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REVIEW

From Revolution to Evolution: The Glutamate Hypothesis of Schizophrenia and its Implication for Treatment

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Glutamate is the primary excitatory neurotransmitter in mammalian brain. Disturbances in glutamate-mediated neurotransmission have been increasingly documented in a range of neuropsychiatric disorders including schizophrenia, substance abuse, mood disorders, Alzheimer's disease, and autism-spectrum disorders. Glutamatergic theories of schizophrenia are based on the ability of N-methyl-D-aspartate receptor (NMDAR) antagonists to induce schizophrenia-like symptoms, as well as emergent literature documenting disturbances of NMDAR-related gene expression and metabolic pathways in schizophrenia. Research over the past two decades has highlighted promising new targets for drug development based on potential pre- and postsynaptic, and glial mechanisms leading to NMDAR dysfunction. Reduced NMDAR activity on inhibitory neurons leads to disinhibition of glutamate neurons increasing synaptic activity of glutamate, especially in the prefrontal cortex. Based on this mechanism, normalizing excess glutamate levels by metabotropic glutamate group 2/3 receptor agonists has led to potential identification of the first non-monoaminergic target with comparable efficacy as conventional antipsychotic drugs for treating positive and negative symptoms of schizophrenia. In addition, NMDAR has intrinsic modulatory sites that are active targets for drug development, several of which show promise in preclinical/early clinical trials targeting both symptoms and cognition. To date, most studies have been done with orthosteric agonists and/or antagonists at specific sites. However, allosteric modulators, both positive and negative, may offer superior efficacy with less danger of downregulation.

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INTRODUCTION

Fifty years ago, when the ACNP was first convened, it was an exciting time for new treatment development in psychiatry. In schizophrenia, a new era of treatment was ushered by the fortuitous finding that chlorpromazine and other phenothiazines showed dramatic effects in control of psychosis. Implementation of these treatments permitted deinstitutionalization of large numbers of patients with schizophrenia, and allowed a substantial number of chronically disabled patients to resume relatively normal lives. This was followed shortly thereafter with seminal findings linking effective doses of antipsychotic drugs to blockade of the recently discovered D2-type dopamine receptor (Seeman *et al*, 1975; Creese *et al*, 1976). Pharmaceutical companies quickly exploited these findings by developing large families of phenothiazine and non-phenothiazine antipsychotics, all showing similar efficacy and with side effect profiles modulated by binding profile across a wide variety of receptor types.

Fifty years later, pharmacological treatment of schizophrenia remains virtually unchanged (Lieberman *et al*, 2005). The most efficacious antipsychotic drug is clozapine, developed in 1961. All attempts to develop an equally effective compound free of its hematological and orthostatic side effects so far have failed. The failure is most obvious in the case of negative symptoms and cognitive deficits, which remain as key predictors of functional disability (Anderson *et al*, 1996; Goldberg and Weinberger, 1996; Green and Nuechterlein, 1999; Goldberg *et al*, 2003; Kirkpatrick *et al*, 2006). However, even positive symptoms of schizophrenia

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persist despite an aggressive antipsychotic treatment in a significant number of individuals (Foussias and Remington, 2010). Perhaps most disappointingly, not only have compounds directed at D2 receptors failed to ameliorate core symptoms of schizophrenia in a great many individuals, but even compounds directed at some of the most obvious closely related sites, such as serotonin 5-HT2A receptors, have no or inferior efficacy compared with typical antipsychotics such as haloperidol (Geyer *et al*, 1999b; Meltzer *et al*, 2004; Marder, 1999). A half century after the initial discovery of antipsychotics, the field finds itself in need not only of alternative medications but also alternative targets (Abbott, 2010). Selection of these targets must be guided by sound etiological theories, as well as by practical considerations such as 'drug ability' and stability of effect.

The glutamate synapse has emerged as one of the most prominent targets in this context (Javitt, 2004; Moghaddam, 2004). This is due in part to the fact that biological evidence at several levels supports an involvement for glutamate neurotransmission in the etiology and pathophysiology of the disease. More importantly, the glutamate synapse is a target-rich environment containing a large number of presynaptic, postsynaptic, and regulatory proteins that represent appropriate targets for drug development (Moghaddam, 2003; Marek *et al*, 2010). Here, we review the progression of scientific discovery and theoretical thinking that has moved the glutamate hypothesis of schizophrenia from a neurotransmitter theory into the practical arena of target identification and animal modeling.

THE REVOLUTION

The dopamine hypothesis of schizophrenia, which remains the most prominent theory in the field, can be seen as originating from the fortuitous discovery of the antipsychotic effects of chlorpromazine in the mid-1950s. So too, glutamatergic theories can be dated to a precise observation, in this case the synthesis in the late 1950s of the dissociative anesthestics phencyclidine (PCP) and ketamine (Chen and Weston, 1960), followed shortly thereafter by the demonstration of their psychotogenic potential in humans (Luby *et al*, 1962), the discovery of the PCP receptor (Zukin and Zukin, 1979), and finally the discovery that these compounds function by blocking the *N*-methyl-D-aspartate receptor (NMDAR) channel (Javitt and Zukin, 1991). PCP and ketamine induced negative symptoms and cognitive dysfunction similar to that of schizophrenia, suggesting that this model may be particularly relevant to persistent, poor-outcome forms of schizophrenia.

Historically, attempts have been made to attribute PCP- and ketamine-induced psychosis to a wide variety of targets including dopaminergic, monoaminergic, cholinergic, GABAergic, opiatergic, sigma (Javitt and Zukin, 1991), and, most recently, to high D2 receptors (Seeman, 2010). However, the behavioral effects of NMDAR antagonists that are relevant to schizophrenia persist in the absence of dopamine activity (Carlsson and Carlsson, 1989; Adams and Moghaddam, 1998) or dopamine antagonists (Krystal *et al*, 1995). Furthermore, both the absolute concentrations and the rank-order potency with which a range of compounds induce psychotomimetic effects in humans and animal models conforms to their rank order of potency at NMDAR but not other receptor types (Javitt and Zukin, 1991; Seeman, 2010).

To date, all compounds that bind to the PCP site of the NMDAR have been found to induce psychosis when given to humans, whereas the same is not true for proposed alternative non-NMDAR-related targets, so that NMDAR blockade by these agents appears both necessary and sufficient to explain their psychotomimetic effects. Moreover, the effects do not appear to be unique to agents that inhibit NMDAR via the PCP site. Thus, antagonists at both the glutamate binding site (eg CGS-19755) and the glycine modulatory site (eg, CP-101,606) also induce psychotomimetic effects when administered clinically (Muir *et al*, 1995; Preskorn *et al*, 2008). Finally, it has been observed recently that psychosis related to systemic lupus erythematous and other autoimmune disorders may be due to the production of CNS-penetrant anti-NMDAR antibodies (Omdal *et al*, 2005), providing unexpected support to NMDAR models of endogenous psychosis.

The NMDAR model may be considered revolutionary, not only because it proposes a different set of targets than would be predicted by more traditional monoaminergic models, but because it proposes a fundamental reconceptualization of what brain regions to target and what assays may be most effective for continued drug development. In particular, dopamine projections in the brain are relatively discrete. In dopaminergic models, therefore, symptoms are seen as arising from dysfunction within a limited number of brain regions, such as dorsolateral prefrontal cortex (Lesh *et al*, 2011) or striatum (Simpson *et al*, 2010), with secondary top-down dysregulation elsewhere in the brain.

In contrast to the limited range of dopamine neurotransmission, all cortical efferents and the majority of cortical afferents, and cortico-cortical connections are glutamatergic. In glutamatergic models, therefore, deficits are seen as distributed throughout cortical and subcortical regions, within involvement of sensory as well as higher cortical brain regions (Javitt, 2009b). For example, mismatch negativity (MMN), an event-related potential that indexes brain function at the level of auditory cortex, has been shown repeatedly to be abnormal in schizophrenia (Javitt *et al*, 1993; Naatanen and Kahkonen, 2009). Deficits similar to those observed in schizophrenia are induced by ketamine administration in normal volunteers (Krystal *et al*, 1994; Umbricht *et al*, 2000; Heekeren *et al*, 2008) and by local administration of NMDAR antagonists into auditory cortex of awake, behaving primates (Javitt *et al*, 1996). Most recently, similar effects have been reported in rodents (Ehrlichman *et al*, 2008; Tikhonravov *et al*, 2008), suggesting that measures such as MMN may be used as translational biomarkers for future drug development research.

Nevertheless, the observation that symptoms, cognitive deficits, and neurophysiological indices of schizophrenia can be reproduced by blocking NMDAR receptors does not, in itself, explain either how such deficits arise or how they best can be treated. In the simplest versions of the NMDAR models, the primary goal of treatment would be the restoration of function at the NMDAR itself. However, in many, if not most, medical conditions, the target of treatment may not be the site of dysfunction. Thus, a goal of ongoing research has been to delineate, not only potential causes of NMDAR dysfunction, but also the steps leading from NMDAR dysfunction to psychosis and cognitive impairment. This research has led to an evolution in the conceptualization of glutamatergic dysfunction over the past 20 years, and elaboration of targets beyond the NMDAR itself.

THE EVOLUTION

Despite the conceptual simplicity of the NMDAR model, ie ongoing NMDAR hypofunction leads to expression of schizophrenia symptoms, two broad classes of questions with regard to NMDAR dysfunction remain unanswered. First, what causes NMDAR dysfunction on an etiological level and second, what approaches may be most effective in reversing underlying abnormalities. Research into both causes and treatments continues to evolve. Here, we divide the current ideas and therapeutic approaches into two broad categories of presynaptic and postsynaptic hypotheses.

Presynaptic Hypotheses

On the presynaptic level, the most obvious potential cause of NMDAR dysfunction would be a reduction in overall glutamatergic tone in the brain, leading to a global deficit in glutamatergic neurotransmission. However, while some findings of reduced CSF glutamate levels were reported (Kim *et al*, 1980), ultimately these were not confirmed (Javitt and Zukin, 1991), suggesting that more complex disturbances in glutamatergic function might be involved. In fact, over the last 20 years, it has been increasingly demonstrated that hyper, rather than hypo, glutamatergic function, potentially mediated through activation of AMPA receptors may be critical in schizophrenia, and that ideal treatment approaches may reduce rather than increase presynaptic glutamate levels (Moghaddam, 2003).

One key finding leading to the glutamate hyperactivity theory was that, in awake animals (but not in brain slice preparations or anesthetized animals), systemic injection of NMDAR antagonists at doses that impaired cognitive functions and produced motor stereotypy increase glutamate efflux in the prefrontal cortex (Liu and Moghaddam, 1995; Moghaddam *et al*, 1997; Moghaddam and Adams, 1998; Lorrain *et al*, 2003). This increase in the extracellular levels of glutamate had functional significance because blockade of AMPA receptors reduced the motoric and cognitive detriments of NMDAR blockade (Moghaddam *et al*, 1997). Thus, NMDAR antagonists appeared to increase the release of glutamate at some synapses, which then abnormally increased glutamate neurotransmission at non-NMDAR, in particular AMPA receptors (Figure 1). This finding, therefore, suggested that behavioral consequences of NMDAR deficiency is not due to a generalized 'glutamate hypofunction' but dysregulation of glutamate neurotransmission that may potentially involve NMDAR hypofunction but excessive activity of non-NMDA receptors.

Two additional lines of evidence from animal and human studies supported this mechanism. One was that neuronal activity in the PFC was generally enhanced by NMDAR antagonists in human fMRI studies measuring metabolic activation in PFC regions (Breier *et al*, 1997; Vollenweider *et al*, 1997) and single unit recordings in awake rodents measuring random spiking of spontaneously active neurons (Jackson *et al*, 2004) (Figure 1). Although this increased activity may be interpreted counterintuitive to a state of 'hypofrontality' in schizophrenia, the enhanced spike activity led to a disorganized pattern of activity essentially adding 'noise' and interrupting the ability of cortical neurons to process relevant information.

A second line of evidence was that reducing the release of glutamate by metabotropic glutamate group 2/3 receptor agonists also reduced the behavioral and cellular effects of NMDAR antagonists (Moghaddam and Adams, 1998; Krystal *et al*, 2005). These glutamate receptors are localized extrasynaptically including on presynaptic terminals. Activation of these receptors by exogenous agonists reduces activated release of glutamate (Battaglia *et al*, 1997; Schoepp *et al*, 1997). Rodent studies showed that these exogenous agonists reduced NMDAR antagonist-activated release of glutamate and cortical hyperactivity at the same time they ameliorated the aberrant behavioral effects of these antagonists, including PCP and MK801 (Moghaddam and Adams, 1998; Cartmell *et al*, 1999; Cartmell *et al*, 2000; Homayoun *et al*, 2005). Proof of concept studies in healthy volunteers showed that mGlu2/3 receptor agonists also reduced some of the cognitive impairing effects of ketamine in healthy volunteers (Krystal *et al*, 2005). Collectively, these studies showed that the excitatory consequences of NMDAR hypofunction may mediate some of the behavioral effects of this treatment and, more importantly, reducing the presynaptic output of glutamate receptors by presynaptic autoreceptors such as mGlu2 receptors or by targeting synthetic enzymes such as glutaminase (Gaisler-Salomon *et al*, 2009) provide novel targets for treatment of symptoms of schizophrenia. Clinical trials based on this concept have so far been encouraging. The first published study demonstrated comparable efficacy between an mGlu2/3 receptor agonist and the atypical antipsychotic drug olanzopine for treating negative and positive symptoms (Patil *et al*, 2007) without the metabolic and motor-related side effects generally associated with antipsychotic drug. A subsequent trial was inclusive because both the mGlu2/3 agonist and olanzopine did not significantly separate from

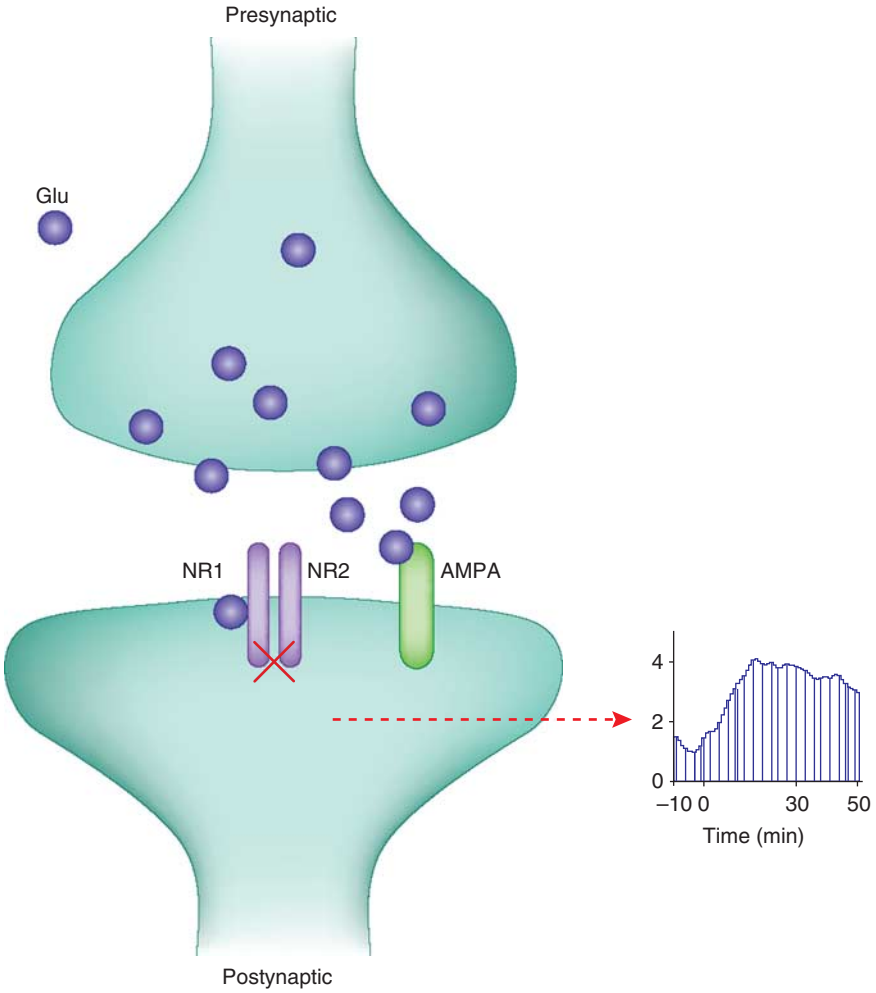

Figure 1. One of the downstream consequences of NMDAR inhibition is increased availability of glutamate (see text and Figure 2 for potential mechansims that can cause this effect). This increase causes excess activity of AMPA receptors and enhanced postsynaptic spiking of cortical principle cells at rest.

placebo (Kinon *et al*, 2011). A recent long-term (6 months) safety trial comparing mGlu2/3 agonist with atypical antipsychotic standard of care showed that the mGlu2/3 agonist is not associated with increased seizure rates and has an efficacy profile that is consistent with an active antipsychotic compound (B Kinon, personal communication). There were, however, more patients who discontinued mGlu2/3 agonist treatment owing to reduced efficacy. This may be expected given that continued use of orthosteric agonist may lead to receptor desensitization and thus lack of efficacy. Therefore, increasing the dose or shifting to targets that allosterically modulate this receptor may be necessary for chronic use.

The critical question in this context has been what is the mechanism by which an NMDAR antagonist increases and thus disorganizes the firing of cortical neurons? The most straightforward mechanism is the so-called cortical disinhibition process (Homayoun and Moghaddam, 2007b). Figure 2 depicts this model at its simplest form. It is well established that the activity of hippocampal and neocortical principal (pyramidal) neurons is under the control of GABA interneurons. Without this GABAergic inhibition, excitatory inputs onto pyramidal neurons would cause a chain reaction of ever increasing activation. The regulation or stabilization of the firing of pyramidal neurons by GABA interneurons is critical for coordination of cell assemblies that support cortical-mediated behaviors (Buzsaki *et al*, 2004). One classic example of GABAergic influence is the feed-forward inhibition where the effect of afferent excitation on a pyramidal neuron is dampened by co-activation of GABA interneurons that synapse onto the same pyramidal neuron. Thus, increased discharge of an interneuron results in decreased discharge of pyramidal neurons. Accordingly, processes that inhibit the discharge of GABA neurons excite or 'disinhibit' pyramidal neurons.

In the neocortex and hippocampus, some subtypes of interneurons have a lower threshold for action potential generation compared with pyramidal cells (Csicsvari *et al*, 1998; Maccaferri and Dingledine, 2002). This more depolarized state of interneurons would dictate that more NMDAR channels, which are voltage gated, are open on these neurons. Given this, when the system is exposed to an

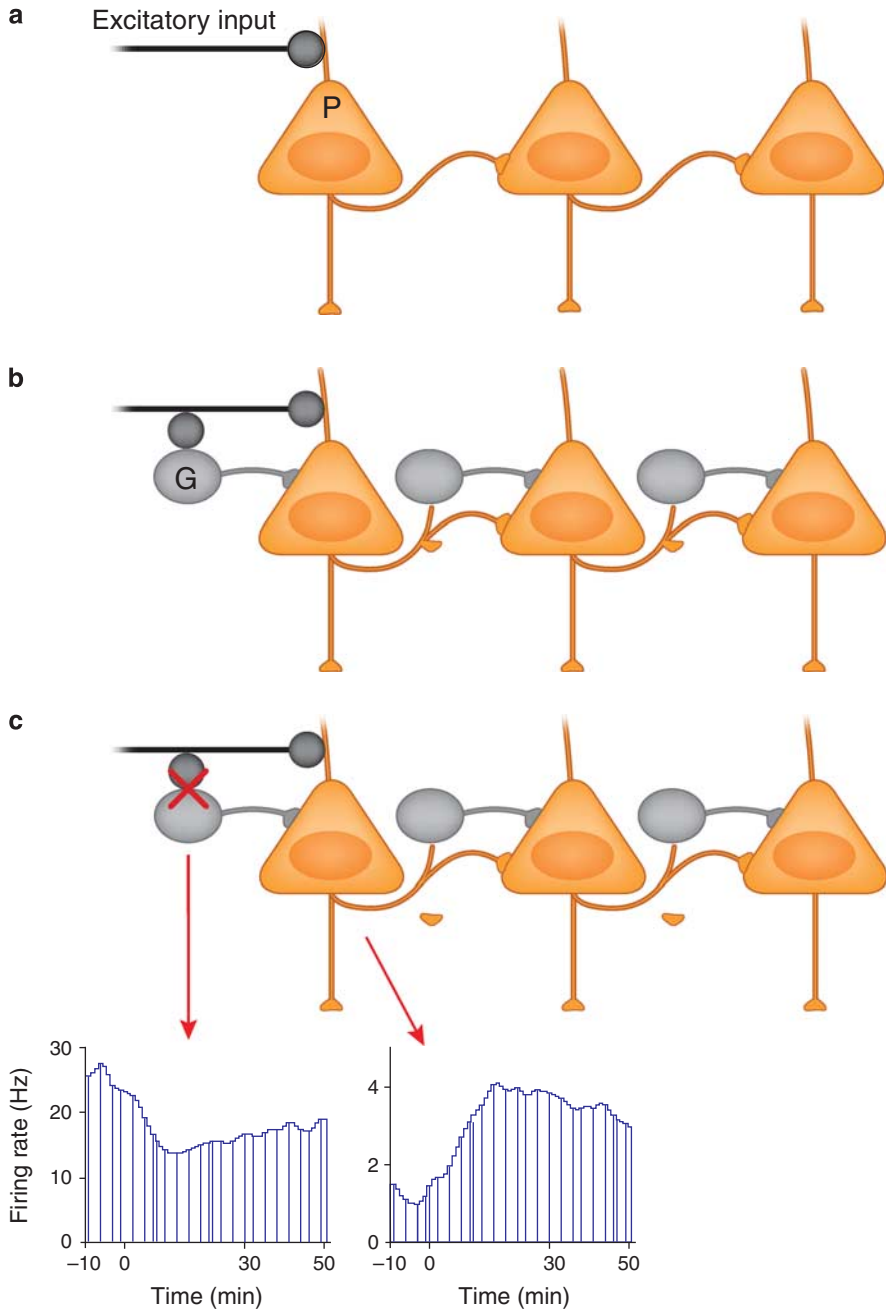

Figure 2. (a) Principal (P) or pyramidal cells in the neocortex and hippocampus, all which use glutamate as their neurotransmitter, receive extensive excitatory input from subcortical and cortical regions. In the absence of a counteracting inhibitory influence, activation of these inputs could cause a chain reaction of ever increasing excitation. (b) The regulation or stabilization of the firing of pyramidal cells is served by GABA (G) interneurons. One classic example of GABAergic influence is the feed-forward inhibition model where the effect of afferent excitation on a pyramidal neuron is dampened by co-activation of GABA interneurons that synapse onto the same pyramidal neuron. (c) The excitatory–inhibitory balance can be disrupted by many factors. An example is exposure to pro-psychotic compounds such as NMDA receptor antagonists. Blockade of NMDA receptors preferentially acts on fast spiking GABA interneurons because these neurons have a more depolarized membrane potential (note the higher firing rate on the recording from a putative GABA interneuron in the prefrontal cortex of an awake rat) and thus contain more open NMDA channels. This preferential inhibition of GABA interneurons creates an artificial state of disinhibition for the pyramidal cells and increases their firing rate.

NMDAR antagonist, there is a preferential effect on inhibiting the excitatory drive of GABA neurons compared with pyramidal neurons. This will produce an artificial state of disinhibition whereby the NMDAR antagonist produces a transient decrease in firing rate of GABA neurons that in turn enhances the firing of pyramidal neurons (Homayoun and Moghaddam, 2007b). The reduced impact of GABA on pyramidal neurons then would lead to an unstable situation where there is an artificial engagement of pyramidal neurons or noise as described by awake animal recordings (Jackson *et al*, 2004). This also may cause a secondary effect of enhanced glutamate release from local pyramidal cells, which then leads to excess activation of AMPA receptors at some synapse (Moghaddam *et al*, 1997). Overall, this process

of NMDAR hypofunction-induced disinhibition may render the pyramidal neurons compromised when it comes to responding to incoming stimuli and passing on properly coordinated activity to subcortical regions.

Postsynaptic Hypotheses

On the postsynaptic level, the last 20 years of research has also led to an evolution in thinking about both causes and targets of NMDAR dysfunction. Functional NMDAR are heteroligomers composed of variable combinations of NR1, NRA-D, and NR3A-B subunits. Complex alterations in NMDAR subunit composition have been reported at both the protein and message level in schizophrenia, along with alterations in specific NMDAR-related postsynaptic proteins (Kristiansen *et al*, 2007). In addition, positive genetic associations are reported between both NR1 (Begni *et al*, 2003) and NR2B polymorphisms and schizophrenia (Martucci *et al*, 2006; Qin *et al*, 2005; Allen *et al*, 2008), suggesting that abnormalities of NMDAR expression or function may directly contribute to schizophrenia. NMDARs are primarily localized to postsynaptic dendritic terminals. However, additional populations exist on presynaptic terminals where they control glutamate release (Javitt *et al*, 1987; Corlew *et al*, 2008; Larsen *et al*, 2011) and growth cones of oligodendrocytes (Matute *et al*, 2005), and where dysfunction may be responsible for the well-replicated white matter abnormalities in schizophrenia (Ardekani *et al*, 2005). Nevertheless, the role of specific NMDAR subunits remains to be determined.

Along with intrinsic NMDAR dysfunction, disturbances in several modulatory mechanisms also have been demonstrated in schizophrenia, and have increasingly become targets of both etiological and therapeutic interventions (Figure 3). These include disturbances in synthesis and degradation of glycine and D-serine, which bind to the glycine modulatory site of the NMDAR (Javitt, 2007), and glutathione, which regulates the redox site (Gysin *et al*, 2007). In addition, endogenous inhibitors, such as kynurenic acid, may have a key role and thus may represent secondary targets for drug development (Wonodi and Schwarcz, 2010).

Glycine metabolism. Glycine in brain is synthesized primarily from L-serine by serine hydroxymethyltransferase, and regulated synaptically by glycine (GlyT1) transporters (Javitt, 2007). To date, there is limited evidence for disturbance of glycine metabolism in schizophrenia. Nevertheless, GlyT1 transporters may be an appropriate target for therapeutic intervention, potentially raising synaptic glycine to super-physiological levels in order to compensate for disturbances elsewhere in the system (Javitt, 2009a).

D-serine metabolism. In contrast, abnormalities in D-serine metabolism have been demonstrated at both the synthetic and degratory level. D-serine is synthesized in brain from L-serine by serine racemase, and degraded by D-amino-acid oxidase, which, in turn, is modulated by the protein G72. Genetic studies have shown associations of both enzymes with schizophrenia. Furthermore, serine racemase knockout mice show behavioral and structural abnormalities similar to those observed in schizophrenia, with a phenotype that can be rescued by crossbreeding with D-amino-acid oxidase (DAAO) knockouts. Perhaps even more important, reductions in D-serine levels have been demonstrated in both plasma and CSF in schizophrenia, suggesting potential physiological relevance to the genetic abnormalities (Labrie and Roder, 2009).

Glutathione/n-acetylcysteine. Genetic studies have implicated impairments in the glutathione system (Gysin *et al*, 2007). As with D-serine, reduced glutathione levels have been demonstrated in schizophrenia using both MRS and CSF measurements. Recently, associations also have been reported for several of the glutathione synthetic enzymes (Rodriguez-Santiago *et al*, 2010). Many factors nonspecifically affect brain glutathione levels. Thus, the glutathione site may represent a point of convergence for nonspecific brain injury mechanisms. Brain glutathione levels may be modulated to some extent by administration of dietary precursors, such as N-acetylcysteine (Dodd *et al*, 2008; Berk *et al*, 2008). To date, however, no high-affinity compounds have been developed that may function via modulation of this site.

ALLOSTERIC MODULATORY SITES

A critical concern with the overall approach of manipulating glutamate neurotransmission at presynaptic or postsynaptic levels is that traditional agonist or antagonist therapy may be detrimental. This is because glutamate synapses are highly dynamic synapses where stimulus-induced release of glutamate causes a rapid postsynaptic response and efficient uptake of either amino acid from the synaptic cleft. A sustained activation of receptors that mediate glutamate neurotransmission could result in neurotoxicity or adaptive responses that may be detrimental to cortical function. Thus, a more practical approach is to modulate the function of these receptors in an activity-dependent manner, ie, to enhance or reduce their function transiently in response to an incoming stimulus. This function is served naturally in the brain by the so-called allosteric modulatory sites on many brain receptors. These sites, when activated, enhance the function of the natural neurotransmitters in stimulating the targeted receptor. In other words, they only work to modulate the function of the receptor when the receptor is stimulated by the natural neurotransmitter.

These targets can be ideal for treatment because, as depicted in Figure 4, unlike a direct agonist that sustains a continuous level of receptor activation, stimulation of these sites by so-called positive allosteric modulator (PAM) only potentiates the function of the natural ligand and thus only

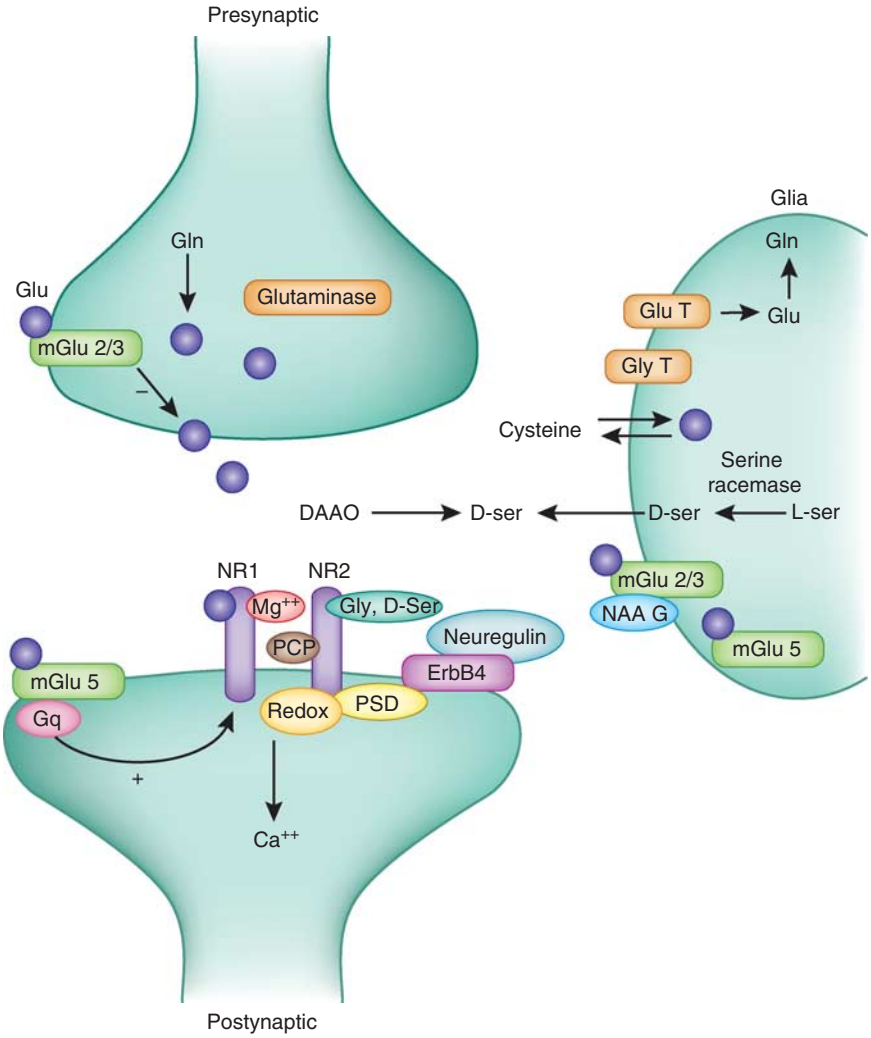

Figure 3. A simplified model of glutamate (Glu) synapse depicting some of the potential targets for manipulating the function of NMDA receptors. The two primary subunits of the receptor (NR1 and NR2) are depicted. On the presynaptic side, excess release of glutamate can be reduced by metabotropic group 2 receptors. Levels of vesicular glutamate also can be manipulated by the activity of the synthetic enzyme glutaminase, which converts glutamine (Gln) to Glu. On the postsynaptic site, several regulatory sites on the NMDA channel itself (eg, magnesium and PCP-binding sites, the D-serine and glycine (Gly) site and the redox (glutathione)) regulate the function of the receptor. In addition, other membrane-spanning receptors, such as the metabotropic group 5 (mGlu5) receptor or the ErbB4 receptor, indirectly influence the function of NMDAR by interacting through postsynaptic density (PSD) or signal transduction mechanisms. The glia includes a large number of proteins that influence both presynaptic and postsynaptic function of this synapse. These include transporters for both Glu and Gly, the D-serine-synthesizing enzyme serine racemase, D-serine transporter, as well as cystine–Glu transporter. In addition, a number of metabotropic Glu receptors including mGluR3 and mGluR5 are expressed by glia.

'stimulates' when it is activated by the natural neurotransmitter released in response to stimuli. In general, two sets of allosteric modulatory sites can be considered. First, those inherent to the NMDAR itself and second, those inherent to receptors that may serve to modulate NMDAR-mediated neurotransmission.

Intrinsic Sites

To date, the sites that have been most investigated in therapeutic trials are the glycine modulatory site and, to a much lesser extent, the redox site. Studies of glycine-site agonists have been possible because the two endogenous ligands for this site, glycine and D-serine, are both natural substances and so can be used in clinical trials even in advance of availability of optimized ligands. D-cycloserine, an antituberculosis drug, fortuitously cross-reacts with NMDAR, but is only a partial agonist. Overall, significant effects have been observed in several single-site studies of both glycine and D-serine (Javitt *et al*, 1994; Heresco-Levy *et al*, 1999; Tuominen *et al*, 2005; Tsai and Lin, 2010). To date, however, multicenter studies have not shown separation *vs* placebo (Buchanan *et al*, 2007; Weiser *et al*, 2008). However, both studies also showed substantial placebo effects. In the absence of an active comparator, therefore, it cannot be determined whether these should be interpreted as negative or failed studies. Caution, moreover, must be exercised in interpreting these studies, however, as none have been conducted with the types of internal controls typical of industry-sponsored studies. Most particularly,

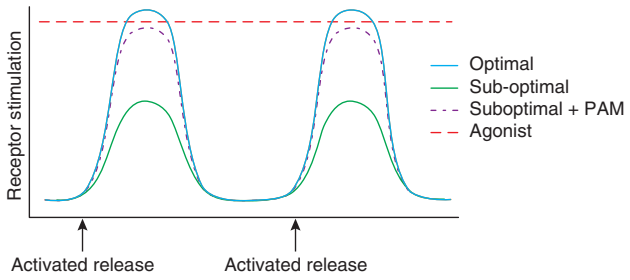

Figure 4. Allosteric modulation of receptors can selectively modulate active synapses. Under normal conditions, postsynaptic receptors are activated phasically when an action potential releases neurotransmitter from presynaptic terminals. A suboptimal activation of these receptors in a disease state is better treated with an allosteric positive modulator (PAM) that enhances the function of the natural neurotransmitter on those receptors as opposed to an agonist that produces constant activation of receptors. The latter could lead to desensitization of receptors, neurotoxicity, and other side effects.

compounds have been used at non-optimized doses. In the case of glycine, the maximum dose use has been limited by general tolerability; in the case of D-serine, by nephrotoxicity. As no target engagement biomarkers have been used in these studies, the degree of target occupancy remains unknown.

Glycine transport inhibitors: basic mechanisms. An alternative approach to glycine modulation was first proposed in the late 1990's based upon analogy to the serotonin system in depression. The goal of this approach was to increase synaptic glycine levels by blocking function of the GlyT1 that are co-localized with NDMAR, thereby permitting a natural increase in synaptic glycine levels (Javitt, 2004). Initial preclinical studies were performed with glycyldodecylamide and related compounds, which were shown to reverse PCP-induced hyperactivity with parallel rank order of potency to their effects on glycine transport (Javitt and Frusciante, 1997; Javitt *et al*, 1999). Subsequently, higher affinity compounds such as N[3-(4′-fluorophenyl)-3-(4′-phenylphenoxy)propyl]sarcosine (NFPS) were found to modulate hippocampal NMDAR function *in vitro* (Bergeron *et al*, 1998), and to modulate striatal dopamine release (Javitt *et al*, 2004) and prefrontal cortical activity (Chen *et al*, 2003) *in vivo*.

Since then, several series of high-affinity glycine transport inhibitors have been developed and shown to be effective in multiple animal models related to schizophrenia (Javitt, 2009a, b). Although initial high-affinity compounds, such as NFPS, showed unexpected toxicity such as compulsive walking ('obstinate progression') and respiratory distress in initial *in vivo* animal studies, these side effects were subsequently shown not to be NMDAR mediated (Kopec *et al*, 2010). In general, recently developed non-sarcosine-based competitive GlyT1 antagonists show greater preclinical safety and tolerability than initial sarcosine-based compounds, such as NFPS, that showed irreversible, noncompetitive inhibition of GlyT1 function (Javitt, 2009a; Wolkenberg and Sur, 2010).

Glycine transport inhibitors: clinical studies. Initial clinical studies were performed with the naturally occurring glycine transport inhibitor sarcosine (N-methylglycine), which has been found to be effective in both acute and chronic schizophrenia in several small-scale studies conducted in Taiwan (Lane *et al*, 2008; Lane *et al*, 2005; Lane *et al*, 2010; Tsai *et al*, 2004). As with glycine and D-serine, no biomarkers were available to demonstrate engagement of the glycine-binding site; therefore, there is no way to know whether the dose used represents a clinically optimal dose.

Most recently, the first selective, high-affinity GlyT1 compound, RG-1678 (Roche), was studied in a phase II program involving 323 subjects (Umbricht *et al*, 2010). As opposed to earlier studies, the clinical dose of RG-1678 was selected based upon a PET study of glycine-site occupancy, with dose chosen to prevent activation-related NMDAR desensitization. The study demonstrated, first, that inhibition of GlyT1-mediated transport does indeed lead to increased CNS glycine levels, and second, that resultant allosteric NMDAR via the glycine modulatory site may be therapeutically beneficial. This compound recently has been entered into definitive phase III trials for treatment of persistent negative symptoms.

DAAO inhibition. In the case of D-serine, a 'second generation' approach also is under development. In this approach, D-serine is combined with a DAAO inhibitor to prevent renal and brain D-serine degradation. Use of this approach produces a 30-fold increase in D-serine potency in animal models (Hashimoto *et al*, 2009), potentially decreasing clinically effective doses of D-serine from gram to milligram levels. As knock out of renal DAAO also prevents D-serine toxicity (Konno *et al*, 2010), it is possible that a combination treatment also will produce greater compound tolerability. To date, however, DAAO inhibitors remain in the preclinical testing stage, so ultimate utility of this approach remains to be determined. Finally, the cystine/glutamate antiporter (xCT) may be crucial in the regulation of brain glutathione levels, and may serve as an additional target for glutamate-related drug development (Shih *et al*, 2006).

Glutamatergic basis of clozapine response. Finally, studies with NMDAR may shed light on the mechanism by which the atypical antipsychotic clozapine is differentiated from other typical and atypical antipsychotics. Clozapine effectively reduces the impact of NMDA receptor antagonists on cortical neuron hyperactivity (Homayoun and Moghaddam, 2007a). Among its many pharmacological effects, clozapine significantly potentiates NMDAR transmission in the brain, by inhibition of system A-type glycine transporters in the brain (Javitt *et al*, 2004). Similarly, clozapine, along with D-serine and GlyT1 inhibitors, block PCP effects on social recognition (Shimazaki *et al*, 2010) and other rodent models (Lipina *et al*, 2005). Finally, while glycine, D-serine, and sarcosine have found to be effective in combination with typical antipsychotics or newer atypicals such as risperidone

or olanzapine, they appear less effective when combined with clozapine (Tsai and Lin, 2010). This lack of effect may reflect that clozapine already functions, at least in part, as a NMDAR/glycine-site agonist.

Extrinsic Sites

A second approach to enhance NMDAR is by targeting metabotropic glutamate receptors, which, in turn, may modulate either glutamate presynaptically or NMDAR postsynaptically. Presynaptic glutamate release is modulated by mGlu2/3 receptors, which serve to limit release. To date, clinical trials have been conducted with the mGlu2/3 full agonist, which has shown promising results. However, as with all full agonists, downregulation of receptors over time also is a concern. As opposed to full agonists, therefore, PAMs for this site may maximize efficacy while limiting side effects and toxicity.

At the postsynaptic level, mGlu 5 receptors may provide an indirect target for modulation of NMDAR. This group of receptors, which at some synapses are localized near NMDARs, modulate the dynamics of NMDAR channels by increasing NMDAR-mediated current (Conn *et al*, 2009). Targeting the mGlu5 receptors with PAMs has shown promise in some preclinical models (Lecourtier *et al*, 2007; Liu *et al*, 2008). A number of mGluR5 agonists and PAMs with appropriate pharmacological properties have been synthesized over recent years, and have been shown to be effective in specific preclinical models of schizophrenia, although issues related to regional expression and potential downregulation during chronic treatment need to be resolved (Parmentier-Batteur *et al*, 2010; Rodriguez-Santiago *et al*, 2010; Spear *et al*, 2010). In addition to direct targeting of the receptor, other novel targets such as Norbin, an endogenous protein that interacts with mGlu5 receptors *in vivo*, have been proposed (Wang *et al*, 2009).

In addition to mGlu receptors, other interesting targets related to cortical circuits have been proposed (Wroblewska and Lewis, 2009; Marek *et al*, 2010). These include agents that stimulate AMPA-type glutamate receptors or 'AMPA-kines' (Arai and Kessler, 2007), although studies to date have not shown efficacy for either symptoms or cognition (Goff *et al*, 2008). Other compounds, such as the M1/M4 muscarinic agent xanomeline (Bridges *et al*, 2010) and neurosteroids such as pregnenolone sulfate (Marx *et al*, 2009; Ritsner, 2010) may also function in part through indirect modulation of NMDAR-mediated neurotransmission. Thus, continued research into mechanisms of normal and abnormal NMDAR regulation may lead to further advances in drug development in schizophrenia.

FUTURE DIRECTIONS

Although the dopamine model of schizophrenia remains heuristically valuable, many aspects of schizophrenia cannot be explained based upon dopaminergic dysfunction alone, and many patients with schizophrenia remain persistently disabled despite treatment with various dopaminergic compounds. Glutamatergic theories of schizophrenia account for negative symptoms and cognitive dysfunction, as well as positive symptoms, and thus may lead to new treatment approaches specifically targeting this unmet medical need. Improving the future treatment of schizophrenia and increasing our biological understanding of the disease will be contingent on development of appropriate models and biomarkers for glutamatergic drug development. In particular, studies focusing on mechanistically based and clinically relevant dynamic circuit models are needed to consolidate the evolving genetic data with translational physiological measures. Although clinical drug development progresses slowly, the field has now progressed to the point where treatment predictions of the glutamate model can be tested.

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DISCLOSURE

Bita Moghaddam declares no conflict of interest. Daniel Javitt holds intellectual property rights for use of glycine, D-serine, and glycine transport inhibitors in treatment of schizophrenia and equity interest in Glytech. Within the past year, Dr Daniel Javitt has served as a consultant to Sepracor, AstraZeneca, Pfizer, Cypress Bioscience, Merck, Sunovion, Eli Lilly, and BMS, and has received research support from Pfizer, Roche, and Jazz Pharmaceuticals. Dr Daniel Javitt serves on the Scientific Advisory Board of Prementis.

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