to exon 1b, the major SRR isoform in the brain (Yamada et al., 2005), suggesting a potential modulation of SRR transcription in the brain.

Pick1, an interacting partner of SRR, has also been described to confer susceptibility to schizophrenia, particularly to the disorganized subtype (Fujii et al., 2006; Hong et al., 2004). The Pick1 gene is found on chromosome 22q13.1, which a genetic locus that has been often linked to schizophrenia (Hong et al., 2004). Pick1 is a scaffolding protein that regulates the subcellular localization and surface expression of a number of binding partners (Dev and Henley, 2006), many of which are relevant to schizophrenia, including glutamate receptors, dopamine transporters, neuregulin, and ErbB tyrosine kinase receptors (Dev and Henley, 2006). Pick1 has also been demonstrated to have an important role in NMDAR-dependent forms of synaptic plasticity (Terasima et al., 2008).

7. Evidence for abnormal modulation of the NMDAR D-serine/glycine site in patients with schizophrenia

Kynurenic acid (KYNA) is the only known endogenous NMDAR D-serine/glycine site antagonist (Erhardt et al., 2009). It also functions as a non-competitive inhibitor of α-7 nicotinic acetylcholine receptors (Hilmas et al., 2001). Elevations in kynurenic acid have been found in the CSF and postmortem brain of schizophrenia patients (Erhardt et al., 2001; Schwarz et al., 2001). Additionally, increased activity of kynurenine aminotransferase-1 (KAT-1), a synthesis enzyme for KYNA, was reported in schizophrenic individuals (Table 1) (Kapoor et al., 2006).

D-Serine levels were found to be reduced in the CSF of drug-naive patients with schizophrenia (Hashimoto et al., 2005b; Bendikov et al., 2007). Serum analysis has also indicated diminished D-serine, along with a concomitant elevation in γ-serine, suggesting a dysfunction of SRR activity (Hashimoto et al., 2003). Indeed, changes in SRR protein expression have been reported in the postmortem hippocampus and cortex of schizophrenic individuals, with some studies indicating a decrease (Bendikov et al., 2007), while others an increase (Table 1) (Stefek et al., 2006; Verrall et al., 2007). Postmortem findings have also demonstrated an elevation in DAO protein in the hippocampus and cerebellum of patients with schizophrenia (Verrall et al., 2007; Bendikov et al., 2007), as well as an increase in cortical DAO activity (Table 1) (Madeira et al., 2008; Kapoor et al., 2006). Further support for abnormalities in the D-serine pathway in schizophrenia is...
indicated by reductions in PICK1 mRNA (Beneyto and Meadow-Woodruff, 2006) and Asc-1 protein (Burnet et al., 2008) in the prefrontal cortex. It has been speculated that lower levels of Asc-1 may be a response to diminished D-serine availability (Burnet et al., 2008), though further investigation is required. Additionally, postmortem findings will benefit from replication with more brain series and regions to determine the extent of the abnormal expression and function of NMDAR δ-serine/glycine site modulators in schizophrenia. Use of biopsied olfactory epithelium from living patients (Sawa and Cascella, 2009) may also reveal molecular changes associated with the NMDAR δ-serine/glycine site, while limiting potential confounds that include the effects of long-term exposure to medications, agonal state, and postmortem interval.

Although conventional antipsychotics do not directly alter δ-serine levels, improvement of schizophrenia symptoms are correlated with an elevation in δ-serine (Ohnuma et al., 2008). A similar effect has been observed for glycine. In medication-free schizophrenia patients, circulating levels of glycine have been found to be reduced and glycine availability inversely correlates with the severity of negative symptoms (Sumiyoshi et al., 2004; Neeman et al., 2005). Treatment efficacy to ameliorate these negative symptoms correlated with a rise in plasma glycine levels (Sumiyoshi et al., 2005). Together, these studies suggest an abnormality in available glycine and/or δ-serine in schizophrenia and predict the potential therapeutic utility of these compounds.

### Table 1

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<tr>
<td>Serine racemase (SRR)</td>
<td>mRNA: NS; protein: ^; NS</td>
<td>mRNA: NS; protein: ^; NS</td>
<td>Protein: ^; ^</td>
<td>mRNA: NS; protein: NS</td>
<td>mRNA: NS; protein: NS</td>
<td>mRNA: NS; protein: NS</td>
<td>Bendikov et al. (2007); Verrall et al. (2007); Steffek et al. (2006); Kapoor et al. (2006)</td>
<td>Bendikov et al. (2007); Verrall et al. (2007); Steffek et al. (2006); Kapoor et al. (2006)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D-amino acid oxidase (DAO)</td>
<td>mRNA: NS; protein: ^; NS</td>
<td>mRNA: NS; protein: ^; NS</td>
<td>Protein: ^</td>
<td>mRNA: NS; protein: NS; activity: ^</td>
<td>mRNA: NS; protein: NS; activity: ^</td>
<td>mRNA: NS; protein: NS; activity: ^</td>
<td>Bendikov et al. (2007); Verrall et al. (2007); Steffek et al. (2006); Kapoor et al. (2006)</td>
<td>Bendikov et al. (2007); Verrall et al. (2007); Steffek et al. (2006); Kapoor et al. (2006)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NS, no significant change; \^, increase; \_\^, decrease; \^\^, trend for increase (near significance).

8. Pharmacological treatments targeting the NMDAR δ-serine/glycine site

Since NMDAR hypoactivity potentiates schizophrenia-like symptoms and is implicated in the pathophysiology of schizophrenia, it may follow that NMDAR activation could alleviate symptoms of this disorder. The NMDAR δ-serine/glycine site has been proposed as a potential therapeutic target, as increasing its activity offers a safer alternative to elevations in glutamate levels that can promote neurotoxicity (Olney, 1994; Coyle, 2006).

To date, clinical trials have been conducted with the partial agonist δ-cycloserine, the full agonists glycine and δ-serine, and the GlyT-1 inhibitor sarcosine (Table 2). Supporting the therapeutic effectiveness of correct modulation of disrupted glutamatergic pathways are clinical reports that activation of mGluR2/3 receptors provides symptomatic improvements in schizophrenia patients without major adverse effects (Patil et al., 2007).

An initial study assessing the clinical efficacy of δ-cycloserine in conjunction with conventional medications observed a U-shaped dose response curve (Goff et al., 1995), since as a partial agonist δ-cycloserine can function as an agonist or antagonist depending on the degree of occupancy at the δ-serine/glycine site (Sheinin et al., 2001). In this study of only 9 patients with doses escalating every 2 weeks, improvements in negative and cognitive deficits were found at an optimal dose (50 mg/day) (Goff et al., 1995). Although the capacity of δ-cycloserine to improve negative symptoms has been replicated in some larger placebo-controlled studies (Heresco-Levy et al., 2002; Goff et al., 1999b), evidence supporting the effectiveness of δ-cycloserine is weak. δ-Cycloserine was not found to be beneficial as adjunctive treatment in a 6-month placebo-controlled trial (Goff et al., 2005), in a large multicenter 16-week trial with a placebo comparison (Buchanan et al., 2007), nor in a systematic review of the literature and meta-analysis (Tuominen et al., 2005; Tuominen et al., 2006). Amidst all these negative findings, one study shows that δ-cycloserine enhances temporal lobe activation in schizophrenia patients during a memory task and that this response is correlated with a significant decrease in negative symptoms (Yurgelun-Todd et al., 2005). However, the overall disparity in findings indicates a limited therapeutic effect of δ-cycloserine in the general patient population.

Clinical trials examining the effects of a high dose of glycine (0.8 g/kg/day) administered as adjuvant treatment have demonstrated promising results, particularly in the amelioration of primary negative symptoms in patients with chronic schizophrenia (Javitt et al., 1994, 2001; Leiderman et al., 1996; Tuominen et al., 2006). Some studies have indicated that glycine may also improve cognitive and positive symptoms (Heresco-Levy et al., 1999, 2004), although these additional benefits are not supported in a meta-analysis (Tuominen et al., 2005, 2006). In contrast, a large multicenter study found that glycine administration did not ameliorate negative or cognitive symptoms compared to placebo treatment (Buchanan et al., 2007). The lack of improvement in this trial may be related to the higher percentage of patients treated with second-generation antipsychotics rather than conventional antipsychotics. Additionally, serum levels of glycine in this trial were lower compared to those in some of the previous positive studies. Though glycine is generally well-tolerated with minimal serious side effects, glycine treatment has been associated with reoccurrent gastrointestinal upset (Heresco-Levy et al., 2004; Diaz et al., 2005). In addition, glycine can be converted to δ-serine, which can subsequently lead to the biosynthesis of δ-serine. Consequently, a rise in δ-serine may contribute to the therapeutic effects of large doses of glycine. Accordingly, clinical trials administering glycine treatments to schizophrenia patients have reported an elevation serum serine levels (Heresco-Levy et al., 1999, 2004; Javitt et al., 2001).
In a preliminary study, D-serine (30 mg/kg) in combination with antipsychotic drugs was found to be therapeutically beneficial, as it considerably reduced positive, negative, and cognitive symptoms of schizophrenia (Tsai et al., 1998). These ameliorative effects were confirmed in a subsequent clinical trial in which D-serine was added to risperidone or olanzapine, and improvements in positive, negative, cognitive, and depressive symptoms were found in treatment-resistant patients (Heresco-Levy et al., 2005). However, in patients with an acute exacerbation of psychosis, D-serine did not produce any benefits beyond risperidone monotherapy (Lane et al., 2005).

### Table 2

Modulators of the NMDAR D-serine/glycine site in clinical trials with schizophrenia patients.

<table>
<thead>
<tr>
<th>Clinical trial</th>
<th>Design</th>
<th>Daily dose</th>
<th>Sample size</th>
<th>Treatment duration</th>
<th>Patient sample</th>
<th>Antipsychotics</th>
<th>Therapeutic effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>D-Cycloserine</td>
<td>Open label</td>
<td>250 mg</td>
<td>7</td>
<td>6 weeks</td>
<td>Chronic</td>
<td>Typical</td>
<td>+, −, gen psychopath</td>
</tr>
<tr>
<td>Goff et al. (1995)</td>
<td>Single blind</td>
<td>5–250 mg</td>
<td>9</td>
<td>10 weeks (2 weeks/dose)</td>
<td>Chronic</td>
<td>Typical</td>
<td>− at 50 mg</td>
</tr>
<tr>
<td>Rosse et al. (1996)</td>
<td>Double-blind, placebo-controlled</td>
<td>10</td>
<td>13</td>
<td>4 weeks</td>
<td>Chronic</td>
<td>Molindone</td>
<td>NS</td>
</tr>
<tr>
<td>van Berckel et al. (1996)</td>
<td>Single blind</td>
<td>15–250 mg</td>
<td>7</td>
<td>24 days (4 days/dose)</td>
<td>Not specified</td>
<td>Drug-free</td>
<td>− at 100 mg</td>
</tr>
<tr>
<td>Heresco-Levy et al. (1998)</td>
<td>Double-blind, placebo-controlled</td>
<td>50 mg</td>
<td>9</td>
<td>6 weeks</td>
<td>Chronic, treatment-resistant</td>
<td>Typical/atypical</td>
<td>−</td>
</tr>
<tr>
<td>Goff et al. (1999b)</td>
<td>Double-blind, placebo-controlled</td>
<td>50 mg</td>
<td>38</td>
<td>8 weeks</td>
<td>Chronic, prominent negative symptoms</td>
<td>Typical</td>
<td>+, gen psychopath</td>
</tr>
<tr>
<td>van Berckel et al. (1999)</td>
<td>Double-blind, placebo-controlled</td>
<td>100 mg</td>
<td>25</td>
<td>8 weeks</td>
<td>Treatment-resistant</td>
<td>Typical</td>
<td>−</td>
</tr>
<tr>
<td>Heresco-Levy et al. (2002)</td>
<td>Double-blind, placebo-controlled</td>
<td>50 mg</td>
<td>16</td>
<td>6 weeks</td>
<td>Prominent negative symptoms</td>
<td>Typical</td>
<td>NS</td>
</tr>
<tr>
<td>Duncan et al. (2004a)</td>
<td>Double-blind, placebo-controlled</td>
<td>50 mg</td>
<td>22</td>
<td>4 weeks</td>
<td>Prominent negative symptoms</td>
<td>Typical</td>
<td>NS</td>
</tr>
<tr>
<td>Goff et al. (2005)</td>
<td>Double-blind, placebo-controlled</td>
<td>50 mg</td>
<td>26</td>
<td>6 months</td>
<td>Prominent negative symptoms</td>
<td>Typical/atypical</td>
<td>−</td>
</tr>
<tr>
<td>Buchanan et al. (2007)</td>
<td>Double-blind, placebo-controlled</td>
<td>50 mg</td>
<td>133</td>
<td>16 weeks</td>
<td>Prominent negative symptoms</td>
<td>Typical/atypical</td>
<td>−</td>
</tr>
<tr>
<td>Goff et al. (2008)</td>
<td>Double-blind, placebo-controlled</td>
<td>50 mg once/week</td>
<td>33</td>
<td>8 weeks</td>
<td>Chronic</td>
<td>Typical/atypical</td>
<td>−, cognitive</td>
</tr>
<tr>
<td>Glycine</td>
<td>Open label</td>
<td>5–25 g</td>
<td>11</td>
<td>8–9 months</td>
<td>Chronic</td>
<td>Drug-free</td>
<td>4 patients</td>
</tr>
<tr>
<td>Rosse et al. (1989)</td>
<td>Open label</td>
<td>10.8 g</td>
<td>6</td>
<td>4 days–8 weeks</td>
<td>Chronic</td>
<td>Typical</td>
<td>2 patients; −</td>
</tr>
<tr>
<td>Costa et al. (1990)</td>
<td>Open label</td>
<td>15 g</td>
<td>6</td>
<td>5 weeks</td>
<td>Treatment-resistant</td>
<td>Typical</td>
<td>−</td>
</tr>
<tr>
<td>Javitt et al. (1994)</td>
<td>Double-blind, placebo-controlled</td>
<td>30 g</td>
<td>14</td>
<td>8 weeks</td>
<td>Chronic</td>
<td>Typical</td>
<td>−</td>
</tr>
<tr>
<td>Leiderman et al. (1996)</td>
<td>Open label</td>
<td>60 g</td>
<td>5</td>
<td>8 weeks</td>
<td>Chronic</td>
<td>Typical</td>
<td>−</td>
</tr>
<tr>
<td>Heresco-Levy et al. (1996)</td>
<td>Double-blind, placebo-controlled</td>
<td>60 g</td>
<td>11</td>
<td>6 weeks</td>
<td>Chronic, treatment-resistant</td>
<td>Typical</td>
<td>−</td>
</tr>
<tr>
<td>Heresco-Levy et al. (1999)</td>
<td>Double-blind, placebo-controlled</td>
<td>60 g</td>
<td>19</td>
<td>6 weeks</td>
<td>Chronic, treatment-resistant</td>
<td>Typical</td>
<td>−</td>
</tr>
<tr>
<td>Javitt et al. (2001)</td>
<td>Double-blind, placebo-controlled</td>
<td>60 g</td>
<td>12</td>
<td>6 weeks</td>
<td>Chronic</td>
<td>Typical</td>
<td>−</td>
</tr>
<tr>
<td>Heresco-Levy et al. (2004)</td>
<td>Double-blind, placebo-controlled</td>
<td>60 g</td>
<td>14</td>
<td>6 weeks</td>
<td>Chronic, treatment-resistant</td>
<td>Olanzapine/risperidone</td>
<td>+, −, cognitive</td>
</tr>
<tr>
<td>Buchanan et al. (2007)</td>
<td>Double-blind, placebo-controlled</td>
<td>60 g</td>
<td>133</td>
<td>16 weeks</td>
<td>Prominent negative symptoms</td>
<td>Typical</td>
<td>−</td>
</tr>
<tr>
<td>D-Serine</td>
<td>Tsai et al. (1998)</td>
<td>Double-blind, placebo-controlled</td>
<td>30 mg/kg</td>
<td>28</td>
<td>6 weeks</td>
<td>Prominent negative symptoms, PDS, treatment-resistant</td>
<td>Typical/atypical/risperidone</td>
</tr>
<tr>
<td>Lane et al. (2005)</td>
<td>Double-blind, placebo-controlled</td>
<td>2 g</td>
<td>57</td>
<td>6 weeks</td>
<td>Acutely ill</td>
<td>Risperidone</td>
<td>NS</td>
</tr>
<tr>
<td>Heresco-Levy et al. (2005)</td>
<td>Double-blind, placebo-controlled</td>
<td>30 mg/kg</td>
<td>38</td>
<td>6 weeks</td>
<td>Treatment-resistant</td>
<td>Risperidone/olanzapine</td>
<td>+, −, cognitive</td>
</tr>
<tr>
<td>Sarcosine</td>
<td>Tsai et al. (2004a)</td>
<td>Double-blind, placebo-controlled</td>
<td>2 g</td>
<td>36</td>
<td>6 weeks</td>
<td>Chronic</td>
<td>Typical/risperidone</td>
</tr>
<tr>
<td>Lane et al. (2005)</td>
<td>Double-blind, placebo-controlled</td>
<td>2 g</td>
<td>57</td>
<td>6 weeks</td>
<td>Acutely ill</td>
<td>Risperidone</td>
<td>−</td>
</tr>
<tr>
<td>Lane et al. (2008)</td>
<td>Double-blind, placebo-controlled</td>
<td>1, 2 g</td>
<td>16</td>
<td>6 weeks</td>
<td>Acutely ill</td>
<td>Drug-free/risperidone</td>
<td>−</td>
</tr>
</tbody>
</table>

| | | | | | | | |
| −, improvement; +, exacerbation; NS, no significant change. | Abbreviations: +, positive symptoms; −, negative symptoms; gen psychopath, general psychopathology; all, +/−/cognitive symptoms/general psychopathology; PDS, primary deficit syndrome. | | | |
| a Number of patients that completed the trial. |
et al., 2005). The lack of beneficial effects could be related to differential treatment responses in acutely ill patients compared to treatment-resistant individuals and indicates that D-serine may be less effective in treating psychotic symptoms. A meta-analysis of clinical trials with D-serine and glycine indicated that these compounds improved negative symptoms of schizophrenia when administered as adjuvant therapies, but determined that these effects were not fully consistent and required further replication (Tuominen et al., 2006). Overall, D-serine was well-tolerated and devoid of significant side effects (Tsai et al., 1998; Lane et al., 2005; Heresco-Levy et al., 2005). One concern with D-serine treatments has been renal toxicity, since large doses of D-serine have been found to cause reversible acute tubular necrosis in rats (Carone et al., 1985). The nephrotoxic effects of D-serine were not observed in mice, guinea pigs, rabbits, dogs, and gerbils (Kaltenbach et al., 1979) and analysis of kidney function parameters did not reveal any abnormalities in the clinical trials (Tsai et al., 1998; Lane et al., 2005; Heresco-Levy et al., 2005).

As an alternative to directly activating the NMDAR D-serine/glycine site, clinical investigations also examined sarcosine, an inhibitor of GlyT-1 that effectively raises synaptic levels of glycine (Bergeron et al., 1998). Sarcosine (2 g/day) cotreatment with conventional medications or risperidone significantly reduced positive, negative, cognitive, and general psychopathology symptoms in patients with stable chronic schizophrenia and in patients with acute exacerbation of schizophrenia (Tsai et al., 2004a; Lane et al., 2005). Recently, the effectiveness of sarcosine was examined in a drug-free cohort displaying acute psychotic symptoms; the first clinical trial examining a glycine reuptake inhibitor in absence of other medications (Lane et al., 2008). Though symptomatic amelioration was observed, it was limited to a small patient subgroup with no previous antipsychotic exposure and no placebo control was conducted (Lane et al., 2008). Larger studies with placebo or active comparators will be necessary to determine the therapeutic benefits of NMDAR D-serine/glycine site agonists as first-line antipsychotics.

A peculiarity of NMDAR D-serine/glycine site agonists is that although ameliorative effects are found in combination with second-generation antipsychotics, such as risperidone and olanzapine, these treatments are ineffective when administered in conjunction with clozapine (Table 3) (Tsai et al., 1999; Lane et al., 2006; Goff et al., 1996). Clozapine is a commonly used atypical antipsychotic with a broad pharmacological profile that includes affinity for D1- and D2-like dopamine receptors and 5-HT2 receptors (Ross et al., 2006). Also, clozapine has been shown to increase extracellular levels of glutamate (Melone et al., 2001), potentiate NMDAR-mediated synaptic transmission (Arvanov et al., 1997), reverse antagonist blockade of NMDAR channels in vivo (Bressan et al., 2005), normalize PCP-induced neuronal hyperactivity in the prefrontal cortex (Kargieman et al., 2008), and attenuate behavioral deficits induced by NMDAR inhibitors (Gaisler-Salomon and Weiner, 2003; Lipina et al., 2005). Thus, clozapine may augment NMDAR activity to an extent that impedes further enhancements with D-serine/glycine site agonists. Additionally, clozapine may augment synaptic levels of glycine by inhibiting glycine reuptake into neurons and glia (Javitt et al., 2005; Williams et al., 2004).

Overall, clinical trials with D-serine/glycine site activators have indicated therapeutic potential, particularly D-serine and sarcosine. However, thus far, studies are generally conducted with a relatively low number of participants (<30) over a 6 week period (Tables 2 and 3). Consequently, longer studies investigating a greater number of patients are required to ascertain the benefits of D-serine/glycine site agonists. Also, since the majority of studies examine treatment-resistant patients with stable chronic symptoms, assessment of these proposed antipsychotics in a more diverse patient population may reveal additional improvements. Regardless, clinical trials to date demonstrate promising ameliorative effects and warrant further investigation with agonists of the NMDAR D-serine/glycine site. Compounds targeting the D-serine/glycine site may offer a novel and well-tolerated alternative for the treatment of schizophrenia.

In addition, investigations that examine how direct and indirect agonists of the NMDAR D-serine/glycine site affect the tonic and phasic components of NMDAR activation will be important in determining the consequences and safety of long-term administration of these compounds. Exogenous D-serine or glycine administration could potentially increase tonic extracellular D-serine/glycine levels. Alternatively, exogenous D-serine or glycine could be rapidly cleared from the synaptic space by reuptake transporters, leading to increased intracellular stores and enhanced agonist release during phasic stimulation. Furthermore, inhibition of GlyT-1 may facilitate tonic NMDAR activation, although this would be dependent on the rate of synaptic efflux.

Table 3
Clinical trials with modulators of the NMDAR D-serine/glycine site added to clozapine treatment.

<table>
<thead>
<tr>
<th>Clinical trial</th>
<th>Design</th>
<th>Daily dose</th>
<th>Sample size*</th>
<th>Duration</th>
<th>Patient sample</th>
<th>Therapeutic effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>D-serine</td>
<td>Single blind</td>
<td>5–250 mg</td>
<td>10</td>
<td>10 weeks (2 weeks/dose)</td>
<td>Primary deficit syndrome</td>
<td>↓: – at 50 mg</td>
</tr>
<tr>
<td>Goff et al. (1996)</td>
<td>Double-blind, placebo-controlled</td>
<td>50 mg</td>
<td>11</td>
<td>6 weeks</td>
<td>Prominent negative symptoms</td>
<td>↓: –</td>
</tr>
<tr>
<td>Glycine</td>
<td>Potkin et al. (1999)</td>
<td>Double-blind, placebo-controlled</td>
<td>30 g</td>
<td>19</td>
<td>12 weeks</td>
<td>Chronic, treatment-resistant</td>
</tr>
<tr>
<td>Evins et al. (2000)</td>
<td>Double-blind, placebo-controlled</td>
<td>60 g</td>
<td>27</td>
<td>8 weeks</td>
<td>Prominent negative symptoms</td>
<td>NS</td>
</tr>
<tr>
<td>Diaz et al. (2005)</td>
<td>Double-blind, placebo-controlled</td>
<td>60 g</td>
<td>12</td>
<td>12 weeks</td>
<td>Treatment-resistant</td>
<td>NS</td>
</tr>
<tr>
<td>Sarcosine</td>
<td>Tsai et al. (1999)</td>
<td>Double-blind, placebo-controlled</td>
<td>30 mg/kg</td>
<td>20</td>
<td>6 weeks</td>
<td>Prominent negative symptoms, PDS, treatment-resistant</td>
</tr>
<tr>
<td>Lane et al. (2006)</td>
<td>Double-blind, placebo-controlled</td>
<td>2 g</td>
<td>20</td>
<td>6 weeks</td>
<td>PDS, treatment-resistant</td>
<td>NS</td>
</tr>
</tbody>
</table>

* Number of patients that completed the trial.
and influx of glycine originating from astroglial and neuronal cells. Passive diffusion of glycine from the CSF could also contribute to increased tonic NMDAR stimulation in the presence of an GlyT-1 antagonist (Supplisson and Bergman, 1997). Prolonged NMDAR stimulation can potentiate neuronal death (Hardingham and Bading, 2003) and studies have demonstrated that excessive D-serine/glycine site activation may contribute to excitotoxicity and neuroinflammation in several neurodegenerative diseases (Sasabe et al., 2007; Wu et al., 2004; Wolosker et al., 2008). However, the possibility that chronic exposure to D-serine/glycine site agonists produce such effects in patients with schizophrenia (that are proposed to have limited D-serine/glycine site occupancy) is presently unknown.

Enhanced activity of the NMDAR D-serine/glycine site may also be beneficial for the treatment of other psychiatric disorders, including several anxiety syndromes and drug addiction, by augmenting the extinction of learned behaviors (Davis et al., 2006). Extinction is considered to be a form of inhibitory learning, whereby acquired behavioral responses are suppressed following the repetitive exposure to a conditioned stimulus in the absence of a reinforcing (unconditioned) stimulus (Davis et al., 2006). Studies of fear extinction in rats found that administration of d-cycloserine accelerated the effects of extinction and that this acceleration could be blocked by an NMDAR D-serine/glycine site antagonist (Walker et al., 2002). Since fear extinction in rodents is similar to exposure-based psychotherapy in the humans, translational research from preclinical to clinical studies was initiated to examine the therapeutic effects of d-cycloserine (Norberg et al., 2008). The initial study examined individuals with a fear of heights (acrophobia) and found that compared to placebo, d-cycloserine enhanced the effects of virtual reality exposure therapy, resulting in a larger reduction of acrophobic symptoms at 1 week and 3 months following treatment (Ressler et al., 2004). d-Cycloserine was effective when given as single doses prior to the exposure sessions (Ressler et al., 2004), consistent with findings in animal studies demonstrating that d-cycloserine is better able to enhance extinction learning when administered acutely (Parnas et al., 2005; Davis et al., 2006). Subsequent placebo-controlled studies in patients with obsessive-compulsive disorder (Kushner et al., 2007; Wilhelm et al., 2008) and social phobia (Hofmann et al., 2006; Guastella et al., 2008) have confirmed the ability of d-cycloserine to augment the effects of exposure therapy. Additionally, extinction of the preference for a cocaine-associated environment was facilitated by d-cycloserine (Botreau et al., 2006), suggesting that this partial agonist of the NMDAR D-serine/glycine site aids in extinguishing craving behaviors related to drug addiction. From these studies with d-cycloserine, it is not clear whether fear extinction and exposure therapy are facilitated by the augmentation of NMDAR responses during extinction or by the reduction of NMDAR activity during the memory consolidation process.

9. Animal models based on diminished NMDAR function

Consistent with the psychotomimetic effects of NMDAR antagonists in humans, non-competitive inhibition of the NMDAR in animals produces a range of behavioral impairments that are reminiscent of the symptoms of schizophrenia (Bubeniková-Valesová et al., 2008). Behavioral abnormalities following acute or chronic treatment with an NMDAR antagonist in rodents include locomotor hyperactivity, information-processing disturbances, social approach impairments, and deficits in reversal learning and working memory (Nilsson et al., 1957; Yee et al., 2004; van der Meulen et al., 2003; Ellenbroek and Cools, 2000; Bubeniková-Valesová et al., 2008). Similarly, administration of PCP in non-human primates produces impairments in prepulse inhibition (Linn et al., 2003), social behaviors (Mao et al., 2008), and working memory (Thompson et al., 1987). Chronic PCP treatment followed by a withdrawal period has also been shown to induce enduring cognitive deficits, along with reduced dopaminergic utilization in the prefrontal cortex (Jentsch et al., 1997). Furthermore, repeated exposure to PCP has been shown to induce neurodegeneration throughout the brain (Ellison and Switzer, 1993), which may resemble some of the neuroanatomical changes associated with schizophrenia (Beckmann, 1999; Harrison and Weinberger, 2005).

To address neurodevelopmental aspects of schizophrenia, models that involve perinatal treatment with NMDAR antagonists have been developed (Bubeniková-Valesová et al., 2008). These models test the hypothesis that viral or environmental insults occurring during the late second trimester of pregnancy, a period important to the development of the fetal CNS, subsequently increases the likelihood of developing schizophrenia in adulthood (Beckmann, 1999). In rats, the corresponding period is in the first 2 weeks of postnatal life (Clancy et al., 2001), and administration of NMDAR antagonists during this time results in an increase in transient neuronal apoptosis (Ikonomidou et al., 1999). Early exposure to NMDAR inhibitors leads to the emergence of several schizophrenia-related behaviors in adult animals, including deficits in information-processing (Wedzony et al., 2008), enhanced sensitivity to NMDAR blockers and dopamine-releasing stimulants (Abekawa et al., 2007; Wedzony et al., 2005), and impairments in working and reference memory (Andersen and Pouzet, 2004). Additionally, perinatal administration of NMDAR antagonists induces enduring alterations in hippocampal NR1 subunit expression (Harris et al., 2003), mesolimbic dopamine receptor binding (du Bois et al., 2008), synaptogenesis in the hippocampus (Bellinger et al., 2002), and decreases the number of cortical parvalbumin-positive GABAergic neurons in adult rats (Abekawa et al., 2007). Recently, oligodendrocyte differentiation and myelination have also been found to be affected by PCP treatment in developing rats (Lindahl et al., 2008), consistent with the white matter abnormalities that have been observed in schizophrenia (Harrison and Weinberger, 2005). Thus, early changes in NMDAR function produce lasting changes relevant to schizophrenia pathophysiology.

Since schizophrenia is a heritable disease proposed to involve abnormalities in the glutamatergic system, genetic animal models of aberrant NMDAR function have been established. These models also have the advantage of reproducing the chronic and developmental nature of NMDAR hypofunction theorized to occur in schizophrenia. Mice with complete and global loss of the NMDA-NR1 subunit die neonatally (Forrest et al., 1994). However, targeted ablation of the NR1 subunit in the dentate gyrus of the hippocampus results in spatial working memory impairments (Niewoehner et al., 2007), while loss of NR1 in hippocampal CA3 pyramidal cells results in deficient associative memory recall in adult mice (Table 4) (Nakazawa et al., 2002). Mice with a 95% reduction in normal levels of NR1 show abnormalities in CNS development, disrupted sensorimotor gating, impaired social and sexual interactions, and increased spontaneous locomotor activity, stereotypy, and sensitivity to amphetamine (Table 4) (Elberger and Deng, 2003; Mohn et al., 1999; Moy et al., 2006). Alternatively, overexpression of the NMDA receptor composed of NR1-NR2B subunits in the mouse forebrain enhances NMDAR-dependent synaptic potentiation and produces improvements in learning and memory (Tang et al., 1999).

Although these studies support that disturbances in the NMDAR produce phenotypes that are potentially relevant to the symptoms of schizophrenia, they do not directly examine the effects of modulating the NMDAR D-serine/glycine site. Considering the growing evidence indicating abnormal D-serine availability and NMDAR D-serine/glycine site function in the pathophysiology
of schizophrenia (as described earlier), animal models investigating the effects of decreased occupancy and activation of the NMDAR D-serine/glycine site may be more proximal to the neural changes proposed to occur in schizophrenia. Furthermore, examination of such animal models may uncover novel targets for the treatment of this disease.

10. Animal models relevant to the NMDAR D-serine/glycine site

Several genetic animal models examining the effects of aberrant function of the NMDAR D-serine/glycine site and its modulatory enzymes have been developed (Table 5). Grin1<sup>D481N</sup> mutant mice with a five-fold reduction in NMDAR D-serine/glycine site affinity display behavioral abnormalities that include persistent latent inhibition (LI) and impairments in sociability, spatial recognition, learning, and memory (Labrie et al., 2008a; Duffy et al., 2008b). LI persistence is also seen in mice administered the highly selective NMDAR D-serine/glycine site antagonist L-701,324 (Labrie et al., 2008a) and the NMDAR channel blocker MK-801 (Lipina et al., 2005). Prolonged LI has been associated with the deficits in information-processing and cognitive flexibility observed in schizophrenia patients (Weiner, 2003; Moser et al., 2000) and has been correlated with the severity of negative symptoms (Rascle et al., 2001; Cohen et al., 2004). Furthermore, sociability deficits in animals, as seen in the Grin1<sup>D481N</sup> mice, resembles the social withdrawal aspect of the negative symptoms of schizophrenia (Ellenbroek and Cools, 2000). Impaired sociability is also seen in mice carrying an N-nitroso-N-ethylurea (ENU)-induced point mutation in the SrrY<sup>269</sup>* gene that results in a loss of DAO activity (Labrie et al., 2008a; Duffy et al., 2008b). DAO inactivation and augmented D-serine concentrations may normalize NMDAR function and cognitive inflexibility in schizophrenia. Moreover, the improvements in reversal learning and extinction imply that DAO inhibition and D-serine may be beneficial for the treatment of many other psychopathologies.
involving persistent maladaptive behaviors, such as obsessive-compulsive disorder and post-traumatic stress syndrome. Though D-serine and similar compounds have demonstrated promising effects in clinical trials with medicated schizophrenia patients (Heresco-Levy et al., 2005; Tsai et al., 1998; Coyle, 2006), there are issues regarding the administration of these compounds, as large doses are required for therapeutic effect. In contrast, DAO inhibitors cross the blood-brain-barrier readily (Adage et al., 2008; Hashimoto et al., 2009). To date several compounds that inhibit DAO have therapeutically potential. However, these investigations are in vitro and in vivo inhibitor of DAO activity, in addition to its established action as a D2 receptor antagonist (Yagi et al., 1956). Interestingly, the typical antipsychotic chlorpromazine has been shown to be a potent in vitro and in vivo inhibitor of DAO activity, in addition to its established action as a D2 receptor antagonist (Yagi et al., 1956). Together, these findings support that inhibition of DAO function may have therapeutically potential. However, these investigations are still at a preclinical stage and whether DAO inhibitors are capable of alleviating disease symptoms in patients has yet to be examined.

Recently, transgenic mice carrying the human G72 genomic region have been developed (Otte et al., 2009). In the brains of these mice, G72 transcripts were most abundant in the cerebellum, hippocampus, cortex, and olfactory bulb, while in the periphery G72 was elevated in the heart and spleen (Otte et al., 2009). Several phenotypes relevant to psychiatric disease were displayed in the G72-expressing mice, including sensorimotor gating disruption, enhanced sensitivity to PCP, and an increase in compulsive behaviors (Otte et al., 2009). Further analysis of these mice may

<table>
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<th>Gene</th>
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<tr>
<td>NR1</td>
<td>NMDAR subunit with D-serine/glycine binding site</td>
<td>Grin1&lt;sup&gt;188fs11&lt;/sup&gt; (5-fold reduction in glycine affinity)</td>
<td>Persistent LI; impairments in sociability, spatial object recognition, spatial reference learning and memory; behavioral deficits reversible by D-serine or clozapine; reduced anxiety; increased startle reactivity</td>
<td>Labrie et al. (2008a), Krew et al. (2000)</td>
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<td>Grin1&lt;sup&gt;188fs11/R449S&lt;/sup&gt; (biphasic NMDAR glycine affinity: 6 and 90-fold reductions)</td>
<td>Hyperactivity and increased stereotypy that is resistant to antipsychotics and zolpidem; resistance to MK-801-induced hyperactivity; increased startle reactivity; impaired nest building and performance in motor tests and in a visible platform learning task; striatal dopaminergic and serotonergic hyperfunction</td>
<td>Ballard et al. (2002)</td>
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<td>Serine racemase (SRR)</td>
<td>D-serine synthesis</td>
<td>Srr&lt;sup&gt;−/−&lt;/sup&gt; (exon 1 KO; loss of SRR and ~89% reduction in D-serine)</td>
<td>Males show open-field hyperactivity and impaired spatial reference memory; females show increased anxiety and startle reactivity</td>
<td>Basu et al. (2009)</td>
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<td>Srr&lt;sup&gt;Y259F&lt;/sup&gt; (loss of SRR activity and ~55% reduction in D-serine)</td>
<td>Deficits in PPI, sociability, spatial object recognition, spatial reference memory; behavioral deficits reversible by D-serine or clozapine; increased sensitivity to MK-801-induced hyperactivity and PPI deficits</td>
<td>Labrie et al. (2009)</td>
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<td>D-amino acid oxidase (DAO)</td>
<td>D-serine catabolism</td>
<td>Dao&lt;sup&gt;C185SR&lt;/sup&gt; (loss of DAO activity)</td>
<td>Improved spatial reversal learning and extinction (C57BL/6J background)</td>
<td>Labrie et al. (2008b)</td>
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<td>G72tg (expression of primate-specific LG72 protein in mouse)</td>
<td>Impaired PPI that is reversible by haloperidol; deficits in motor coordination and olfactory discrimination; increased compulsive behaviors (more grooming and females exhibit increased digging behavior); decreased aggression in males; increased sensitivity to PCP-induced hyperactivity</td>
<td>Otte et al. (2009)</td>
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<tr>
<td>G72</td>
<td>Regulator of D-amino acid oxidase</td>
<td>G72tg (expression of primate-specific LG72 protein in mouse)</td>
<td>Improved spatial reversal learning and extinction (C57BL/6J background)</td>
<td>Labrie et al. (2008b)</td>
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<td>Glycine transporter 1 (GlyT-1)</td>
<td>Glycine reuptake transporter</td>
<td>GlyT1&lt;sup&gt;+/−&lt;/sup&gt; (50% loss of GlyT-1 function and glycine uptake)</td>
<td>Enhanced spatial reference memory; resistance to amphetamine-induced PPI deficit; increased sensitivity to MK-801-induced PPI disruption</td>
<td>Tsai et al. (2004b)</td>
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<td>CamKIIserCre:Glyt1tm1.2fl/fl (GlyT-1 KO in forebrain neurons)</td>
<td>Improved associative learning, LI, spatial and object recognition memory; resistance to PCP-induced hyperactivity</td>
<td>Yee et al. (2006), Singer et al. (2007)</td>
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<td>Alanine-serine-cysteine transport (Asc-1)</td>
<td>D-serine reuptake</td>
<td>Asc&lt;sup&gt;1&lt;/sup&gt;-/- (Asc-1 KO)</td>
<td>Tremors, ataxia, seizures, and early postnatal death</td>
<td>Xie et al. (2005)</td>
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Abbreviations: KO, knockout; PPI, prepulse inhibition; LI, latent inhibition.
uncover the molecular and cellular functions of G72 in vivo and clarify the mechanism by which G72 induces behavioral disturbances in the mouse.

Homoygous deletion of all GlyT-1 subtypes in mice results in severe motor and respiratory deficits leading to death on the first postnatal day (Gomez et al., 2003). However, heterozygote GlyT-1 knockout mice are fully viable (Tsai et al., 2004b), as are mice with a selective elimination of GlyT-1 in the forebrain (Yee et al., 2006). Reduced GlyT-1 expression led to a corresponding increase in glycine availability and augmented NMDAR-evoked excitatory postsynaptic currents in hippocampal slices (Yee et al., 2006; Tsai et al., 2004b). Lower levels of GlyT-1 also enhanced cognitive performance in several behavioral tasks. Heterozygote GlyT-1 knockout mice demonstrated greater spatial memory retention and a decreased sensitivity to amphetamine-induced disruption of sensorimotor gating (Tsai et al., 2004b). Additionally, mice with a loss of forebrain GlyT-1 showed improvements in LI, spatial and object recognition memory, and a resistance to PCP-induced hyperactivity (Yee et al., 2006; Singer et al., 2007). The procognitive object recognition memory, and a resistance to PCP-induced loss of forebrain GlyT-1 showed improvements in LI, spatial and a selective elimination of GlyT-1 in the forebrain (Yee et al., 2006). Knockout mice are fully viable (Tsai et al., 2004b), as are mice with postnatal day (Gomeza et al., 2003). However, heterozygote GlyT-1 severe motor and respiratory deficits leading to death on the first sensorimotor gating (Tsai et al., 2004b). Additionally, mice with a loss of forebrain GlyT-1 showed improvements in LI, spatial and object recognition memory, and a resistance to PCP-induced hyperactivity (Yee et al., 2006; Singer et al., 2007). The procognitive object recognition memory, and a resistance to PCP-induced loss of forebrain GlyT-1 showed improvements in LI, spatial and a selective elimination of GlyT-1 in the forebrain (Yee et al., 2006). Knockout mice are fully viable (Tsai et al., 2004b), as are mice with postnatal day (Gomeza et al., 2003). However, heterozygote GlyT-1 severe motor and respiratory deficits leading to death on the first sen

11. Comparison of animal models examining genes related to the NMDAR $\delta$-serine/glycine site and other genetic models based on schizophrenia susceptibility genes

Since schizophrenia is a complex disorder involving both genetic and environmental factors, manipulation of one genetic or neurochemical pathway is unlikely to replicate all aspects of the disease. Instead, targeting specific pathways may reveal their importance in specific clinical features associated with schizophrenia. Overall, marked reduction in the function of the NMDAR $\delta$-serine/glycine site and in the activity of $\delta$-serine modulatory enzymes appear to consistently recapitulate some of the neurocognitive abnormalities and negative symptoms characteristic of schizophrenia. Deficits in spatial learning and memory as well as social interactions are particularly apparent, although this may reflect the choice in assessed behavior and the limitations in valid behavioral paradigms. Models affecting NMDAR $\delta$-serine/glycine site activity also demonstrate an altered sensitivity to psychotropic agents and are responsive to antipsychotic treatment. However, consistent with being a genetically heterogeneous disease, many other candidate susceptibility genes have been implicated in the pathophysiology of schizophrenia (Harrison and Weinberger, 2005; Ross et al., 2006), suggesting the involvement of a synergistic interaction of several risk genes and neurotransmitter systems.

Discoveries in human genetic studies have fueled the development of several genetic mouse models based on schizophrenia susceptibility genes. Among the candidate risk genes, catechol-O-methyltransferase (COMT) is the only one known associate a functional mutation with increased vulnerability for schizophrenia. COMT is involved in dopamine metabolism and the val158met polymorphism in COMT affects enzymatic activity (Lachman et al., 1996; Chen et al., 2004a). Carriers for the Met allele have the lowest COMT activity, resulting in reduced dopamine degradation (Chen et al., 2004a). In addition, the Met allele has been associated with a more efficient activation of the prefrontal cortex and improved cognitive performance in tasks dependent on prefrontal cortical function in healthy and schizophrenic individuals (Sheldrick et al., 2008; Heinz and Smolka, 2006). Mice that overexpress the human COMT–Val variant demonstrated deficits in attentional set-shifting and impairments in object recognition and working memory, along with diminished stress responses and pain sensitivity (Papaleo et al., 2008). In contrast, COMT knockout mice have an increase in dopamine in the frontal cortex and improved working memory, but elevated stress responses and pain sensitivity (Papaleo et al., 2008; Gogos et al., 1998). Consequently, these findings indicate that the COMT val158met polymorphism may be involved in abnormal cognitive processing and stress reactivity in diverse clinical disorders and may influence natural variation in cognition and stress in healthy populations.

The COMT gene is located on the 22q11 locus, which is a region that has been linked to greater schizophrenia risk (Ross et al., 2006; Harrison and Weinberger, 2005). Deletions in this chromosomal region are associated with velocardiofacial syndrome, a disease accompanied with an increased prevalence of psychotic symptoms (Ross et al., 2006; Harrison and Weinberger, 2005). The proline dehydrogenase (PRODH) gene is also located in the 22q11 region. Studies have identified a significant association of PRODH with schizophrenia vulnerability (Li et al., 2004; Kempf et al., 2008) and the presence of missense mutations in this gene in patients with schizophrenia (Jacquet et al., 2002). PRODH is involved in the catalysis of proline, which in turn affects glutamate availability and acetylcholine function in the cortex (Delwingle et al., 2007, 2003). Mice with a deficiency in PRODH exhibit aberrant glutamatergic transmission, deficits in sensorimotor gating and associative memory, and exaggerated responses to stress and amphetamine (Paterlini et al., 2005; Gogos et al., 1999). Additionally, an epistatic interaction between the PRODH and COMT genes was demonstrated in these mice, as reductions in PRODH resulted in an upregulation of COMT mRNA in the frontal cortex and altered behavioral responses to a COMT inhibitor (Paterlini et al., 2005).

Neuregulin-1 (NRG1) was originally identified as a candidate gene following fine-mapping of a locus on chromosome 8p, a region that has been frequently linked to schizophrenia (Stefansson et al., 2002). Numerous studies have since confirmed the association of NRG1 with greater schizophrenia risk (Harrison and Weinberger, 2005) and a variant in the NRG1 promoter region has been associated with decreased cortical activation, the development of psychotic symptoms, and reduced premorbid IQ (Hall et al., 2006). NRG1 has a range of roles in the development and function of the nervous system, affecting neuronal migration and differentiation, synapse formation, glial proliferation, synaptic plasticity, and neurotransmitter receptor expression and function (Esper et al., 2006). Furthermore, the NRG1 receptor ErbB4 is critically involved in glutamatergic synapse maturation and signaling (Li et al., 2007a). Mice with heterozygous deletions in selective domains of NRG1 demonstrate locomotor hyperactivity, altered exploratory behaviors, disrupted sensorimotor gating and
L1, and are responsive to antipsychotics (Stefansson et al., 2002; Duffy et al., 2008a; Rimer et al., 2005). In rodents, locomotor hyperactivity and disrupted L1 are considered to be models relevant to the positive symptoms of schizophrenia, corresponding respectively to the psychomotor agitation and loss of L1 observed in patients displaying acute psychotic symptoms (Bubeniková-Valesová et al., 2008; Weiner, 2003). Reductions in the ErbB4 receptor similarly induce hyperactivity in mice, as well as impairments in prepulse inhibition and spatial learning and memory (Stefansson et al., 2002; Golub et al., 2004). Together, these results suggest an important role for NRG1 and ErbB4 in the biological mechanism underlying the positive and cognitive symptoms of schizophrenia. Interestingly, a recent study indicates that NRG1-ErbB4 signaling may contribute to NMDAR hypofunction in schizophrenia, as NRG1-induced activation of ErbB4 was found to be elevated in the prefrontal cortex of schizophrenia patients and increased NRG1 stimulation led to the suppression of NMDAR activation in human cortical tissue (Hahn et al., 2006).

Dysbindin (dystrobrevin binding protein 1) is another susceptibility gene that was initially associated with schizophrenic pathology, through linkage to chromosome 6p (Straub et al., 2002). The positive association of this locus with schizophrenia has been replicated in several subsequent independent studies, some of which show that genetic variation in the dysbindin gene influences general cognitive ability and prefrontal brain function in healthy populations, as well as negative symptoms and cognitive decline in schizophrenia patients (Burdick et al., 2006; Fallgatter et al., 2006; Fanous et al., 2005; Burdick et al., 2007). The functions of dysbindin are not well understood, although growing evidence supports a role in neurotransmitter release, affecting the kinetics, amount, and probability of presynaptic vesicular release and the morphology of vesicles (Chen et al., 2008). In primary neuronal cultures dysbindin has been shown affect extracellular glutamate release and promote neuronal viability (Numakawa et al., 2004). Additionally, dysbindin binds to β-dystrobrevin, a member of the dystrophin protein complex that affects synaptic structure and signaling (Benson et al., 2001). Studies of the homozygous sandy mice that have a loss in dysbindin protein show behavioral disturbances that include impairments in object recognition memory, social interactions, long-term and spatial working memory (Feng et al., 2008; Takao et al., 2008; Bhardwaj et al., 2009). Pharmacological responses to amphetamine and pain sensitivity are also abnormal in the sandy mice (Bhardwaj et al., 2009). Thus, results demonstrating social withdrawal and cognitive deficits in mice lacking dysbindin protein are consistent with association studies indicating that aberrant dysbindin activity may contribute to the negative symptoms and cognitive dysfunction in schizophrenia. Furthermore, the behavioral abnormalities in the sandy mice are surprisingly similar to mice with aberrant NMDAR α-serine/glycine site function and this may reflect converging functions of these genes. Indeed, epistatic effects and common protein–protein interactions have been reported for schizophrenia susceptibility genes, suggesting some overlap of common biological processes (Nicodemus et al., 2007; Camargo et al., 2007; Paterlini et al., 2005).

Disrupted-in-schizophrenia 1 (DISC1) was first identified at the breakpoint of a balanced chromosomal translocation t(1;11) (q42.1;q14.3) which cosegregated in a large Scottish family with schizophrenia and other major psychiatric disorders (Millar et al., 2000). Multiple independent studies have since shown association between polymorphisms in DISC1 and schizophrenia susceptibility in diverse population samples (Chubb et al., 2008), along with several reports indicating that allelic variation in DISC1 is associated with abnormal cortical and hippocampal gray matter volume and function, positive symptoms, cognitive impairments, and social anhedonia (Di Giorgio et al., 2008; Szeszko et al., 2008; Li et al., 2007b; Tomppo et al., 2009). DISC1 plays an important role in CNS development and neuronal functions that includes involvement in neuronal migration and maturation, neurite outgrowth, cytoskeletal function, synaptic transmission, and plasticity (Chubb et al., 2008; Ross et al., 2006). Mice with altered DISC1 function display phenotypes relevant to schizophrenia and mood disorders (Clapcote et al., 2007; Hickuda et al., 2007; Pletnikov et al., 2008; Shen et al., 2008; Li et al., 2007b). Examination of mice with ENU-induced missense mutations in exon 2 of the DISC1 gene revealed that a mutation at amino acid position 100 (L100P) resulted in schizophrenia-like behavioral abnormalities that were normalized by typical and atypical antipsychotics, while a mutation at amino acid position 31 (Q31L) produced depressive-like phenotypes that were reversible by antidepressant treatment (Clapcote et al., 2007). Both the L100P and Q31L mutant mice demonstrated disrupted L1, working memory impairments, and a decrease in brain volume; however, the L100P mutant mice also demonstrated locomotor hyperactivity and severe sensorimotor gating deficits, while the Q31L showed additional phenotypes related to depression, anhedonia, and social withdrawal (Clapcote et al., 2007). Expression of truncated DISC1 protein on a background of endogenous mouse DISC1 protein led to numerous structural, cellular, and behavioral perturbations (Hickuda et al., 2007; Pletnikov et al., 2008; Shen et al., 2008). These included an enlargement of the lateral ventricles, reductions in cortical thickness, neurite outgrowth, parvalbumin GABAergic neurons in the hippocampus and cortex, as well as hyperlocomotion, sensorimotor gating deficits, spatial memory impairments, and augmented depressive-like behaviors (Hickuda et al., 2007; Pletnikov et al., 2008; Shen et al., 2008). Furthermore, expression of a C-terminal portion of DISC1 in mice at postnatal day 7, but not in adulthood, produced social impairments, depressive-like behaviors, spatial working memory deficits, and decreased dendritic branching in the hippocampus (Li et al., 2007b). Overall, studies in genetic mouse models investigating the effects of altered DISC1 function indicate a broad spectrum of abnormalities pertinent to schizophrenia and affective disorders, suggesting that DISC1 is a major component in a multidimensional risk pathway for psychiatric illness.

Several other candidate genes involved in schizophrenia risk have been identified and described in recent reviews (Harrison and Weinberger, 2005; Ross et al., 2006). For some genes, preliminary characterization of genetic mouse models have indicated phenotypes that are relevant to schizophrenia and other psychiatric conditions. In contrast, a lack of overt behavioral phenotypes has been observed in some candidate gene mouse models. For example, regulator of G-protein signaling 4 (RGS4) has been associated with schizophrenia in multiple studies (Harrison and Weinberger, 2005) and decreased RGS4 transcripts have been reported in a number of cortical regions in schizophrenia patients (Mirnics et al., 2001). However, mice with a RGS4 deletion show intact locomotor activity, prepulse inhibition, and working memory (Grillet et al., 2005).

Absence of behavioral abnormalities in the RGS4 null mice and other candidate gene mouse models may be related to developmental compensatory effects, the obscuring of phenotypes by a mixed genetic background, procedural limitations, or a lack of involvement of the gene in the assessed behaviors or schizophrenia. Hence, it is crucial that behavioral phenotyping strategies take into account the influences of sex and background strain, in addition to undertaking a comprehensive approach that assesses multiple endophenotypes related to psychiatric illness and controls for sensory, motor, visual, olfactory, and other functions that may influence behavioral performance. Since conventional knockout and certain transgenic technologies induce gene mutations from conception, the lifelong absence of a gene will often
induce compensatory processes and developmental defects that contribute to the adult phenotype in tandem with the targeted mutation. Consequently, mutations that have regional and temporal specificity may be of interest, as this strategy may notably limit widespread compensatory changes. Inducible and region-specific mutations will also allow the study of alterations that more closely reflect the subtle perturbations found in schizophrenia and other psychiatric disorders. Furthermore, assessment of mice with single nucleotide substitutions similar to those reported in schizophrenia will be required to study the effects of variations at risk alleles. Many of the polymorphisms related to schizophrenia affect regulatory elements of genes and examination of animal models with these genetic abnormalities will help clarify the effects these mutations have on gene processing and regulation, as well as their role in the more complex molecular processes involved in schizophrenia. An extension to this approach is to eliminate the mouse gene of interest and replace it with the human ortholog (knock-in) containing the risk variant, in an effort to better predict the effects of the polymorphism in humans. Since schizophrenia likely arises from the simultaneous disruption of several genes, compound mutant mice in which several candidate genes are targeted may offer the greatest potential for future genetically modified mice. Partial gain- or loss-of-function mutations in several risk genes within the same animal would most closely replicate the etiological mechanism of schizophrenia and would be highly valuable for the development of novel therapeutic interventions.

12. The future of NMDAR δ-serine/glycine site modulators in the treatment of schizophrenia

Overall findings from preclinical and clinical studies demonstrate apparent beneficial effects of NMDAR δ-serine/glycine site activators, particularly on the negative and cognitive symptoms domains. Though administration of direct agonists, such as δ-serine and glycine, are of therapeutic value, such treatments require large doses and exhibit difficulties in penetrating the blood–brain-barrier. Consequently, agents targeting proteins involved in δ-serine metabolism may be a more effective strategy, providing selective modulation of the NMDAR δ-serine/glycine site. Inhibition of DAO activity in the brain is of particular interest as it would circumvent any nephrotoxicity associated with the catabolism of high levels of systemic δ-serine (Maekawa et al., 2005b). Development of DAO antagonists has recently begun (Adage et al., 2008; Hashimoto et al., 2009; Smith et al., 2008). Intravenous injections of the DAO inhibitor AS057278 were found to readily cross the blood–brain-barrier and enhance δ-serine contents in the rat brain (Adage et al., 2008). Chronic administration of AS057278 in mice was shown to normalize PCP-induced deficits in behavioral tasks relevant to the cognitive and positive symptoms of schizophrenia (Adage et al., 2008). However, elevations in δ-serine following DAO inhibition are considerably lower than with exogenous δ-serine treatments (Smith et al., 2008; Ferraris et al., 2008) and since DAO is predominantly located in caudal brain regions (Moreno et al., 1999) it remains to be determined if DAO inhibition can effectively elevate δ-serine in frontal brain areas. This limitation may be overcome by combining DAO inhibition with a low dose of exogenous δ-serine. Indeed, coadministration of δ-serine and a DAO antagonist were shown to produce greater ameliorative effects in animals treated with an NMDAR antagonist than either compound applied alone (Hashimoto et al., 2009). Thus, DAO inhibition in combination with δ-serine administration may be a valuable therapeutic approach for the treatment of schizophrenia. Activators of SRR function may also provide beneficial effects, however such compounds have yet to be developed. Targeting SRR function may be a highly effective means of modulating synaptic δ-serine availability due to the prevalence of SRR in the forebrain (Xia et al., 2004; Schell et al., 1995), its colocalization with NMDARs (Schell et al., 1997), and its prominent capacity to regulate δ-serine abundance (Wolosker et al., 1999; Foltyn et al., 2005; Strîsovsky et al., 2005). In sum, agents targeting the NMDAR δ-serine/glycine site are examples of fundamentally novel therapies that may deliver symptomatic improvement with substantially fewer side effects than current antipsychotics.

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