Beyond the dopamine receptor: novel therapeutic targets for treating schizophrenia

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Limitations of antipsychotic medications in schizophrenia

To this day, the pharmacological management of schizophrenia is based upon the serendipitous discovery, over 50 years ago, of the antipsychotic effects of chlorpromazine. Subsequent drug discovery for schizophrenia treatments was directed at identifying agents with comparable properties inferred by quite indirect criteria such as protection against apomorphine-induced canine vomiting or improvement in the conditioned avoidance response, while at the same time seeking increased potency and attenuated neurologic side effects. Carlson proposed that antipsychotic drugs produced their therapeutic effects by blocking dopamine receptors. Advances in ligand-binding techniques led Snyder and Seemen to demonstrate that there was a specific and highly robust correlation between the clinical potencies of antipsychotics and their ability to block the dopamine D2 receptor. With the target of therapeutic action clearly identified, pharmacologists could then “build” into new agents other neurotransmitter receptor interactions to minimize side effects. However, these modifications, while virtually eliminating extrapyramidal side effects, introduced other serious problems including weight gain, hyperlipidosis, and glucose intolerance.

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differences in effectiveness between first-generation antipsychotics and second-generation antipsychotics in non-refractory patients. As pointed out by Lieberman, both the CATIE and the CUtLASS studies are “effectiveness” studies, which examine the therapeutic response in real-world clinical situations. This design is markedly different from the randomized clinical trial of “efficacy,” in which a new drug is compared with placebo in a very select group of patients subject to a myriad of exclusionary criteria. Thus, basing a drug discovery effort for schizophrenia on the assumption that it is primarily a disorder of dopaminergic dysfunction has led to the introduction of antipsychotics that are marginally more efficacious than their “progenitors,” chlorpromazine and haloperidol.

Starting about 20 years ago, psychopharmacologists began to focus on other components of schizophrenia rather than just the antipsychotic responsive positive symptoms (ie, hallucinations, delusion, and thought disorder). Negative symptoms including apathy, poverty of thought, anhedonia, lack of drive, disorganization, and social isolation were observed to covary independently of positive symptoms, be much more enduring, and correlate inversely with outcome. With advances in neuropsychology, much more rigorous testing delineated the specific impairments in memory, problem-solving, and executive functions, which were noted a century ago with the designation of “dementia praecox.” At the same time, progress in both structural and functional brain imaging revealed substantial cortical involvement in schizophrenia. On average, cortical volume is reduced and lateral ventricular volume is increased in individuals with a first episode of schizophrenia. While meta-analyses comparing the first-generation antipsychotics to the second-generation antipsychotics do suggest some modest superiority of the second-generation antipsychotics, these effects are limited to positive symptoms known to be sensitive to D2 receptor antagonism. In the large-scale CATIE (Clinical Antipsychotic Trials of Intervention Effectiveness) trial, Lieberman et al compared several second-generation antipsychotics with a first-generation antipsychotic, perphenazine. The majority of patients in each group discontinued their antipsychotics owing to inefficacy or intolerable side effects. When clozapine was compared with other second-generation antipsychotics, it did exhibit modest but significant superiority over these other drugs. A separate study carried out in England, Cost Utility of the Latest Antipsychotic Drugs and Schizophrenia Study (CUtLASS 1), also found few
The N-methyl-D-aspartate receptor and schizophrenia

Dissociative anesthetics such as ketamine and phencyclidine (PCP) have been known since their introduction a half-century ago to produce in adults a syndrome difficult to distinguish from schizophrenia. While these drugs have complex interactions in the nervous system, Javitt and Zukin noted that the psychotomimetic effects of PCP occurred at plasma concentrations that cause a noncompetitive, use-dependent antagonism of N-methyl-D-aspartate (NMDA) receptors. Ketamine infused in normal volunteers at doses that do not cause delirium/dementia produced the full range of signs and symptoms of schizophrenia, with positive symptoms, negative symptoms, and the selective cognitive deficits. Subsequent studies showed that low-dose ketamine caused in normal volunteers the physiologic abnormalities associated with schizophrenia, including abnormal event-related potentials, eye-tracking abnormalities and enhanced subcortical dopamine release. Individuals with stabilized schizophrenia exhibited marked sensitivity to ketamine with recurrence of individual specific symptoms.

With a greater availability of brain tissue for histologic and neurochemical analyses, a number of findings have crystallized over the last 15 years as they have been confirmed in different laboratories using a variety of techniques including quantitative neurochemistry, immunocytochemistry, in situ hybridization, and DNA chip arrays. One of the first neurochemical abnormalities described in postmortem studies in schizophrenia was a reduction in the cortical activity of glutamate decarboxylase (GAD), the enzyme that synthesizes γ-amino butyric acid (GABA), in the cortex. More recent studies have revealed a much more selective effect primarily on the parvalbumin (PV)-expressing, fast-firing GABAergic interneurons in the intermediate layers of the cortex and in subsectors of the hippocampus that provide recurrent inhibition to the pyramidal cells. Thus, the reduction in the expression of GAD67, PV, and the GABA transporter has been demonstrated in this neuronal population. That the downregulation of these presynaptic markers reflects reduced activity of these GABAergic neurons is inferred by the compensatory upregulation of postsynaptic GABA receptors and its α2-containing subunit. Another recurrent finding from Golgi-stain studies and more recent immunocytochemistry of spinophilin, a protein enriched in dendritic spines, is the reduction in dendritic complexity and spine density on pyramidal neurons in several cortical regions, consistent with the overall cortical atrophy in schizophrenia.

These core pathologic features of schizophrenia have been linked to NMDA receptor hypofunction. Several studies have demonstrated that subacute treatment of rats with dissociative anesthetics results in a downregulation of GAD67 and PV expression in the GABAergic neurons in the intermediate layers of the cortex and a consequent disinhibition of pyramidal neuronal firing. This disinhibition of the pyramidal neurons is consistent with the results of functional imaging studies in the hippocampus, as well as the elevated evoked subcortical dopamine release in normal individuals challenged with ketamine. The paradoxically reduced firing of the PV-GABAergic interneurons may be secondary to the decreased flux of calcium through their NMDA receptors, which causes a misperception of reduced excitatory drive. NMDA receptors also play an important role in dendritic elaboration and spine development. Mice that are homozygotes for a null mutation of serine racemase, the enzyme that synthesizes D-serine, exhibit marked reduction in NMDA receptor function. Cortical pyramidal neurons of these serine racemase knockout mice have significantly reduced dendritic complexity and spine density, as compared with their wild-type littermates, with the pathology quite similar to that observed in schizophrenia.

Schizophrenia is a disorder with a high degree of heritability, and recent genetic studies have provided support for a role for NMDA receptors in this disorder. Most of the evidence is derived from association studies, although that strategy has come under criticism by advocates of “unbiased” genome-wide association study (GWAS) strategy. Meta-analysis has strongly implicated the gene encoding D-amino acid oxidase (DAAO), which regulates the availability of D-serine, as well as G72, a gene encoding a protein that binds to and inhibits DAAO (for review, see ref 42). Meta-analysis has also pointed to NR2B, a component of the NMDA receptor, as a risk gene for schizophrenia. Other risk genes include neuregulin 1, which among other actions directly modulates NMDA receptor activity, and dysbindin, which is concentrated in glutamatergic terminals. Integrating the postmortem, genetic, and animal modeling results has suggested a plausible pathologic circuit in schiz-
ophrenia (Figure 1). Hypofunction of corticolimbic NMDA receptors on the fast-firing PV+GABAergic interneurons in the intermediate layers of the cortex results in down-regulation of GAD67 and PV expression, reduced inhibitory postsynaptic potentials (IPSPs), and disinhibition of the postsynaptic pyramidal cells. NMDA receptor hypofunction can be due to elevated endogenous inhibitors such as kynurenic acid or N-acetyl aspartyl glutamate (NAAG), reduced availability of the endogenous co-agonist D-serine, or heritable abnormalities in NR2B expression or function. Electrophysiological correlates include loss of gamma-band responses to sensory stimuli and elevated neuronal activity in the default mode. Disinhibition of glutamatergic output from the ventral hippocampus would drive the firing of dopaminergic neurons in the ventral tegmental area and enhanced subcortical dopamine release, which in PET studies correlates with psychosis. Thus, in this model, psychosis is a downstream event. Hypofunction of NMDA receptors could account for other aspects of the disorder. First, given the role of

**Figure 1.** Schematic representation of the synaptic circuitry relevant to the pathophysiology of schizophrenia. NMDA receptor hypofunction can be produced by exogenous antagonists such as ketamine, endogenous antagonists such as N-acetyl aspartyl glutamate (NAAG) or kynurenic acid, reduced availability of D-serine due to increased activity of D-amino acid oxidase (DAAO) or mutant NR2B. This results in dendritic dysplasia on pyramidal neurons and reduced activity of the parvalbumin positive GABAergic interneurons. Reduced recurrent inhibition disrupts cortical processing, causing cognitive impairment and negative symptoms and increased excitatory drive to the ventral tegmental area (VTA), leading to psychosis. An allelic variant of the gene encoding the α7 nicotinic receptor causes reduced expression and disrupts sensory gating. NMDA, N-methyl-D-aspartate; GABA, γ-aminobutyric acid; DA, dopamine; NBM, nucleus basalis of Meynert; nAChR, nicotinic acetylcholine receptor; Glu, glutamate.
NMDA receptors in neuronal migration, it could account for the finding of abnormal distribution of cortical GABAergic interneurons in some cases. Secondly, persistent hypofunction of NMDA receptors is consistent with the reduced pyramidal neuron dendritic complexity, reduced spine density, and net compaction of the neuropil in schizophrenia. Obviously, the pathophysiology of schizophrenia is much more complex and nuanced than suggested by this simplified model. Indeed, a number of putative risk genes encode transcriptional factors that affect brain development. Other risk genes encode products involved in myelination. Furthermore, in recognition of the variation in symptoms among patients who satisfy the diagnostic criteria for schizophrenia and its complex genetics, where literally hundreds of genes of modest effect might be involved, the proposed “pathologic circuit” represents at best a crude first approximation of the pathophysiology of schizophrenia. Nevertheless, it does yield a host of potential targets for therapeutic intervention.

Figure 2. Potential pharmacologic interventions to treat schizophrenia: (i) Enhance NMDA receptor function by increasing synaptic glycine concentrations with an inhibitor of GlyT1, administering exogenous D-serine, inhibiting D-amino acid oxidase or by treating with an mGluR5 agonist that augments NMDA receptor function; (ii) Increase the excitability of the parvalbumin-positive GABAergic interneurons with a α7nicotine receptor-positive modulator; (iii) Reduce pyramidal neuron excitability with GABA receptor-positive modulator; (iv) Decrease disinhibited pyramidal neuron glutamate release with an mGluR2/3 agonist. NMDA, N-methyl-D-aspartate; GABA, γ-aminobutyric acid; DA, dopamine; NBM, nucleus basalis of Meynert; mAChR, metabotropic acetylcholine receptor; Glu, glutamate; DAAO, D-amino acid oxidase.
and many of these are under investigation by the pharmaceutical industry. It is these potential therapeutic targets related to this circuit that are the subject of this review (Figure 2). Of particular interest is the fact that these targets would intervene in the primary cortical pathology of schizophrenia and thus potentially treat the negative symptoms and cognitive deficits.

Targeting the glutamatergic synapse

Structure and function of the NMDA receptor

The NMDA receptor, with its triple gate for activation, is a critical postsynaptic mediator of activity-dependent synaptic plasticity. Throughout most of the brain, the heterotetrameric receptor is composed of two NR1 subunits and two NR2 subunits, all of which contribute transmembrane domains to the pore of the ion channel. The NR1 subunit has eight different splice variants, which may affect channel function differently by associating with different intracellular signaling pathways. NR2 subunits may be expressed in four different forms (NR2A-D), and in some regions of the nervous system may be substituted by two different forms of NR3 subunits, each of which confer different biophysical and pharmacologic properties to the channel. Mg\(^{2+}\) occludes the ion channel at resting membrane potential. Hence, opening of the “voltage gate” by expelling Mg\(^{2+}\) with depolarization of the postsynaptic cell is one requirement for conductance through the channel. A second requirement is opening of the “ligand gate” by agonist binding at glutamate binding sites on the NR2 subunits. A third requirement is agonist binding at glycine modulatory sites (GMS, also the Glycine B receptor) on the NR1 channel-encoding subunit. Endogenous polyamines also modulate NMDA receptors by potentiating the action of glutamate. Dissociative anesthetics gain access to and bind within the NMDA receptor channel pore when the channel is open, and as such are both noncompetitive and use-dependent antagonists.

The key roles that the NMDA receptor is known to play in neurodevelopment and in activity-dependent plasticity make it all the more plausible as a contributor to the pathophysiology of schizophrenia, particularly deficits in cognitive function. Because it opens only when the postsynaptic neuron receives several simultaneous excitatory inputs to sufficiently depolarize it so as to relieve the Mg\(^{2+}\) blockade, the NMDA receptor functions as a molecular coincidence detector. The NMDA receptor ion channel is characterized by high Ca\(^{2+}\) permeability, and the influx of Ca\(^{2+}\) triggers a cascade of intracellular events that mediate local, acute synaptic plasticity as well as changes in gene expression that influence long-term neural plasticity and have trophic effects. Whether or not symptoms of schizophrenia are caused in part by hypofunctional signaling through NMDA receptor-mediated pathways, enhancing NMDA receptor-mediated activity may improve cognition and neural plasticity, thereby reducing the debilitating negative and cognitive symptoms. On the other hand, a significant risk in pursuing NMDA receptor activation as a therapeutic pathway is that of excitotoxic damage to the brain, which can result from excessive activation of NMDA receptors. With this caveat in mind, efforts to treat symptoms of schizophrenia through the NMDA receptor have focused on positive modulation of the receptor rather than increasing agonist binding at the glutamate site.

The glycine modulatory site

The GMS of the NMDA receptor is a potentially rich target for therapeutics. Despite the presence of endogenous high potency agonists glycine and D-serine, the GMS is not saturated in vivo, supporting the idea that administration of GMS agonists could benefit patients by enhancing activation of NMDA receptors. Furthermore, evidence of reduced cerebrospinal fluid (CSF) and serum D-serine levels in schizophrenic patients as well as evidence of elevated levels of the endogenous GMS antagonist kynurenate in postmortem brain and CSF suggest that the GMS occupancy is downshifted or shifted toward antagonism in the disease state. There have been more than 80 clinical trials of agents that increase agonist occupancy of the GMS in schizophrenia, including D-serine, glycine, D-cycloserine, D-alanine, and sarcosine. Several of these studies have reported significant improvements over multiple symptom domains while others have not. Aside from intrinsic differences in efficacy between candidate GMS regulators, methodological factors likely contribute to the variability in results among these trials, most notably small sample sizes, variability in concomitant typical and atypical antipsychotic use, and subject compliance. Also, important to consider from the point of view of evaluating the promise of the GMS strategy, the majority of these trials have been conducted using glycine and/or
the partial GMS agonist D-cycloserine, which are not the most potent agonist of the site. Studies employing cloned NMDA receptors expressed in a Xenopus oocyte system suggest the potency of D-serine is about three times that of glycine,\(^72\) and D-cycloserine is a partial agonist with only about half the efficacy of glycine at the GMS.\(^73\) Still, glycine and D-cycloserine have been more widely tested than D-serine due to historical approval of these agents for human use, glycine as a nonessential amino acid, and D-cycloserine as a second-line antibiotic effective against Mycobacterium tuberculosis.

A recent meta-analysis of strategies to enhance NMDA receptor-mediated neurotransmission in schizophrenia reported the striking finding that NMDA-enhancing molecules as a whole exerted statistically significant effects on total psychopathology, depressive symptoms, negative symptoms, cognitive symptoms, positive symptoms, and general psychopathology in descending order of effect size.\(^74\) The meta-analysis included results from 26 double-blind, placebo-controlled clinical trials in which the treatment lasted at least 4 weeks. Agents tested were glycine, D-cycloserine, D-serine, sarcosine, and D-alanine. Pooling of data from different studies was made possible by including only those for which enough data were available to calculate a standardized metric of the degree of improvement seen in a particular symptom domain relative to placebo, or the effect size (ES). There was some heterogeneity in the trials that were included, in that patients enrolled were administered concomitant typical or atypical antipsychotics and in others were not. Also, trials of chronic stable and acutely exacerbated schizophrenia were included. When the effects of different molecules were assessed separately, glycine was found to have significant effects on total psychopathology, positive symptoms, and depressive symptoms. D-serine was found effective on total psychopathology, negative symptoms, and cognitive symptoms. Sarcosine, an endogenous inhibitor of the glycine transporter, was effective on total psychopathology, negative symptoms, and general psychopathology. D-cycloserine was not effective on any domain of schizophrenia symptoms. However, if the trials that use clozapine as the antipsychotic are excluded, the duration of exposure restricted and compliance controlled the data suggest that D-cycloserine significantly reduces negative symptoms.\(^75\)

The findings of the meta-analysis by Tsai and Lin\(^76\) provide some interesting new illumination for the results of the largest individual study to date of glycine and D-cycloserine, a multicenter trial called the Cognitive and Negative Symptoms in Schizophrenia Trial (CONSIST). The CONSIST study found no statistically significant effects of either glycine or D-cycloserine on negative symptoms or cognitive performance in patients with chronic schizophrenia. Previous smaller studies of high doses of glycine administered concurrently with typical and atypical antipsychotics had reported improvements negative and cognitive symptoms.\(^76\) High doses were purported to be required to achieve sufficiently high serum glycine levels for clinical efficacy, and difficulty with compliance was noted. The CONSIST study did report a significant effect of site (P<0.01), as well as lower serum levels of glycine than was achieved in previous studies. Thus, one of the concerns raised about the interpretation of this study with respect to its results with glycine was that variability in patient compliance between inpatient and outpatient clinics could account for the negative result. Indeed, restricting the results to those obtained with inpatients, for whom compliance was not in question, both glycine and D-cycloserine significantly (P<0.03) reduced negative symptoms. However, the results of the meta-analysis, which includes the CONSIST study, showed that when double-blind, placebo-controlled trials with glycine were considered together, glycine still had no significant effect on negative symptoms, but rather was effective on positive and depressive symptoms, which were not assessed by the CONSIST. Nonetheless, as serum levels of glycine were not part of the meta-analysis, the lack of any significant dose-response with glycine on negative symptoms, positive symptoms, or total psychopathology is still open to the question of whether compliance is a major issue in evaluating outcomes. Despite the negative findings of glycine efficacy with respect to negative and cognitive symptoms, the issues of compliance and serum levels may still be highly relevant to schizophrenia therapy, given the significant effects of glycine on positive and depressive symptoms, particularly for patients who do not respond to antipsychotics or are experiencing adverse side effects of clozapine.

Consistent with its action as a partial agonist, an initial dose-finding study with D-cycloserine added on to conventional neuroleptics reported a U-shaped dose-response curve with an intermediate dose that improved negative as well as cognitive symptoms.\(^78\) Given that the GMS is not saturated in vivo, one might speculate that a
partial agonist would augment the activity of the NMDA receptor up to a point and then actually begin to compete with the endogenous agonist. Furthermore, this point of inflection in the nature of the D-cycloserine effect may vary depending on the individual patient’s level of GMS saturation. D-cycloserine may in any case be an impractical approach for prolonged treatment, as NMDA receptor desensitization has been observed with chronic administration. The apparent lack of consistent success of D-cycloserine use in schizophrenia stands in contrast to the positive results observed with it in extinction therapy for specific phobias. The extinction of a conditioned fear memory is an NMDA-dependent process, which can be enhanced by positive modulation of the GMS. D-cycloserine has been effective in treating acrophobia in combination of a virtual reality-based cognitive behavioral therapy. Thus, a key difference between the successful application of D-cycloserine in anxiety disorders and the unsuccessful application in schizophrenia may be the that in the former it is used acutely or subchronically as an adjunct to concomitant activation of specific brain circuitry related to specific fear or phobia.

D-serine itself is a potential therapy, as it has been shown in rodents to be relatively efficient at crossing the blood-brain barrier upon peripheral administration compared to glycine, and can persist in cortex thereafter. In contrast to other GMS agonists, there is direct indication that D-serine is affected in schizophrenia, as it is decreased in CSF and serum from patients. The significant effects of D-serine on total psychopathology, negative symptoms, and cognitive symptoms found in the Tsai and Lin meta-analysis are based only on small trials that tested it as an add-on therapy to typical or atypical antipsychotics. In the case of D-serine as well as other agents, testing in conjunction with typical or atypical antipsychotics may occlude potential effects on positive symptoms, which are relatively well controlled with available antipsychotics. Large Phase II trials of D-serine in schizophrenia and schizophrenia prodrome are currently underway, both as an add-on to antipsychotics and as a monotherapy.

An intriguing pattern in the literature on GMS agonists, corroborated by the meta-analysis, is that they are ineffective when combined with clozapine as opposed to other antipsychotics. These results together could be explained by an effect of clozapine on GMS occupancy. The mechanism of putative clozapine interaction with NMDA receptors is not yet known, but increased NMDA-mediated currents have been observed in the presence of clozapine in rat frontal cortex, and effects on glycine transport have been proposed. If clozapine, which is superior to other atypical antipsychotics in treating negative and cognitive symptoms, works through the GMS, it may be possible to achieve comparable benefits without the troubling side effects of clozapine such as agranulocytosis, weight gain, and metabolic syndrome by using other agents that enhance GMS occupancy.

D-amino acid oxidase

The peroxisomal enzyme D-amino acid oxidase (DAAO) converts D-serine to hydroxy-pyruvate in the brain, yielding hydrogen peroxide as a by-product. DAAO expression was originally believed to be restricted to astrocytes in the mammalian cerebellum, but has since been observed in neurons. Inhibitors of DAAO would be expected to increase D-serine in the brain, and could thereby increase GMS occupancy. Direct evidence of involvement of DAAO in schizophrenia is somewhat controversial. DAAO has been implicated as a putative schizophrenia gene by linkage and association methods, but meta-analyses have revealed that the disease-associated variants of the gene are different across studies, precluding a simple functional hypothesis based on the findings. Postmortem studies of brain DAAO expression in schizophrenia have reported elevated transcript levels and enzyme activity. G72, a mysterious putative interacting protein of DAAO, is coded for in a linkage region identified for schizophrenia by multiple studies, and considered one of the strongest genetic risk factors for schizophrenia identified using linkage analysis. The link between G72 and DAAO originates from a yeast 2-hybrid study from which DAAO emerged as a G72 interactor. An in vitro functional assay suggested that G72 protein is an activator of DAAO; but more recent studies demonstrate that it inhibits DAAO. According to this conceptualization, mutations in G72 would result in disinhibition of DAAO, thereby reducing the availability of D-serine. However, despite significant attention paid to it pursuant to its repeated appearance in the schizophrenia genetic literature, to date the protein has been observed only in heterologous expression systems. It should be noted that DAAO activity is not specific to D-serine, so manipulating the activity of this enzyme can affect the levels of other D-amino acids.
Several pharmaceutical companies have established DAAO inhibitor programs. While there are no published clinical data, preclinical studies have revealed promising behavioral effects. Adage et al.\(^9\) reported that DAAO inhibitor, AS057278, significantly increased cortical D-serine, corrected PCP induced prepulse inhibition (PPI) deficits and normalized PCP-induced hyperactivity, a behavioral surrogate for psychosis. Hashimoto et al.\(^9\) found that combining D-serine with the DAAO inhibitor, 5-chloro-benzo[\text{d}]isoxazol-3-ol (CBIO), markedly increased cortical D-serine levels and corrected dizocilpine-induced (MK801) PPI deficits. However, another DAAO inhibitor, in spite of elevating CSF D-serine levels, failed to normalize amphetamine-induced hyperactivity and MK801-induced disruption of cognition. As D-serine treatment was effective, it appears that DAAO inhibition must be greater than 80%, the upper limit achieved by their drug.\(^1\)

**D-Serine synthesis and transport**

D-serine, the highest-affinity endogenous GMS agonist, is synthesized from L-serine by the pyridoxal 5'-dependent enzyme serine racemase. Polymorphisms in the 5' untranslated region of the serine racemase gene, which may be functionally related to levels of its promoter activity, have been associated with schizophrenia.\(^1\) Like DAAO, serine racemase was originally believed to be restricted to astrocytes in its localization\(^1\) but has since been observed in neurons.\(^1\) Genetic knockout of serine racemase leads to a reduction of 80% to 90% in brain D-serine in mice.\(^1\) The origin of the remaining 10% to 20% is unknown but may be diet and/or bacterial flora. D-serine levels within the synapse are regulated by the arginine-serine-cysteine transporter, ASC-1,\(^1\) which is localized to neuronal somata and dendrites.\(^1\) Inhibitors of ASC-1 have been proposed as therapeutics in schizophrenia, as they would presumably elevate levels of extracellular brain D-serine. On a cautionary note, constitutive ACS-1 gene deletion in mice has been shown to cause tremors, seizures, and early postnatal death.\(^1\)

**GlyT1 inhibitors**

The concentration of glycine in mammalian CSF is high relative to its dissociation constant (K_d) for the GMS, but local glycine levels are functionally regulated at the synapse by the sodium-dependent glycine transporter-1 (GlyT1) expressed in astrocytes.\(^1\) The activity of GlyT1 is itself endogenously regulated by sarcosine (N-methylglycine), an intermediate and byproduct in glycine synthesis and degradation. Electrophysiological studies in rodents suggest that inhibition of GlyT1 is more effective than exogenous application of glycine at potentiating NMDA receptor-mediated neurotransmission. For example, in an acute hippocampal slice preparation, NMDA receptor-mediated excitatory postsynaptic potentials (EPSPs) in CA1 hippocampal pyramidal neurons were potentiated robustly by the sarcosine analog N\[3-(4’-fluorophenyl)-3-(4’-phenylphenoxy)-propyl\] sarcosine (NFPS), whereas perfusion with high concentrations of glycine (1 or 10 µM) had relatively little effect.\(^6\) Similar findings have been reported in acute frontal cortical slices.\(^1\) Systemic treatment with NFPS increased NMDA receptor currents and LTP in the dentate gyrus and enhanced prepulse inhibition of the acoustic startle response.\(^1\)

Sarcosine administered to patients in conjunction with antipsychotics has shown some promise for treatment of schizophrenia. A meta-analysis of clinical trials testing the efficacy of GMS-enhancing agents found it effective on total psychopathology, negative symptoms, and general psychopathology.\(^7\) However, undesirable side effects of sarcosine-derived GlyT1 inhibitors have also been noted, including ataxia, hypoactivity, and decreased respiration, prompting the development of novel classes of non-sarcosine-based inhibitors of GlyT1.\(^1\) Several GlyT1 inhibitors are in the early stages of clinical trials; and Hoffman-LaRoche has reported that their GlyT1 inhibitor caused significant reductions in overall symptoms and especially negative symptoms in a Phase-II clinical trial in schizophrenia.

**Metabotropic glutamate receptors (mGluRs) as therapeutic targets**

**Characteristics of mGluRs**

While ionotropic glutamate (iGlu) receptors (AMPA, kainate and NMDA subtypes) serve as the mediators of excitatory (glutamatergic) signaling, G-protein coupled metabotropic glutamate (mGlu) receptors act as modulators of excitatory signaling. Given the increased interest in the pathophysiological impact of dysfunctional glutamate signaling and their role as modulatory recep-
Pharmacological aspects

Editors, mGluRs have become a major target for the development of therapeutics for schizophrenia and other psychiatric disorders. The mGluRs are members of Class C of the G-protein coupled receptor superfamily. Eight subtypes of mGluRs have been identified and divided into three groups, based upon pharmacology, sequence homology, and G protein coupling: Group I (mGlu1 and mGlu5), Group II (mGluR2 and mGluR3), and Group III (mGluR4, mGluR6, mGluR7, and mGluR8) (for review, see ref 122). Each of these receptors possesses a distinct expression pattern that relates to physiological control over glutamatergic neurotransmission at various levels including neurotransmitter release, function of postsynaptic iGluRs, glial function, and neuroplastic changes in postsynaptic neurons. These discrete functions make these receptors very attractive targets for pharmacological intervention. Group I and II receptors have notably risen in interest as potential treatments for schizophrenia because of their ability to normalize dysfunctional glutamatergic neurotransmission thought to be a core feature of the disorder.

Group II mGluRs

Group II mGluRs are promising therapeutic targets because of their role as autoreceptors in the regulation of glutamate release from nerve terminals. Activation of Gαi/o-coupled mGluR2/3 receptors attenuate electrically evoked excitatory neurotransmission. Pharmacologically evoked and spontaneous excitatory currents are attenuated by mGluR2/3 activation, with effects predominantly on the frequency of currents, supporting a presynaptic mode of activity. Preclinical observations have been made that psychotomimetic drugs that act as noncompetitive blockers of NMDA receptors (eg, PCP, ketamine, MK801) cause an increase in synaptic glutamate levels in the prefrontal cortex (PFC). As reviewed above, the deficient PV-GABAergic neuron function found in postmortem studies in schizophrenia has led to the hypothesis that the pathophysiology of schizophrenia involves a disinhibition of cortical glutamatergic neurons and that group II mGluR-mediated reduction in glutamate release account for their antipsychotic action. The development of ligands selective for mGlu2/3 receptors has allowed for the examination of this hypothesis in preclinical models of schizophrenia.

(1R,4R,5S,6R)-4-Amino-2-oxabicyclo[3.1.0]hexane-2,6-dicarboxylic acid (LY379268) and (1S,2S,5R,6S)-2-Aminobicyclo[3.1.0]hexane-2,6-dicarboxylic acid (LY354740) are highly selective agonists of mGlu2/3 receptors possessing >100-fold selectivity over other subtypes of mGluRs. These ligands have been shown to reverse the behavioral disruptive effects of the psychotomimetic PCP in numerous paradigms including stereotypy and hyperactivity, social interactions and cognition. These ligands also display apparent antipsychotic efficacy by inhibiting the behavioral effects of psychodelic hallucinogens that influence glutamatergic signaling via serotonin 2A receptors, an effect linked to the inhibition of glutamate release from nerve terminals. A structurally related compound, (1R,4S,5S,6S)-4-amino-2-sulfonylbicyclo[3.1.0]hexane-4,6-dicarboxylic acid (LY404039), administered via a prodrug form, exhibited promising efficacy in a Phase II clinical trial, reversing positive and negative symptoms in schizophrenic patients as a standalone therapy. This therapeutic efficacy was similar to that of olanzapine and was achieved without any of the side effects of commonly prescribed antipsychotics such as elevated prolactin, weight gain, and extrapyramidal symptoms. The achievement of this clinical trial is twofold; it: (i) provides proof of concept for the development and application of glutamatergic based therapeutics and (ii) demonstrates the predictive validity of the PCP/ketamine model of schizophrenia. This second point was initially supported by research demonstrating that the cognitive-disruptive effects of ketamine in humans were indeed attenuated by an mGlu2/3 receptor agonist.

The work with mGluR2/3 receptor agonists also highlights another key mechanistic point about potential schizophrenic therapies: they need not reverse hyper-dopaminergic neurotransmission. All current therapies block D2 receptors to some degree, which has been assumed to be necessary for therapeutic efficacy. The work of Moghaddam and Adams illustrates that the major element of psychotomimetic drug (PCP or ketamine) action is to stimulate glutamatergic neurotransmission (paradoxical to the action of these drugs as NMDA receptor blockers), with dopamine release coincidental. Notably, mGluR2/3 agonists achieve behavioral effects that are paralleled by inhibition of drug-induced glutamate efflux without affecting drug-induced increases in extracellular dopamine levels measured by in vivo microdialysis. Single-unit recordings in awake rats are further illustrative; mGluR2/3 receptor agonists reversed NDMA receptor blocker-induced disinhibition.
and dysregulation of prefrontal pyramidal neuron firing. The results of these studies suggest that antipsychotic efficacy can be achieved in the absence of a direct effect on forebrain dopamine, an effect alluded to in earlier research showing a temporal disconnect between the behavioral effects of PCP and modulation of DA, but not glutamate, brain levels.

**Positive allosteric modulation of mGlu2 receptors**

Efforts to refine the mGlu2/3 agonists have focused upon finding a ligand that selectively activates mGlu2 receptors. Discriminating between mGluR2 and mGluR3 subtypes has been difficult, as they share >90% sequence homology. Expression studies suggest mGluR2 are predominately localized to presynaptic sites,

while mGluR3 are localized more postsynaptically and in glial cells. Using mGluR2-deficient mice, the apparent antipsychotic effects of mGluR2/3 agonists have been attributed to mGluR2 activation. These studies demonstrate the potential for selective activation of mGluR2; however, efforts to develop agonists of the glutamate-binding (orthosteric) site have not surprisingly fallen short. Recently, greater efforts have been undertaken to pursue ligands that activate the receptor through sites other than agonist binding site, termed allosteric sites. The success of these efforts illustrate that while the orthosteric site is highly conserved between the two receptors, allosteric sites are located in less conserved regions of the receptor and can be selectively targeted to modulate agonist-induced signaling.

Allosteric modulators can be either positive or negative in direction of activity, causing an increase or decrease, respectively, in the activity of orthosteric ligand induced signaling by altering agonist affinity and/or efficacy of G-protein coupling. In the case of mGlu2 receptors, efforts have been directed towards identifying positive allosteric modulators (PAMs). To date, numerous PAMs haven been identified and shown to possess selective efficacy to enhance agonist activity at mGlu2 receptors with dramatic selectivity over other targets. These ligands increase the ability of endogenous glutamate and exogenous agonists to reduce evoked excitatory postsynaptic potentials in brain slice preparations. Behavioral studies show that mGlu2 receptor PAMs possess efficacy similar to that of mGluR2/3 agonists, reducing PCP induced locomotion, decreasing fear-potentiated startle, and diminishing hallucinogen-induced stereotypies.

Interestingly, one study showed that one PAM, biphenyl-idanone A (BINA), was capable of uniquely reducing PCP-induced deficits in PPI. These studies demonstrate the validity and therapeutic potential of selectively targeting mGluR2. While issues of in vivo potency remain for currently available ligands, PAMs possess potential benefits. The selective potentiation of endogenous signaling would work to enhance the activity-dependent neurotransmission, while avoiding the potentially deleterious effects of persistent receptor activation, notably desensitization and tolerance.

**Cystine-glutamate exchanger**

Discussion of mGluR2 activation has focused on the development of ligands that directly target these receptors with either direct agonists or PAMs that require endogenous glutamate for activity. An additional way to enhance activity at these receptors is through the modulation of extrasynaptic glutamate levels. The cystine-glutamate exchanger maintains 60% of the extra-synaptic glutamate concentration. The exchanger is located on the glial cell membrane and releases glutamate in a 1:1 ratio with the import of cystine. Activation of this exchanger produces a reduction in excitatory neurotransmission by mGluR2/3-dependent mechanism. The compound N-acetylcysteine (NAC) is a substrate for this exchanger that substitutes for cystine. The work of Kalivas and colleagues has done much to demonstrate the behavioral effects of NAC in a preclinical model of drug-seeking behavior used to understand addiction. In these studies, NAC reduces drug-seeking behaviors and reinstatement of drug consumption after extinction in a manner that is similar to the effects of an mGluR2/3 agonist.

More germane to the current review, recent work has demonstrated the ability of NAC to reverse PCP-induced deficits in cognition and social interactions, as well as PCP-induced activation of glutamate release in the prefrontal cortex of rodents.

These findings provide an additional context for the interpretation of results of a recent clinical trial with NAC (or placebo control) given as an adjunct therapy to schizophrenic patients. The investigators saw a significant improvement over placebo in Positive and Negative Symptom Scale (PANSS) negative scale and overall Clinical Global Impression (CGI-S) after 24 weeks of treatment. General functioning (Scale of Global Assessment of Functioning) improved within a
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comparison of NAC-treated patients, but not as a comparison to placebo treatment (NB, within-group comparison of placebo treatment was not significant). The investigators undertook this clinical trial to test the effect of restoring glutathione deficiency in schizophrenia as a treatment. Cystine and correspondingly NAC are precursors to the production of glutathione, a molecule necessary for the protection against the effects of reactive oxygen species.\textsuperscript{105} Is the physiological effect of NAC treatment prevention of oxidative damage or a restoration of glutamatergic tone on presynaptic mGluR2/3? In spite of preclinical studies demonstrating that mGluR2/3 antagonists block the effects of NAC, further studies need to be done to clarify this point. Perhaps the beneficial effects of NAC are twofold at the molecular level. Given the sensitivity of NMDA receptor function to redox state,\textsuperscript{109,110} this might be an ideal way to use a single therapy to target NMDA receptor hypofunction and oxidative stress.

Activation of mGlu5 receptors

While development of ligands targeting group II mGluRs is focused on reversing excessive, dysfunctional glutamate release downstream of cortical disinhibition, mGluR5 selective activators are sought to directly reverse NMDA receptor hypofunction though enhancement of the ionotropic receptor activity. A functional link is formed between \( \alpha_\text{Glu}\)-coupled postsynaptic mGlu5 receptors and NMDA receptors by the scaffolding protein Homer and Shank interacting with the postsynaptic density.\textsuperscript{111} NMDA receptor signaling in hippocampal slices is selectively potentiated by the mGluR5 agonist (RS)-2-Chloro-5-hydroxyphenylglycine (CHPG).\textsuperscript{112,113} The specificity for mGluR5 versus mGluR1, of this effect on NMDA receptor currents is further demonstrated by the absence of potentiated signaling in the presence of mGluR5 (but not mGluR1) antagonists.\textsuperscript{115,116} Available mGluR5 agonists suffer from poor brain penetration. As a result, much of the in vivo preclinical work demonstrating the role of mGlu5 receptors was done using the centrally active mGluR5-selective antagonist 2-Methyl-6-\((\text{phenylethynyl})\)pyridine hydrochloride (MPEP). MPEP potentiates the locomotor hyperactivity\textsuperscript{115,116} and PPI disruption\textsuperscript{115,117} caused by either PCP or MK801. These effects were seen without any effect on activity or PPI in the absence of PCP/MK801. MPEP also enhances the detrimental effects of PCP/MK801 in cognitive tasks of working memory and instrumental learning.\textsuperscript{118,119} In vivo single-unit recordings show that MPEP enhances the MK801-induced increase in neuronal activity, thereby linking the behavioral findings back to the electrophysiology.\textsuperscript{120}

Like the Group II mGluRs, recent research demonstrates that the most effective strategy to selectively activate mGlu5 versus mGlu1 may be through the use of PAMs. Two unique PAMs, 3-Cyano-N-(1,3-diphenyl-1H-pyrazol-5-yl)benzamide (CDPPB) and (S)-(4-fluorophenyl)-(3-[3-(4-fluoro-phenyl)-[1,2,4]-oxadiazol-5-yl]piperidin-1-yl)methanone (ADX47273), have been developed and shown to display dramatic mGluR5-selectivity and the ability to increase the efficacy of glutamate to activate mGlu5-mediated potentiation of NMDA receptor signaling.\textsuperscript{120,121} Furthermore, the PAMs are systematically active and display antipsychotic-like properties, blocking amphetamine-induced hyperactivity,\textsuperscript{120,123,124} PCP-induced hyperactivity,\textsuperscript{120} and amphetamine/apomorphine-induced disruption of PPI.\textsuperscript{120,121} In the 5-choice serial reaction time task, ADX47273 reduced impulsive errors.\textsuperscript{170} Taken together these results demonstrate the potential antipsychotic-like ability of mGlu5 receptor PAMs to reduce the behavioral effects of multiple classes of psychotomimetics as well as produce procognitive effects. The efficacy in models of disrupted PPI is a divergence from that of mGluR2/3 agonists and suggests that these two approaches might have distinct therapeutic profiles. The limited preclinical testing and absence of any clinical demonstration of mGluR5 activation as a therapeutic target in schizophrenia temper enthusiasm. However, the demonstrated ability to enhance NMDA receptor signaling at the neuronal level will encourage the future development and testing of mGluR5 ligands.

GABA\textsubscript{A} receptors as therapeutic targets for schizophrenia

GABAergic pathology in schizophrenia

There is now substantial evidence that GABA signaling is deficient in corticolimbic regions, particularly in the dorsal lateral prefrontal cortex (DLPFC) and hippocampus, of patients with schizophrenia. One of the most consistent postmortem findings in schizophrenia is a reduction in the mRNA expression level of GAD\textsubscript{67} in PV\textsuperscript{-}-GABAergic interneurons, as well as reductions in PV expression itself.\textsuperscript{172} PV\textsuperscript{-} interneurons exhibit fast-
spiking firing properties and target the spike-initiating region of pyramidal neuron axons, and are therefore thought to play a key role in controlling the overall firing properties of brain networks. Recent pharmacological, immunological, and genetic evidence from animal models suggests that inflammatory cytokine exposure (increased oxidative stress) and NMDA receptor hypofunction occurring during cortical development leads to permanent disturbances in neuronal circuits, specifically in the population of PV-containing interneurons. The reduced GABA signaling by PV-ergic neurons could contribute to the working memory deficits observed in schizophrenia. PV-ergic interneurons control the rate of pyramidal cell firing, thereby synchronizing oscillatory activity of cortical pyramidal neurons in the gamma band range (30 to 80 Hz). Gamma oscillations regulate working memory and the transmission of information between cortical regions. Therefore, it is hypothesized that the asynchronous pyramidal neuronal activity resulting from aberrant PV-ergic GABAergic signaling contributes to the cognitive dysfunction observed in schizophrenia. It is because of this hypothesis that GABA_A receptors are now being considered a viable pharmacologic target for treating the cognitive disturbances associated with schizophrenia.

GABA_A receptors are membrane proteins that form a heteropentameric GABA-gated chloride channel, which mediate largely tonic and phasic inhibition. They are composed of several classes of subunits (α1-6, β1-3, γ1-3, δ, ε, θ, ρ1-3), but generally consist of three types of subunits (α, β, γ). The majority of GABA_A receptors are characterized by their sensitivity to benzodiazepines. These receptors contain subunits (α1, α2, α3, or α5), a β subunit (mainly β2 or β3), and in almost all cases the γ2 subunit in a 2:2:1 stoichiometry. Benzodiazepine-insensitive receptors contain α4, α6, or δ instead of γ2. In addition to their structural diversity, GABA_A receptor subtypes have different expression patterns, pointing to unique roles for these receptor subtypes in regulating neuronal activity.

Postmortem and genetic evidence suggest that α2/α3-containing GABA_A receptors are the most relevant targets for the treatment of cognitive dysfunction in schizophrenia. It is the α2-containing GABA_A receptors that are up regulated on the postsynaptic axon initial segments of pyramidal neurons in schizophrenia. In mice, deletion of the α3 subunit results in mild hyperactivity and a pronounced deficit in PPI of the acoustic startle response, suggesting a hyperdopaminergic phenotype. Targeting these specific receptor subtypes would circumvent the adverse cognitive and sedative effects associated with nonspecific agonists, like benzodiazepines, which are attributable to their affinity for α1 and/or α5-containing GABA_A receptors.

α2-GABA_A receptors

A recent proof-of-concept trial was conducted with MK-0777, a benzodiazepine-like compound selective for GABA_A receptors containing α2 or α3 subunits, to determine whether selective enhancement of GABAergic transmission would improve cognitive functions and gamma oscillations in patients with schizophrenia. MK-0777 improved the performance of patients in several working memory tasks, and was associated with increased gamma band power in the frontal cortex during task performance. However, MK-0777 did not significantly alter scores on the Brief Psychiatric Rating Scale (BPRS) or Repeatable Battery for the Assessment of Neuropsychological status, except for improvement in the delayed memory index in the latter test.

α3-GABA_A receptors

Mouse genetics supports the hypothesis that α3-GABA_A receptors are involved in sensorimotor gating, a process that is disrupted in schizophrenia. Compounds that would selectively augment signaling through these receptors would be potentially beneficial in treating this schizophrenia endophenotype. As the α3-containing GABA_A receptor is the major subtype expressed on dopaminergic and other monoaminergic neurons, agonists at this receptor might augment the inhibitory tone of these neurons and reverse their hyper-functioning state in psychosis.

Cholinergic therapeutic targets

Muscarinic receptors

Muscarinic acetylcholine (mACH) receptors are widely distributed throughout the neocortex and are promising targets for numerous neurological and psychiatric disorders. Five isoforms (M1-M5) of these G-protein coupled metabotropic receptors have been identified and
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characterized. The therapeutic potential for muscarinic receptor activation in schizophrenia is fueled in large part by the efficacy of acetylcholine esterase inhibitors, which elevate synaptic acetylcholine levels, in reducing behavioral disturbances in Alzheimer’s disease patients that are reminiscent of symptoms of schizophrenia. These effects are in addition to the primary cognitive enhancement due to the therapy. Efficacy of these treatments could likely be due to downstream activation of mACh receptors. Consistent with the hypothesized therapeutic impact of mACh receptor activation is a small clinical trial in schizophrenia showing antipsychotic efficacy of the putative M1/M4 selective mACh receptor agonist xanomeline.

Current cholinergic therapeutics are limited in their applicability because of aversive side-effect profiles that are attributed to peripheral activation of M2 and M3 mACh receptors. For this reason, the development of subtype selective ligands has been a major interest. M1 and M4 subtypes are of greatest interest in schizophrenia, given the efficacy of xanomalone (an M1/M4-preferring agonist) and postmortem findings of reduced M1 and M4 receptor densities in schizophrenia. Studies with mutant mice support the targeting of M1 and M4 receptors. Null deletion mutants of M1 receptors display deficits in working memory and social memory, as well as elevated baseline dopamine turnover and increased sensitivity to the behavioral and neurochemical effects of amphetamine. Likewise M4 null mutant mice display hypersensitivity to amphetamine and PCP-induced increases in nucleus accumbens dopamine, consistent with an involvement of NMDA receptors.

In the absence of selective pharmacological tools, mutant animal studies have been used to improve our understanding of the neurophysiological role of mACh receptors. M4 null mutant mice display enhanced baseline ACh efflux with in vivo dialysis in various brain regions, consistent with a prominent role as an autoreceptor. The finding that M1 null mutation abolishes ACh-mediated LTP of pyramidal neurons in the hippocampus complements earlier work suggesting a similar role for M1 receptors in the potentiation of NMDA receptor currents. Taken together, these studies suggest that M1 mACh receptors possess activity similar to that of mGlu5 receptors, modulating NMDA receptor signaling postsynaptically. mACh receptors, like mGluRs, have proven to be difficult to selectively target at the orthosteric site. The agonist xanomalone, though often touted as M1/M4-selective, possesses prominent affinity for other subtypes. Recent progress has been made in the development of M1 and M4 PAMs and allosteric agonists for mACh receptors. As with mGlu receptors, allosteric modulation appears to be a promising route for achieving pharmacological selectivity. Recent studies describe the activity of a M1-selective allosteric agonist, 1-(1’-2-methylbenzyl)-1,4’-bipiperidin-4-yl)-1H-benzo[d]imidazol-2(3H)-one (TBPB) and a PAM, benzylquinolone carboxylic acid (BQCA).

These PAMs display in vivo efficacy, reducing amphetamine-induced hyperactivity and BQCA enhanced reversal learning in a murine transgenic model of Alzheimer’s disease. Likewise the selective targeting of M4 receptors has proven successful via allosteric modulation. These limited pharmacological studies serve as merely a proof of concept. As these compounds (and others with optimized pharmacokinetics) are more widely tested, we are likely to gain a better understanding of the function of and therapeutic potential for targeting M1 and M4 ACh receptors.

Nicotine and schizophrenia

The involvement of nicotinic acetylcholine receptors (nAChRs) in the pathophysiology of schizophrenia was initially suggested by behavioral and biochemical data. People with schizophrenia, in both inpatient and outpatient settings, smoke cigarettes at a rate (80%) more than threefold higher than the general population smoking rate in the United States. They are also heavier smokers and extract more nicotine per cigarette smoked than the general population. Their motivation to quit smoking is low and the smoking cessation rates are lower than the rates of the general population. Furthermore, in schizophrenic patients, cigarette smoking normalized their deficits in sensory gating. Patients with schizophrenia also have reductions in the numbers of [3H]-cytisine and [3H]-a-bungarotoxin binding sites in the hippocampus as well as elevated serum levels of nAChR antibodies compared with controls.
The high rate and heavy level of smoking in schizophrenic subjects suggest that they might be medicating themselves with nicotine to reduce cognitive impairments associated with the disorder and/or antipsychotic treatment. Patients report that they smoke as a sedative, to reduce negative symptoms, and to counteract medication side effects. Studies have demonstrated that nicotine administration produces positive effects on sensory gating, eye movements, negative symptoms, some cognitive tasks, and movement disorders. Although nicotine is therapeutic for certain aspects of schizophrenia, it has several limitations that hinder its clinical utility. Nicotine induces tachyphylaxis and carries abuse liability. The long-term risks of chronic treatment are unknown but might include carcinogenic features and cerebro- or cardiovascular risks. Therefore, novel nicotinic agonists have been developed that are more selective than nicotine for particular nAChR subtypes, and may provide cognitive benefits similar to nicotine, with fewer adverse side effects.

nAChRs

Neuronal nAChRs are widely expressed in the central nervous system and mediate fast synaptic signaling and the release of other neurotransmitters. They are involved in numerous physiological functions including cognition (attention and working associative memory performance), neuronal development, particularly in the sensory cortex, and reward mechanisms via the mesocorticolimbic system. Cholinergic modulation also plays a critical role in the functioning of neural circuits, including those involving glutamatergic, GABAergic, and dopaminergic innervations.

nAChRs are excitatory neurotransmitter-gated ion channels that belong to a superfamily that includes other ionotropic receptors for 5-HT, glycine, and GABA. This family of receptors is comprised of 16 different subunits in humans (α1-7, α9-10, β1-4, δ, ε, γ). This wide variety of subtypes of nAChRs arising from combinations of subunits displays a range of different functional and pharmacological properties. Neuronal nAChRs are assembled from five transmembrane subunits that are arranged around a central water-filled pore. Neuronal subunits that form nAChRs in αβε combinations include α2–α6 and β2–β4. Although most nAChRs subunits assemble only into heteropentameric receptor ion channel combinations, the α7 subunits are able to generate functional homomeric nAChRs. nAChRs composed of α4β2 and α7 subunits make up the majority of the nAChRs in the brain. There are two ACh binding sites per receptor. Mammalian nAChRs are cation-selective, being permeable to small monovalent and divalent cations like Ca²⁺. Nicotinic receptor activity causes depolarization, and the divalent cation permeability plays an important physiological role by supplying ionic signals, including Ca²⁺.

α7nAChRs

α7 nAChRs are abundantly expressed in the hippocampus and cortex. They have distinct characteristics due to their homopentameric composition that distinguishes them from the other nAChR subtypes. α7 nAChRs are rapidly desensitizing, are of order of magnitude less sensitive to nicotine than an agonist, and have a higher calcium permeability than other nAChRs. Because cholinergic innervation arises from projections that send diffuse afferents to a broad range of brain areas, nicotinic activity is a modulatory signal that subtly influences many neurotransmitter systems and contributes to the overall efficiency of various neural circuits. Cholinergic fibers innervate the entire hippocampus with synaptic contacts made onto granule cells, pyramidal cells, interneurons, and neurons of the hilus. The hippocampus expresses a wide variety of nAChR subunits, but the α7, α4, and β2 subunits predominate. The GABAergic interneurons more densely express nAChRs than do the glutamatergic cells. Activation of α7nAChRs on presynaptic terminals of glutamatergic pyramidal neurons increases intraterminal Ca²⁺ levels to facilitate glutamate release. α7nAChRs are also present in high density at postsynaptic sites on PV⁺-GABAergic interneurons that are vulnerable in schizophrenia, where they mediate fast cholinergic excitatory transmission. In the cortex, cholinergic innervation sparsely reaches all layers, but layer V is the most heavily innervated, especially in the motor and sensory areas. The manner in which nicotinic signaling affects cortical activity is dependent on which part of the pyramidal cell the nAChRs are activated. Activation of nAChRs on distal apical dendrites depolarizes the cell and promotes action potential firing, while activation on proximal apical dendrites reduces membrane impedance and shunts signals from the apical tuft. Midbrain dopamine neurons in the substan-
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tia nigra and ventral tegmental area (VTA) express a variety of nAChR subunits (α4-α7 and β2), with β2 subunit containing nAChRs dominating (~40%) of rat dopaminergic VTA neurons express the α7 nAChR subunit.214 Cholinergic afferents into the midbrain enhance glutamate transmission via mainly presynaptic α7 nAChRs on glutamatergic terminals,215 thereby influencing the firing frequency and firing modes of DA neurons.216

Association of α7nAChRs with schizophrenia

α7 nAChRs have been associated with schizophrenia across several domains. A linkage was found between the α7 nAChR and schizophrenia on chromosome 15q13-14,217 a region containing the gene that encodes for the α7 nAChR (CHRNA-7). Although no amino acid-coding region polymorphisms have been found, multiple single-nucleotide polymorphisms (SNPs) in the promoter region of CHRNA-7 as well as a partial duplication of CHRNA-7, have been characterized, with certain alleles more frequently present in people with schizophrenia.218 Reduced α7 receptor binding was found in the reticular nucleus of the thalamus,219 hippocampus,220 and cingulate cortex.221 Moreover, there were reduced α7 subunit levels in the DLPFC,222 as well as reduced mRNA expression of α7 in peripheral blood lymphocytes223 of patients with schizophrenia. In addition to the clinical data, preclinical evidence implicates α7nAChR function in regulating cognition. Mice deficient in α7nAChRs have impaired sustained attention,224 while administration of α7nAChR antagonists225 and agonists226 impair and enhance, respectively, working memory in rodents.

α7nAChR full agonists

The α7nAChR agonist, (-)-spiro[1-azabucyclo[2,2,2]octane-3,5’-oxazolidin-2’-one] (ARR 17779), significantly improved learning and memory in rats,227 while an α7nAChR agonist with 5-HT4 receptor antagonist properties, improved the inhibition of the P50 response in schizophrenia.228 A novel selective α7nAChR agonist, 5-morpholin-4-yl-pentaic acid (4-pyridin-3-yl-phenyl)-amide (SEN12333), with only weak antagonist activity at α3-containing receptors, was shown to have procognitive properties in rats across several domains, including episodic memory, attention, and perceptual processing.229

α7nAChR partial agonists

3-(2,4 Dimethoxy)benzylidene-anabaseine (DMXBA) is one of a series of compounds derived from anabaseine, an alkaloid found in marine worms. DMXBA is a partial agonist at the α7nAChR and is a weak competitive antagonist at the α4/β2 nAChR and at the 5-HT3 receptor. The metabolites of DMXBA are also active at these receptors, but their biological effect may be limited due to their greater polarity, and therefore greater difficulty in crossing the blood-brain barrier. In preclinical animal models, DMXBA was shown to improve learning and memory-related behaviors in multiple paradigms, including nonspatial avoidance task,226 delayed matching to sample,227 Morris water maze,228 and classic eye-blink conditioning.229 DMXBA also normalizes auditory gating in the DBA/2 mouse, a strain with no sensory inhibition under routine experimental conditions.230 Because of the success of DMXBA in preclinical trials, its effects on cognition were initially evaluated in normal subjects.231 DMXBA significantly improved simple reaction time, correct detection during digit vigilance, both immediate and delayed word recall, word and picture recognition memory, and performance speed on a numeric and spatial working memory task.232 A second Phase I trial was conducted in persons with schizophrenia.233 This double-blind study found that DMXBA normalized auditory evoked responses in both the P50 ratio and the test wave amplitude in patients. DMXBA also improved performance on the Repeatable Battery for the Assessment of Neuropsychological Status (RBANS) and the Attention subscale, with effect sizes more favorable when compared with second-generation antipsychotics. However, DMXBA did not produce changes in the BPRS and therefore did not affect positive, negative, or anxiety related symptoms. An initial Phase II trial recently assessed the clinical effects of DMXBA on the Measurement and Treatment Research to Improve Cognition in Schizophrenia (MATRICS) Consensus Cognitive Battery, as well as the Scale for the Assessment of Negative Symptoms (SANS) and Brief Psychiatric Rating Scale (BPRS).234 Although DMXBA did not significantly improve MATRICS cognitive measures, patients reported significant improvements on the SANS total score, most notably on the anhedonia and alogia subscales. fMRI was also conducted in this trial to ascertain if DMXBA would have an effect on hippocampal activity.235 In schizophrenia, increased hip-
pocampal hemodynamic activity is often observed during many tasks, including smooth pursuit eye movements, and is thought to be the result of hippocampal interneuron dysfunction. DMXBA (150 mg) reduced hippocampal activity in patients during pursuit eye movements consistent with the established function of α7nAChRs on hippocampal inhibitory interneurons. R3487/MEM3454 is a partial α7nAChR agonist and a 5-HT3 receptor antagonist. R3487/MEM3454 has been shown to be efficacious in multiple animal behavioral paradigms that evaluate episodic, spatial, and working memory function as well as sustained attention.\(^{247}\) 4-bromophenyl-1,4-diazabicyclo[3,2,2]nonane-4-carboxylate-hydrochloride (SSR180711) is a partial α7 nAChR agonist, with no significant binding and/or functional activity at other human nAChRs. This compound produced electrophysiological, biochemical, and behavioral effects predictive of cognitive benefit in schizophrenia.\(^{238,239}\) SSR180711 also normalized abnormally persistent latent inhibition produced by an acute pharmacologic model (MK801) and a neurodevelopmental model (inhibition of nitric oxide production during the very early postnatal period), which are used as models of impaired cognitive flexibility in schizophrenia.\(^{240}\) Moreover, SSR180711 reversed amphetamine-induced disruption of latent inhibition, an effect considered to be predictive of activity against the positive symptoms of schizophrenia.\(^{240}\)

**Positive allosteric modulators of α7nAChRs**

Positive allosteric modulators of α7nAChRs have attracted interest as potential compounds for the treatment of cognitive deficits associated with schizophrenia. α7nAChR PAMs have been classified as either type I or type II compounds. Type I compounds mainly affect the peak current response, while type II compounds affect both the peak current response, as well as the kinetics of agonist-evoked responses.\(^{241}\) 1-(5-chloro-2, 4-dimethoxy-phenyl)-3-(5-methyl-isoxazol-3-yl)-urea (PNU-120956) is a prototypical type II PAM with little or no activity on most other nAChR subtypes.\(^{242}\) LY-2087101 is a recently discovered allosteric potentiator of nAChRs that is less selective for α7 nAChRs than PNU-120956, with properties similar to type I PAMs.\(^{243}\) There are five amino acids in three α-helical transmembrane regions of the α7nAChR that are critical in facilitating the potentiation of agonist evoked responses by PNU-120956 and LY-2087101.\(^{244}\) In addition to amplifying or unmasking α7nAChR responses to exogenous agonist, PAMs can potentially augment the effects of endogenous agonist, especially PNU-120956, since it reduces α7nAChR desensitization.\(^{245}\) Genetic, biochemical, and behavioral findings have linked α7nAChRs to schizophrenia, particularly the cognitive and sensory processing components of the disease.\(^{246}\) The ability of α7nAChR agonists (partial and full) and PAMs to improve a wide range of cognitive processes preclinically, and to a lesser extent clinically, makes them attractive targets for mitigating the cognitive deficits associated with schizophrenia that are not responsive to current first- and second-generation antipsychotics.

**Conclusion**

While this review is hardly exhaustive, it does identify a number of potential drug discovery targets that could address the symptoms most resistant to current treatments available for schizophrenia. As psychosis is a downstream consequence of a primary cortical dysfunction, it is possible that some of these interventions might not only affect the cognitive deficits and negative symptoms, but also positive symptoms. In this regard, the mGluR2/3 agonist, LY2140023, which has no direct effects on dopaminergic neuronal function, exhibited antipsychotic effects comparable to the positive control, olanzapine.\(^{246}\) Alternatively, other interventions might have only selective effects on negative symptoms and/or cognition, and thus would require the coadministration of an antipsychotic to reduce positive symptoms, much in the way that the combination of a mood stabilizer and an antipsychotic are used to treat bipolar disorder.

As the complex genetics of schizophrenia are resolved, it may be possible in the future to link risk genes to drugs that directly address their mechanisms. For example, an α7nAChR positive modulator might be particularly effective in those patients found to have an allelic variant of the CHRNA7 promoter that is associated with reduced expression.\(^{246}\) Genetic studies indicate that individual risk genes such as common alleles of GABA\(_A\) receptors are associated with elevated risk for schizophrenia, bipolar disorder, and autism-spectrum disorders.\(^{247}\) Such shared risk genes or shared copy number variants provide face validity for the conviction that drug discovery around these targets may yield a much broader therapeutic impact than just in schizophrenia. However, in keeping
with the complex genetics of neuropsychiatric disorders, drugs targeting these pathways will likely be useful only in particular subgroups of patients with schizophrenia, bipolar disorder, and autism-spectrum disorders.

**REFERENCES**

1. Delay J, Denicker P. Neuroleptic effects of chlorpromazine in therapeutics of neuropsychiatry. J Clin Exp Psychopharmacol. 1955;16:104-112.
2. Janssen PA, Awouters FH. Is it possible to predict the clinical effects of neuroleptics from animal data? Part V: from haloperidol and pipamperone to risperidone. Arzneimittelforschung. 1994;44:269-277.
3. Carlson A. Does dopamine play a role in schizophrenia? Psychol Med. 1977;7:583-597.
4. Creese I, Burt DR, Snyder SH. Dopamine receptor binding predicts clinical and pharmacological potencies of antischizophrenic drugs. Science. 1976;192:481-483.
5. Seeman P, Lee T, Chau-Wong M, Wong K. Antipsychotic drug doses and neuroleptics atopicoto receptors. Nature. 1976;261:717-719.
6. Meltzer HY. Illuminating the molecular basis for some antipsychotic drug-induced metabolic burden. Proc Natl Acad Sci U S A. 2007;104:3019-3020.
7. Lehman AF, Cordray DS. Prevalence of alcohol, drug and mental disorders among the homeless: one more time. Contemporary Drug Problems. 1993;20:355-384.
8. Hartvig P, Kjelsberg E. Penrose’s law revisited: the relationship between mental institution beds, prison population and crime rate. Nord J Psychiatry. 2009;63:51-56.

**Más allá del receptor de dopamina: nuevos blancos terapéuticos para tratar la esquizofrenia**

Todos los fármacos actuales aprobados para tratar la esquizofrenia parecen ejercer sus efectos antipsicóticos a través del bloqueo del receptor de dopamina D2. Recientes estudios de meta-análisis y de eficacia comparativa muestran diferencias menores entre la eficacia de los antipsicóticos atípicos más nuevos y los más antiguos, y escasos efectos sobre los síntomas negativos y cognitivos. Esta revisión integra los hallazgos de estudios postmortem, de imágenes y de pruebas con fármacos para aclarar un “circuito patológico” córtico-límbico en la esquizofrenia que pueda ser de particular importancia para los síntomas negativos y los deterioros cognitivos de la esquizofrenia. Se discuten los potenciales sitios para la intervención farmacológica de blancos en la neurotransmisión glutamatergica, gabaérgica y colinérgica para tratar estos síntomas de la esquizofrenia.

**Au-delà du récepteur dopaminergique : de nouvelles cibles thérapeutiques pour traiter la schizophrénie.**

Tous les médicaments actuels autorisés dans le traitement de la schizophrénie exercent leur action antipsychotique en bloquant le récepteur D2 à la dopamine. Selon des métaanalyses récentes et des études d’efficacité comparatives, les différences d’efficacité entre les antipsychotiques atypiques les plus récents et les médicaments les plus anciens sont marginales et les effets sur les symptômes négatifs et cognitifs sont faibles. Cet article présente les résultats d’études d’imagerie, d’études postmortem, et de tests de compétition pharmacologique afin de trouver un « circuit pathologique » corticolumbique dans la schizophrénie, qui serait particulièrement pertinent pour les symptômes négatifs et les déficits cognitifs dans cette maladie. Les sites potentiels de l’action pharmacologique visant la neurotransmission glutamatergique, GABAérgique et choliner- gique pour traiter ces symptômes sont analysés.

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16. Mesholam-Gately RI, Giuliani AJ, Goff KP, Faraone SV, Seidman LJ. Neurocognition in first-episode schizophrenia: a meta-analytic review. *Neuropsychology*, 2009;23:315-336.
17. Heinrichs RW, Zakianz KK. Neurocognitive deficit in schizophrenia: a quantitative review of the evidence. *Neuropsychology*, 1998;12:426-445.
18. Velakoulis D, Wood SJ, Wong MT, et al. Hippocampal and amygdala volumes according to psychosis stage and diagnosis: a magnetic resonance imaging study of chronic schizophrenia, first-episode psychosis, and ultra-high-risk individuals. *Arch Gen Psychiatry*, 2006;63:139-149.
19. Salisbury DF, Kuroki N, Kasi N, Sheronit ME, McCarley RW. Progressive and interrelated functional and structural evidence of post-onset brain reduction in schizophrenia. *Arch Gen Psychiatry*, 2007;64:521-529.
20. Pearlson GD, Calhoun VD. Convergent approaches for defining functional imaging endophenotypes in schizophrenia. *Front Hum Neurosci.*, 2009;3:37.
21. Kyriakopoulos M, Frangou S. Recent diffusion tensor imaging findings in early stages of schizophrenia. *Curr Opin Psychiatry*, 2009;22:168-176.
22. Camchong J, Macdonald RW 3rd, Bell MJ, Mueller BA, Lim KO. Altered functional and anatomical connectivity in schizophrenia. *Schizophren Bull.*, 2009 Nov 17. [Epub ahead of print]
23. Javitt DC. Sensory processing in schizophrenia: neither simple nor intact. *Schizophren Bull.*, 2009;35:1059-1064.
24. Itil T, Keskiner A, Kiremitci N, Holden JM. Effect of phencyclidine in the rat. *Biol Psychiatry*, 1998;46:589-599.
25. Luby ED, Cohen BD, Rosenbaum G, Gottlieb JS, Kelley R. Study of a new schizophrenomimetic drug; senylm. *AMA Arch Neurol Psychiatry* 1959;81:363-369.
26. Javitt DC, Zukin, SR. Recent advances in the phencyclidine model of schizophrenia. *Arch Gen Psychiatry*, 1991;48:1301-1308.
27. Anis NA, Berry SC, Burton NR, Lodge D. The dissociative anesthetic, ketamine and phencyclidine, selectively reduce excitation of central mammalian neurons by N-methyl-aspartate. *Br J Pharmacol.*, 1983;79:565-575.
28. Krystal JH, Karper LP, Seibyl JP, et al. Subanesthetic effects of the non-competitive NMDA antagonist, ketamine, in humans: *Psychotomimetic, perceptual, cognitive, and neuroendocrine responses*. *Arch Gen Psychiatry* 1994;51:199-214.
29. Newcomer JW, Faber NB, Jevtic-Todorovic V, et al. Ketamine-induced NMDA receptor hypofunction as a model of memory impairment and psychosis. *Neuropsychopharmacology*. 1999;20:106-118.
30. Umbricht D, Schmid L, Koller R, Vollenweider FX, Hell D, Javitt DC. Ketamine-induced deficits in auditory and visual context-dependent processing in healthy volunteers: implications for models of cognitive deficits in schizophrenia. *Arch Gen Psychiatry*, 2000;57:1139-1147.
31. Radant AD, Bovvilte TA, Cowley DS, Kharasch ED, Roy-Byrne PP. Does ketamine-induced N-methyl-D-aspartate receptor antagonist cognition cause schizophrenia-like oclomotor abnormalities? *Neuropsychopharmacology* 1998;19:434-444.
32. Kegeles LS, Abi-Dargham A, Zea-Ponce Y, et al. Modulation of amphetamine-induced striatal dopamine release by ketamine in human chronic schizophrenics. *Neuropsychopharmacology*. 2001;25:425-467.
33. Erck JS, Kasten MJ, Siegel JD, et al. Dopamine antagonists differentially attenuate serotonin-induced striatal dopamine release. *Psychopharmacology* 2004;173:353-361.
34. Lahti AC, Weiler MA, TamaraMichaelidis BA, Parwani A, Tamminga CA. Effects of ketamine in normal and schizophrenic volunteers. *Psychopharmacology* 2001;152:353-364.
35. Lewis DA, Hashimoto T, Volk DW. Cortical inhibitory neurons and schizophrenia. *Nat Rev Neurosci*. 2005;6:312-324.
36. Benes FM. Evidence for altered trisynaptic circuitry in schizophrenia hippocampus. *Biochim Biophys Acta*, 1999;1421:45-59.
37. Hashimoto T, Buzm HH, Mirkics K, Wu Q, Sampson AR, Lewis DA. Conserved regional patterns of GABA-related transcript expression in the necortex of subjects with schizophrenia. *Am J Psychiatry*, 2008;165:479-488.
38. Volk DW, Pierri JN, Frischy JM, Auh S, Sampson AR, Lewis DA. Reciprocal alterations in pre- and post-synaptic inhibitory markers at chandelier cell inputs to pyramidal neurons in schizophrenia. *Cereb Cortex*. 2002;12:1063-1070.
39. Glantz LA, Lewis DA. Decreased dendritic spine density on prefrontal cortical pyramidal neurons in schizophrenia. *Arch Gen Psychiatry*. 2000;57:65-73.
40. Sweet RA, Henteleff RA, Zhang W, Sampson AR, Lewis DA. Reduced dendritic spine density in auditory cortex of subjects with schizophrenia. *Neuropsychopharmacology*. 2009;34:374-389.
41. Behrens MM, Ali SS, Doo DN, Lucero J, Shekhtman G, Quick KL, Dugan LL. Ketamine-induced loss of phenotype of fast-spiking interneurons is mediated by NADPH-oxidase. *Science*, 2007;318:1645-1647.
42. Zhang Y, Behrens MM, Lisman JE. Prolonged exposure to NMDAR antagonist suppresses inhibitory synaptic transmission in prefrontal cortex. *J Neurophysiol.*, 2008;100:959-965.
43. Lisman JE, Coyle JT, Green RW, et al. Circuit-based framework for understanding neurotransmitter and risk interactions in schizophrenia. *Trends Neurosci.*, 2008;31:234-242.
44. Ullman SK, Kim JE, Hall BJ, Deerinck T, Eillman M, Ghosh A. Regulation of spine morphology and spine density by NMDA receptor signaling in vivo. *Proc Natl Acad Sci U S A.* 2007;104:19553-19558.
45. Basu AC, Tsai GE, Ma CL, et al. Targeted disruption of serine racemase affects glutamatergic neurotransmission and behavior. *Mol Psychiatry*, 2009;14:719-727.
46. Balu D, Basu AC, Coyle JT. Altered cortical dendritic morphology in serotonin acenoxame knockout mice, a genetic model of NMDA receptor hypofunction. *Soc Neurosci.*, 2009. Abs. No. 443.16.
47. Allen NC, Bagade S, McQueen MB, et al. Systematic meta-analyses and field synopsis of genetic association studies in schizophrenia: the SzGene database. *Nat Genet.*, 2008;40:827-834.
48. Hahn CG, Wang HY, Cho DS, et al. Altered neuregulin 1-erbB4 signaling contributes to NMDA receptor hypofunction in schizophrenia. *Nat Med.*, 2006;12:824-828.
49. Jentsch JD, Tranham-Davidson H, Jairil C, Tinsley M, Cannon TD, Lavin LL. Ketamine-induced loss of phenotype of fast-spiking interneurons is mediated by NADPH-oxidase. *Soc Neurosci.*, 2008;35:185-186.
50. Whitfield-Gabrielson S, Thermenos HW, Milonovic S, et al. Hyperactivity and hyperconnectivity of the default network in schizophrenia and in first-degree relatives of persons with schizophrenia. *Proc Natl Acad Sci U S A.* 2009;106:1279-1284.
51. Grace AA. Dopamine system dysregulation by the ventral subcircuit as the common pathophysiological basis for schizophrenia psychosis, psychostimulant abuse, and stress. *Neurotox Res.*, 2010 (Epub ahead of print).
52. Komuro H, Rakic P. Modulation of neuronal migration by NMDA receptors. *Science*, 1993;260:95-97.
53. Akbarian S, Bunney WE Jr, Potkin SG, Wigal SB, Hallman JO, Sandman CA, Jones EG. Altered distribution of nicotinamide-adenine dinucleotide phosphate-diaphorase cells in frontal lobe of schizophrenics implies disturbances of cortical development. *Arch Gen Psychiatry*, 1993;50:169-177.
54. Margolis RL, Ross CA. Neuronal signaling pathways: genetic insights into the pathophysiology of major mental illness. *Neuropsychopharmacology*. 2010;35:350-351.
55. Rietkerk T, Boks MR, Sommer IE, de Jong S, Kahn RS, Ophoff RA. Network analysis of positional candidate genes of schizophrenia highlights myelin-related pathways. *Mol Psychiatry*, 2009;14:335-335.
56. Bradley J, Carter SR, Rao VR, Wang J, Finkbeiner S. Spike variants of the NR1 subunit differentially induce NMDA receptor-dependent gene expression. *J Neurosci.*, 2006;26:1065-1076.
57. Lynch DR, Guttmann RP. NMDA receptor pharmacology: Perspectives from molecular biology. *Curr Drug Targets*. 2001;2:215-231.
58. Berger AJ, Diedonne S, Ascher P. Glycine uptake governs glycine site occupancy at NMDA receptors of excitatory synaptic pathways. *J Neurophysiol*. 1998;80:3336-3340.
59. Ransom RW, Stee NL. Cooperative modulation of the N-methyl-D-aspartate receptor-ion channel complex by L-glutamate, glycine, and polyanolmes. *J Neurochem* 1988;51:830-836.
60. Foster AC, Wong EH. The novel anticonvulsant MK-801 binds to the activated state of the N-methyl-D-aspartate receptor in rat brain. *Br J Pharmacol*. 1987;91:403-409.
61. Wong EH, Kemp JA, Priestley T, Knight AR, Woodruff GN, Iversen LS. The anticonvulsant MK-801 is a potent N-methyl-D-aspartate antagonist. *Proc Natl Acad Sci U S A.* 1986;83:7104-7108.
Pharmacological aspects

62. Hong SJ, Li H, Becker KG, Dawson VL, Dawson TM. Identification and analysis of plasticity-induced late-response genes. Proc Natl Acad Sci U S A. 2004;101:145–215.
63. Liu L, Wong TP, Pozza MF, et al. Role of NMDA receptor subtypes in governing the direction of hippocampal synaptic plasticity. Science 2004;304:1021–1024.
64. Coyle JT. Glutamate and schizophrenia: beyond the dopamine hypothesis. Cell Mol Neurobiol. 2006;26:365-384.
65. Johnson JW, Ascher P. Glycine potentiates the NMDA response in cultured mouse brain neurons. Nature 1987;325:529-531.
66. Kleckner NW, Dingledine R. Requirement for glycine in activation of NMDA-receptors expressed in Xenopus oocytes. Science. 1988;241:835-837.
67. Thomson AM, Walker VE, Flynn DM. Glycine enhances NMDA-receptor mediated synaptic potentials in neocortical slices. Nature. 1989;338:422-424.
68. Bergeron R, Meyler TM, Coyle JT, Greene RW. Modulation of N-methyl-D-aspartate receptor function by glycine transport. Proc Natl Acad Sci U S A. 1998;95:15730–15734.
69. Hashimoto K, Fukushima T, Shimizu E, et al. Decreased serum levels of D-serine in patients with schizophrenia: evidence in support of the N-methyl-D-aspartate receptor hypofunction hypothesis of schizophrenia. Arch Gen Psychiatry. 2003;60:572-576.
70. Goff DC, Tsai G, Manoach DS, Coyle JT. Increased cortical kynurenic acid content in schizophrenia. Biol Psychiatry. 2010;1:521–530.
71. Nilsson LK, Linderholm KR, Engberg G, et al. Elevated levels of kynurenic acid in the cerebrospinal fluid of male patients with schizophrenia. Schizophr Res. 2005;15:80:315-322.
72. Matsui T, Sekiguchi M, Hashimoto A, Tomita U, Nishikawa T, Wada, K. Functional comparison of D-serine and glycine in rodents: the effect on cloned NMDA receptors and the extracellular concentration. J Neurochem. 1995;65:454-458.
73. Watson GB, Bolanowski MA, Baganoff MP, Deppeler CL, Lanthorn TH. D-cycloserine acts as a partial agonist at the glycine modulatory site of the NMDA receptor expressed in Xenopus oocytes. Brain Res. 2001;900;26:510-186.
74. Tsai GE, Lin PY. Strategies to enhance N-methyl-D-aspartate receptor-mediated neurotransmission in schizophrenia, a critical review and meta-analysis. Curr Pharm Des. 2010;16:522-537.
75. Buchanan RW, Javitt DC, Marder SR, et al. The Cognitive and Negative Symptoms in Schizophrenia Trial (CONSIST): the efficacy of glutamatergic agents for negative symptoms and cognitive impairments. Am J Psychiatry. 2007;164:1003-1005.
76. Heresco-Levy, U, Javitt D C, Emerlov M, Mordey, C, Silipo, G, Lichtenstein M. Efficacy of high-dose glycine in the treatment of enduring negative symptoms of schizophrenia. Arch Gen Psychiatry. 1999;56:29–36.
77. Javitt DC, Silipo G, Cienfuegos A, Shelley AM, Bark N, Park M et al. Adjunctive high-dose glycine in the treatment of schizophrenia. Int J Neuropsychopharmacol. 2001;4:385-391.
78. Goff DC, Tsai G, MANOACH DS, COYLE JT. Dose-finding trial of D-cycloserine added to neuroleptics for negative symptoms in schizophrenia. Arch Gen Psychiatry. 2001;152:1213-1215.
79. Quartermain D, Mower J, Rafferty MF, Herting RL, Lanthorn TH. Acute but not chronic activation of the NMDA-coupled glycine receptor with D-cycloserine facilitates learning and retention. Eur J Pharmacol. 1994;257:7-12.
80. Baker JD, Azorlosa J. The NMDA antagonist MK-801 blocks the extinction of Pavlovian fear conditioning. Behav Neurosci. 1996;110:618-627.
81. Walker DL, Ressler KJ, Lu KT, Davis M. Facilitation of conditioned fear extinction by systemic administration or intra-amygldal infusions of D-cycloserine in mice assessed with fear-potentiated startle in rats. J Neurosci. 2002;22:2343-2351.
82. Myers KM, Carlezen WA Jr. D-cycloserine facilitates extinction of naloxy-induced conditioned place aversion in morphine-dependent rats. Biol Psychiatry. 2010;67:85-87.
83. Ressler KJ, Rothbaum BO, Tannenbaum L, et al. Cognitive enhancers as adjuncts to psychotherapy: use of D-cycloserine in phobic individuals to facilitate extinction of fear. Arch Gen Psychiatry. 2004;61:1136-1144.
84. Norberg MM, Krystal JH, Tolin DF. A meta-analysis of D-cycloserine and the facilitation of fear extinction and exposure therapy. Biol Psychiatry. 2008;63:1118-1126.
85. Oldendorf WH. Uptake of radiolaabeled essential amino acids by brain following arterial injection. Proc Soc Exp Biol Med. 1971;136:385–386.
86. Hashimoto A, Chiba S. Effect of systemic administration of D-serine on the levels of D- and L-serine in several brain areas and periphery of rat. Eur J Pharmacol. 2004;495:153–158.
87. Ninan I, Jardemark KE, Wang RY. Differential effects of atypical and typical antipsychotic drugs on N-methyl-D-aspartate- and electrically evoked responses in the piramidal cells of the rat medial prefrontal cortex. Synapse. 2003;48:66-79.
88. Verrall L, Burnet PW, Bristow GC, Godlewska BR, Sikka P, Walker M. The neurobiology of D-cycloserine and its involvement in schizophrenia. Mol Psychiatry. 2010;15:122-137.
89. Detera-Wadleigh SD, McMahon FJ. G72/G30 in schizophrenia and bipolar disorder: review and meta-analysis. Biol Psychiatry. 2006;60:106–114.
90. Shi J, Badner JA, Gershon ES, Liu C. Allelic association of G72/G30 with schizophrenia susceptibility in humans and in a mouse model. Mol Psychiatry. 2007;12:234-241.
91. Norberg MM, Krystal JH, Tolin DF. A meta-analysis of D-cycloserine and the facilitation of fear extinction and exposure therapy. Biol Psychiatry. 2008;63:1118-1126.
92. Nagata Y. Involvement of D-amino acid oxidase in elimination of D-serine in mouse brain. Experation. 1992;48:753-755.
93. Heresco-Levy, U, Javitt, D C, Emerlov M, Mordey, C, Silipo, G, Lichtenstein M. Efficacy of high-dose glycine in the treatment of enduring negative symptoms of schizophrenia. Arch Gen Psychiatry. 1999;56:29–36.
94. Addante A, Mentis KA, Sotiriou M, et al. The cognitive and negative symptoms in schizophrenia trial (CONSIST): the efficacy of glutamatergic agents for negative symptoms and cognitive impairments. Am J Psychiatry. 2007;164:1003-1005.
95. Madeira C, Freitas ME, Vargas-Lopes C, Wolosker H, Panizutti R. Increased brain D-amino acid oxidase activity (DAOA) activity in schizophrenia. Schizophr Res. 2008;101:76-83.
96. Burnet PW, Eastwood SL, Bristow GC, Godlewska BR, Sikka P, Walker M, Harrison PJ. D-amino acid oxidase activity and expression are increased in schizophrenia. Mol Psychiatry. 2003;8:658-660.
97. Chumakov I, Blumenfeld M, Guerassimenko O, Cavares L, Palicio M, Abderhamih H, et al. Genetic and physiological data implicating the new human gene G72 and the gene for D-amino acid oxidase in schizophrenia. Proc Natl Acad Sci U S A. 2002;99:13675–13680.
98. Adage T, Trillat AC, Quattropani A, et al. In vitro and in vivo pharmacological profile of AS957278, a selective D-amino acid oxidase inhibitor with potential anti-psychotic properties. Eur Neuropsychopharmacol. 2008;18:200-214.
99. Hashimoto K, Fujita Y, Horio M, et al. Co-administration of a D-amino acid oxidase inhibitor potentiates the efficacy of D-serine in attenuating prepulse inhibition deficits after administration of dizocilpine. Biol Psychiatry. 2005;64:85-92.
100. Smith SM, Uslaner JM, Yao L, et al. The behavioral and neurochemical effects of a novel D-amino acid oxidase inhibitor compound 8 [4H-thieno[3,2-b]pyrrolo-5-carboxylic acid] and D-serine. J Pharmacol Exp Ther. 2009;328:921-930.
101. Goltsov AV, Loseva JG, Andreeva TV, e al. Polymorphism in the 5'-promoter region of the serine racemase gene is associated with schizophrenia. Hum Mol Genet. 2002;11:325-326.
102. Morita Y, Ujike H, Tanaka Y, Otani K, et al. A genetic variant of the serine racemase gene is associated with schizophrenia. Biol Psychiatry. 2007;61:1200–1215.
103. Labrie V, Fukumura R, Rastogi A, et al. Serine racemase is associated with schizophrenia susceptibility in humans and in a mouse model. Hum Mol Genet. 2009;18:3227-3234.
II metabotropic glutamate receptors reduce stimulated and spontaneous
potentiates NMDA receptor-mediated responses in vivo and produces an
inhibitor N-[3-(4_-fluorophenyl)-3-(4_-phenylphenoxy)propyl]sarcosine
potentiates NMDA-mediated responses in rat prefrontal cortical neurons
GLYT2 in the rat CNS.

Matsuo H, Kanai Y, Tokunaga M, et al. High affinity D- and L-serine
reuptake in the mouse CNS. 

Rutter AR, Fradley RL, Clusmann H, Friedl M, Schramm J. Presynaptic group
activation of the disruptive effects of the NMDA glutamate receptor antago-
nist, ketamine, on working memory by pretreatment with the group II
metabotropic glutamate receptor agonist, LY354740, in healthy human
subjects. 

Kalivas PW. The glutamate homeostasis hypothesis of addiction.

Wolosker H, Blackshaw S, Snyder SH. Serine racemase: A glial enzyme
synergizing D-serine to regulate glutamate-N-methyl-D-aspartate neuro-
transmission. 

Kartvelishvily E, Sheper M, Balan L, Dumin E, Wolosker H. Neuron-
derived D-serine release provides a novel means to activate N-methyl-D-
aspartate receptors. 

Miyazaki K, Inoue R, Takata Y, et al. Serine racemase is predominantly
localized in neurons in mouse brain. 

Llabre V, Fukumura R, Rastogi A, Fick L, Wang W, Boutros PC, Kennedy JL, Semerat A, MO, Lee FH, Baker GB, Belsham DD, Barger SW, Gondo Y, Wang AH, Roder JC. Serine racemase is associated with schizo-
phrenia susceptibility in humans and in a mouse model. 

96:13409–13414.
105. Wolosker H, Blackshaw S, Snyder SH. Serine racemase: A glial enzyme
synergizing D-serine to regulate glutamate-N-methyl-D-aspartate neuro-
transmission. Proc Natl Acad Sci U S A. 1999;96:13409-13414.
106. Kartvelishvily E, Sheper M, Balan L, Dumin E, Wolosker H. Neuron-
derived D-serine release provides a novel means to activate N-methyl-D-
aspartate receptors. J Biol Chem. 2006;281:14151-14162.
107. Miyazaki K, Inoue R, Takata Y, et al. Serine racemase is predominantly
localized in neurons in mouse brain. J Comp Neurol. 2008;20;510:641-
654.
108. Llabre V, Fukumura R, Rastogi A, Fick L, Wang W, Boutros PC, Kennedy JL, Semerat A, MO, Lee FH, Baker GB, Belsham DD, Barger SW, Gondo Y, Wang AH, Roder JC. Serine racemase is associated with schizo-
phrenia susceptibility in humans and in a mouse model. 

II metabotropic glutamate receptors reduce stimulated and spontaneous
potentiates NMDA receptor-mediated responses in vivo and produces an
inhibitor N-[3-(4_-fluorophenyl)-3-(4_-phenylphenoxy)propyl]sarcosine
potentiates NMDA-mediated responses in rat prefrontal cortical neurons
GLYT2 in the rat CNS.

Matsuo H, Kanai Y, Tokunaga M, et al. High affinity D- and L-serine
reuptake in the mouse CNS. Eur J Neurosci. 2007;25:1757-1766.

Helboe L, Egebjerg J, Møller M, Thomsen C. Distribution and pharma-
cology of alanine-serine-cysteine transporter 1 (asc-1) in rodent brain. 

Ahn JG, Yang CR. Glycine transporter-1 blockade is temporally dissociated from the cognitive and locomotor effects of
ic glutamate 2/3 receptors reverses the effects of NMDA receptor hypo-
thesis of addiction. 

Trends Pharmacol Sci. 2008;31:234-242.
125. Gallici R, Engelmann NG, Rodriguez AL, Conn PJ. A selective allosteric
potentiator of metabotropic glutamate (mGLu) 2 receptors has effects similar
to an orthosteric mGlu2/3 receptor agonist in mouse models predictive of
antipsychotic activity. 

Psychol Exp Ther. 2005;315:1181-1187.
126. Gallici R, Jones CK, Hemstapat K, Nong Y, Engeshemdia NG, Williams LC, de Paulis T, Conn PJ. Biphenyl-indanone A, a positive allosteric modulator of the metabotropic glutamate receptor subtype 2, has antipsychotic- and anxiolytic-like effects in mice. J Pharmacol Exp Ther. 2006;318:173-185.
127. Rorick-Kehn LM, Hart JC, McKinzie DL. Pharmacological characteriza-
tion of stress-induced hyperthermia in DBA2 mice using metabotropic and ionotropic glutamate receptor ligands. Psychopharmacology (Berl). 2005;183:226-240.
128. Greco B, Invernizzi RW, CarlI. Phencyclidine-induced impairment in attention and response control depends on the background genotype of mice: reversal by the mGLU2/3 receptor agonist LY373626. Psychopharmacology (Berl). 2005;179:68-76.
129. Adams B, Moghadam B. Corticobranchial dopamine neurotransmission is temporally dissociated from the cognitive and locomotor effects of phencyclidine. J Neurosci. 1998;15:1545-1554.
130. Benneyworth MA, Xiang Z, Smith RL, Garcia EE, Conn PJ, Sanders-Bush E. A selective positive allosteric modulator of metabotropic glutamate receptor subtype 2 blocks a hallucinogenic drug model of psychosis. Mol Pharmacol. 2007;72:477-484.
131. Patil ST, Zhang L, Martenyi F, et al. Activation of mGLU3/2 receptors as a new approach to treat schizophrenia: a randomized Phase 2 clinical trial. Nat Med. 2007;13:1102-1107.
132. Krystal JH, Abi-Saab W, Perry E, et al. Preliminary evidence of attenu-
ation of the disruptive effects of the NMDA glutamate receptor antago-
nist, ketamine, on working memory by pretreatment with the group II
metabotropic glutamate receptor agonist, LY354740, in healthy human
subjects. Psychopharmacology (Berl). 2005;179:303-309.
133. Homayoun H, Jackson ME, Moghadam B. Activation of metabotropic
 glutamate receptor 2/3 receptors reverses the effects of NMDA receptor hypo-
thesis of addiction. 

Trends Pharmacol Sci. 2009;30:25-31.
134. Conn PJ, Pin J. Pharmacology and functions of metabotropic gluta-
mate receptors. Annu Rev Pharmacol Toxicol. 1997;37:205-237.
135. Scanzani M, Salin PA, Vogt KE, Malenka RC, Nissel RA. Use-dependent
gains in glueid milieu concentration activate premetabotropic glutamate receptor superfamilies. Nature. 1997;385:630-634.
136. Marek OJ, Wright RA, Schoepf DD, Monn JA, Moghadam B, Aghajanian GK. Physiological antagonism between 5-hydroxytryptamine 2A and group II metabotropic glutamate receptors in prefrontal cortex. J Pharmacol Exp Ther. 2000;292:76-87.
137. Dietrich D, Kral T, Clusmann H, Fried M, Schramm J. Presynaptic group II metabotropic glutamate receptors reduce stimulated and spontaneous
transmitter release in human dentate gyrus. Neuropharmacology. 2002;42:297-305.
Pharmacological aspects

147. Spooren WP, Gasparini F, van der Putten H, Koller M, Nakanishi S, Kuhn R. Lack of effect of LY314582 (a group 2 metabotropic glutamate receptor agonist) on phenycyclidine-induced locomotor activity in metabotropic glutamate receptor 2 knockout mice. *Eur J Pharmacol*. 2000;397:R1-R2.

148. Conn PJ, Lindsay CW, Jones CK. Activation of metabotropic glutamate receptors as a novel approach for the treatment of schizophrenia. *Trends Pharmacol Sci*. 2009;30:25-31.

149. Conn PJ, Christopoulos A, Lindsay CW. Allosteric modulators of GPCRs: a novel approach for the treatment of CNS disorders. *Nat Rev Drug Discov*. 2009;8:41-54.

150. Schaffhauser H, Rowe BA, Morales S, et al. Pharmacological characterization and identification of amino acids involved in the positive modulation of metabotropic glutamate receptor subtype 2. *Mol Pharmacol*. 2003;64:798-810.

151. Johnson MP, Baiz M, Jagdmann GE, et al. Discovery of allosteric potentiators for the metabotropic glutamate 2 receptor: synthesis and subtype selectivity of N-(4-[2-methoxyphenyl]phenoxy)-N-(2,2,2-trifluoroethyl)sulfonyl)glycid-3-l-methylamine. *J Med Chem*. 2003;46:3189-3192.

152. Johnson MP, Barba D, Britton TC, et al. Metabotropic glutamate 2 receptor potentiation: receptor modulation, frequency-dependent synaptic activity, and efficacy in preclinical anxiety and psychosis models. *Psychopharmacology*. 2009;197:271-283.

153. Baker DA, McFarland K, Lake RW, Shen H, Toda S, Kalivas PW. N-acetyl cysteine-induced blockade of cocaine-induced reinstatement. *Ann N Y Acad Sci*. 2003;1030:349-357.

154. McNeeley GJ. Cerebral cystine uptake: a tale of two transporters. *Trends Pharmacol Sci*. 2002;23:299-302.

155. Moran MM, McFarland K, Melendez RJ, Kalivas PW, Seams JK. Cystine/glutamate exchange regulates metabotropic glutamate receptor presynaptic inhibition of excitatory transmission and vulnerability to cocaine seeking. *J Neurosci*. 2005;25:6389-6393.

156. Zhou W, Kalivas PW. N-acetylcycteine reduces extinction responding and induces enduring responses in cue- and heroin-induced drug-seeking. * Biol Psychiatry*. 2008;63:338-340.

157. Peters J, Kalivas PW. The group II metabotropic glutamate receptor agonist, LY379268, inhibits both cocaine- and food-seeking behavior in rats. *Psychopharmacology (Berl)*. 2006;186:143-149.

158. Baker DA, Madayag A, Kristiansen LV, Meador-Woodruff JH, Haroutunian V, Raju I. Contribution of cystine-glutamate antiporters to the psychotomimetic effects of phenycyclidine. *Neuropsychopharmacology*. 2008;33:1769-1772.

159. Berk M, Kopolov D, Dean O, et al. N-acetyl cysteine as a gluthathione precursor for schizophrenia—a double-blind, randomized, placebo-controlled trial. *Biol Psychiatry*. 2008;64:361-368.

160. Behrens MM, Grossberg GT. Effect of rivastigmine in the treatment of behavioral disturbances associated with dementia: review of neuropsychiatric impairment. *Curr Opin Pharmacol*. 2008;8:157-166.

161. Behrens MM, Sejnowski TJ. Does schizophrenia arise from oxidative dysregulation of parvalbumin-interneurons in the developing cortex? *Neuropsychopharmacology*. 2009;34:2112-2124.

162. Yue BK, Keist R, von Bohmier L, et al. A schizophrenia-related senso- rineuron deficit links alpha 3-containing GABAA receptors to a dopamine hyperfunction. *Proc Natl Acad Sci U S A*. 2005;102:17154-17159.

163. Langer O, Mohler H GABA-based therapeutic approaches: GABAA receptor subtype functions. *Curr Opin Pharmacol*. 2006;6:18-23.

164. Baskin JS. Therapeutic modulators of GABA receptors. *Trends Pharmacol Sci*. 2008;29:33-39.

165. Mohler H Role of GABAA receptors in cognition. *Biochem Soc Trans*. 2009;37:1328-1333.

166. Cruz DA, Weaver CL, Lovallo EM, Melchitzky DS, Lewis DA. Selective alterations in postsynaptic markers of chandelier cell inputs to cortical pyramidal neurons in subjects with schizophrenia. *Neuropsychopharmacology*. 2009;34:112-2124.

167. Kinney GG, Burno M, Campbell UC, et al. Metabotropic glutamate subtype 5 receptors modulate locomotor activity and sensorimotor gating in rodents. *J Pharmacol Exp Ther*. 2003;306:116-123.

168. Homayoun H, Stefani MR, Adams BW, Tamagad M, Moghadam B. Functional interaction between NMDA and mGlur5 receptors: effects on working memory, instrumental learning, motor behaviors, and dopamine release. *Neuropsychopharmacology*. 2004;29:1259-1269.

169. Campbell UC, Lalwani K, Hernandez L, Kinney GG, Conn PJ, Bristow LJ. The mGlur5 antagonist 2-methyl-6-(phenylethynyl)-pyridine (MPEP) potentiates PCP-induced cognitive deficits in rats. *Psychopharmacology (Berl)*. 2004;175:310-318.

170. Homayoun H, Moghadam B. Bursting of prefrontal cortex neurons in awake rats is regulated by metabotropic glutamate 5 (mGlur5) receptors: rate-dependent influence and interaction with NMDA receptors. *Cereb Cortex*. 2006;16:93-105.

171. Liu F, Grauer S, Kelley C, Navarra R, et al. ADX47273 [5-(4-fluoro- phenyl)-3-[3-(4-fluoro-phenyl)-1,2,4]-azadiazol-5-yl]-piperidin-1-yl]- methanone; a novel metabotropic glutamate receptor 5-selective positive allosteric modulator with preclinical antipsychotic-like and proognitive activities. *J Pharmacol Exp Ther*. 2008;327:827-839.

172. Schlumberger C, Pietraszek M, Gravus A, et al. Comparison of the mGlur5 receptor positive allosteric modulator ADX47273 and the mGlur2(3) receptor agonist LY354740 in tests for antipsychotic-like activity. *Eur J Pharmacol*. 2009;623:73-83.

173. Hashimoto T, Baxzi HH, Mirkes M, Wu Q, Sampson AR, Lewis DA. Conserved regional patterns of GABA-related transcript expression in the neocortex of subjects with schizophrenia. *Am J Psychiatry*. 2008;165:479-489.

174. Behrens MM, Ali SS, Dungan LL. Interleukin-6 mediates the increase in NADPH-oxidase in the ketamine model of schizophrenia. *J Neurosci*. 2008;28:13957-13966.

175. Whittington MA, Traub RD. Interneuron diversity series: inhibitory interneurons and network oscillations in vitro. *Trends Neurosci*. 2003;26:676-682.

176. Mohler H Role of GABA receptors in cognition. *Biochem Soc Trans*. 2009;37:1328-1333.

177. Cruz DA, Weaver CL, Lovallo EM, Melchitzky DS, Lewis DA. Selective alterations in postsynaptic markers of chandelier cell inputs to cortical pyramidal neurons in subjects with schizophrenia. *Neuropsychopharmacology*. 2009;34:1212-2124.

178. Yee BK, Keist R, von Bohmier L, et al. A schizophrenia-related senso- rineuron deficit links alpha 3-containing GABAA receptors to a dopamine hyperfunction. *Proc Natl Acad Sci U S A*. 2005;102:17154-17159.

179. Rohrer U, Mohler H GABA-based therapeutic approaches: GABAA receptor subtype functions. *Curr Opin Pharmacol*. 2006;6:18-23.

180. Lewis DA, Cho RY, Carter CS, et al. Subunit-selective modulation of GABA type A receptor neurotransmission and cognition in schizophrenia. *Am J Psychiatry*. 2008;165:1585-1593.

181. Okada H, Matsuhashita N, Kobayashi K, Kobayashi K. Identification of GABAA receptor subunit variants in midbrain dopaminergic neurons. *J Neurochem*. 2004;89:7-14.

182. Langmead CJ, Watson J, Reavill C. Muscarinic acetylcholine receptors as CNS drug targets. *Pharmacol Ther*. 2008;117:232-247.

183. Caulfield MF, Birdsell NJ. International Union of Pharmacology. XVIII. Classification of muscarinic acetylcholine receptors. *Pharmacol Rev*. 1998;50:279-290.

184. Grossberg GT. Effect of rivastigmine in the treatment of behavioral disturbances associated with dementia: review of neuropsychiatric impairment in Alzheimer’s disease. *Curr Med Res Opin*. 2005;21:1631-1639.

185. Figiel G, Sadowsky C. A systematic review of the effectiveness of rivastigmine for the treatment of behavioral disturbances associated with dementia and other neurological disorders. *Curr Med Res Opin*. 2008;24:157-166.

186. Shekhar A, Potter WZ, Lightfoot J, et al. Selective muscarinic receptor agonist xanomeline as a novel treatment approach for schizophrenia. *Am J Psychiatry*. 2008;165:1033-1039.

187. Bymaster FP, Carter PA, Yamada M, et al. Role of specific muscarinic receptor subtypes in cholinergic parasympathomimetic responses, in vivo phosphoinositide hydrolysis, and pilocarpine-induced seizure activity. *Eur J Neurosci*. 2003;17:1403-1410.
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188. West J, Egnlen GM, Gautam D. Muscarinic acetylcholine receptors: mutant mice provide new insights for drug development. Nat Rev Drug Discov. 2007;6:721-733.
189. Crook JM, Tomaskovic-Crook E, Copolov DL, Dean B. Low muscarinic receptor binding in prefrontal cortex from subjects with schizophrenia: a study of Brodmann's areas 8, 9, 10, and 46 and the effects of neuroleptic drug treatment. Am J Psychiatry. 2001;158:918-925.
190. Dean B, McLeod M, Keriaous D, McKenzie J, Scarf E. Decreased muscarinic receptors in the dorsolateral prefrontal cortex of subjects with schizophrenia. Mol Psychiatry. 2002;7:1083-1091.

191. Anagnostaras SG, Murphy GP, Hamilton SE, et al. Selective cognitive dysfunction in acetylcholine M1 muscarinic receptor mutant mice. Nat Neurosci. 2003;6:51-58.
192. Gerber DJ, Sotnikova TD, Gainetdinov RR, Huang SY, Caron MG, Tonegawa S. Hyperactivity, elevated dopaminergic transmission, and response to amphetamine in M1 muscarinic acetylcholine receptor-deficient mice. Proc Natl Acad Sci U S A. 2001;98:15312-15317.
193. Tzavara ET, Bymaster FR, Davis RJ, et al. M4 muscarinic receptors regulate the dynamics of cholinergic and dopaminergic neurotransmission: relevance to the pathophysiology and treatment of related CNS pathologies. FASEB J. 2004;18:1410-1412.

194. Tzavara ET, Bymaster FR, Felder CC, et al. Dysregulated hippocampal acetylcholine neurotransmission and impaired cognition in M2, M4 and M2/M4 muscarinic receptor knockout mice. Mol Psychiatry. 2003;8:673-679.
195. Shinoe T, Matsui M, Taketo MM, Manabe T. Modulation of synaptic plasticity by physiological activation of M1 muscarinic acetylcholine receptors in the mouse hippocampus. J Neurosci. 2005;25:11194-11200.

196. Marino MJ, Rouse ST, Levey AI, Potter LT, Conn PJ. Activation of the genetically defined m1 muscarinic receptor potentiates N-methyl-D-aspartate (NMDA) receptor currents in hippocampal pyramidal cells. Proc Natl Acad Sci U S A. 1998;95:11465-11470.
197. Conn PJ, Jones CK, Lindsley CW. Subtype-selective allosteric modulators of muscarinic receptors for the treatment of CNS disorders. Trends Pharmacol Sci. 2009;30:148-155.

198. Shirey JK, Xiang Z, Orton D, et al. An allosteric potentiator of M4 mAChR modulates hippocampal synaptic transmission. Nat Chem Biol. 2008;4:42-50.
199. Jones CK, Brady AE, Davis AA, et al. Novel selective allosteric activator of the M1 muscarinic acetylcholine receptor regulates amyloid processing and produces antipsychotic-like activity in rats. J Neurosci. 2008;28:10422-10433.
200. Shirey JK, Brady AE, Jones PJ, et al. A selective allosteric potentiator of the M1 muscarinic acetylcholine receptor increases activity of medial prefrontal cortical neurons and restores impairments in reversal learning. J Neurosci. 2009;29:14271-14278.
201. Chan WY, McKinzie DL, Bettegowda C, et al. Allosteric modulation of the muscarinic M4 receptor as an approach to treating schizophrenia. Proc Natl Acad Sci U S A. 2008;105:10978-10983.

202. Brady AE, Jones CK, Bridges TM, et al. Centrally active allosteric potentiators of the M4 muscarinic acetylcholine receptor reverse amphetamine-induced hyperlocomotor activity in rats. J Pharmacol Exp Ther. 2008;327:941-953.
203. Hughes JR, Hatsuukami DK, Mitchell JE, Dahlgren LA. Prevalence of smoking among psychiatric outpatients. Am J Psychiatry. 1989;146:933-937.
204. Kelly C, McCreadie RG. Smoking habits, current symptoms, and pre-morbid characteristics of schizophrenic patients in Nithsdale, Scotland. Am J Psychiatry. 1959;116:1751-1757.

205. Strand JE, Niyak J. Tobacco use in schizophrenia: a study of cotinine concentrations in the saliva of patients and controls. Eur Psychiatry. 2005;20:50-54.
206. Addington J, el-Guebaly N, Addington D, Hodges D. Readiness to stop smoking in schizophrenia. Can J Psychiatry. 1997;42:49-52.
207. Adler LE, Olincy A, Waldo M, et al. Schizophrenia, sensory gating, and nicotinic receptors. Schizophr Bull. 1999;24:189-202.
208. Mahadik SP, Korenovsky A, Lauw H, Schnur DB, Reddy R. Serum antibodies to nicotinic acetylcholine receptors in schizophrenic patients. Schizophr Res. 1994;12:131-136.
209. Forchuk C, Norman R, Malia A, et al. Schizophrenia and the motivation for smoking. Perspect Psychiatr Care. 2002;38:41-49.
210. Dani JA, Bertrand D. Nicotinic acetylcholine receptors and nicotinic cholinergic mechanisms of the central nervous system. Annu Rev Pharmacol Toxicol. 2007;47:699-729.
211. Frotscher M, Leranth C. Cholinergic innervation of the rat hippocampus as revealed by choline acetyltransferase immunocytochemistry: a combined light and electron microscopic study. J Comp Neurol. 1985;239:237-246.

212. Gray RA, Rajan AS, Radcliffe KA, Yakheiro M, Dani JA. Hippocampal synaptic transmission enhanced by low concentrations of nicotine. Nature. 1996;383:713-716.

213. Krenz J, Kalkan D, Wevers A, et al. Parvalbumin-containing interneurons of the human cerebral cortex express nicotinic acetylcholine receptor proteins. J Chem Neuroanat. 2001;21:239-246.
214. Frazier CJ, Buhler AV, Weiner JL, Dunwiddie TV. Synaptic potentials mediated via alpha-bungarotoxin-sensitive nicotinic acetylcholine receptors in rat hippocampal interneurons. J Neurosci. 1998;18:8228-8235.

215. Klink R, de Kerchove d’Exaerde A, Zoli M, Changeux JP. Molecular and physiological diversity of nicotinic acetylcholine receptors in the midbrain dopaminergic nuclei. J Neurosci. 2001;21:1452-1463.
216. Mansvelder HD, Keath JR, McGhee DS. Synaptic mechanisms underlying nicotine-induced excitability of brain reward areas. Neuron. 2002;33:905-919.
217. Scholstrom B, Fagerquist MV, Zhang X, et al. Putative role of presynaptic alpha7 nicotinic receptors in nicotine stimulated increases of extracellular levels of glutamate and aspartate in the ventral tegmental area. Synapse. 2000;38:375-383.

218. Gault J, Robinson M, Berger R, et al. Genomic organization and partial duplication of the human alpha7 neuronal nicotinic acetylcholine receptor gene (CHRNA7). Genomics. 1998;62:173-185.
219. Court J, Spurden D, Lloyd S, et al. Neuronal nicotinic receptors in dementia with Lewy bodies and schizophrenia: alpha-bungarotoxin and nicotine binding in the thalamus. J Neurochem. 1999;73:1590-1597.

220. Freedman R, Hall M, Adler LE, Leonard S. Evidence in postmortem brain tissue for decreased numbers of hippocampal nicotinic receptors in schizophrenia. Biol Psychiatry. 1995;38:22-33.
221. Marutle A, Zhang X, Court J, et al. Laminar distribution of nicotinic receptor subtypes in cortical regions in schizophrenia. J Chem Neuroanat. 2001;22:115-126.

222. Martin-Ruiz CM, Haroutunian VH, Long P, et al. Dementia rating and nicotinic receptor expression in the prefrontal cortex in schizophrenia. Biol Psychiatry. 2003;54:1222-1233.
223. Perl O, Iliani T, Strous RD, Lapidus R, Fuchs S. The alpha7 nicotinic acetylcholine receptor in schizophrenia: decreased mRNA levels in peripheral blood lymphocytes. FASEB J. 2003;17:1948-1950.

224. Young JW, Crawford N, Kelly JS, et al. Impaired attention is central to the cognitive deficits observed in alpha 7 deficient mice. Eur Neuropsychopharmacol. 2007;17:145-155.
225. Levin ED, Bradley A, Addy N, Sigurni N. Hippocampal alpha 7 and alpha 4 beta 2 nicotinic receptors and working memory. Neuroscience. 2002;109:757-765.

226. Levin ED, Bettegowda C, Blasser J, Gordon J. AR-R17779, and alpha7 nicotinic agonist, improves learning and memory in rats. Behav Pharmacol. 1999;10:675-680.
227. Kolle K, Hashimoto K, Takai N, et al. Tropisetron improves deficits in auditory P50 suppression in schizophrenia. Schizophr Res. 2005;76:61-72.
228. Roncarati R, Scali C, Comery TA, et al. Procognitive and neuroprotective activity of a novel alpha7 nicotinic acetylcholine receptor agonist for treatment of neurodegenerative and cognitive disorders. J Pharmacol Exp Ther. 2009;329:459-468.
229. Meyer EM, Taylor ET, Patel RL, Meyers C, Huang GL, de Fiebre CM. 3-(2,4-Dimethoxybenzylidene)anabaseine (DMXB) selectively activates rat alpha7 receptors and improves memory-related behaviors in a memacylamine-sensitive manner. Brain Res. 1997;768:49-56.

230. Briggs CA, Anderson DJ, Brioni JD, et al. Functional characterization of the novel neuronal nicotinic acetylcholine receptor ligand GTS-21 in vitro and in vivo. Biochem Pharmacol. 1997;57:231-241.
231. Woodruff-Pak DS. Mecamylamine reversal by nicotine and by a partial alpha7 nicotinic acetylcholine receptor agonist (GTS-21) in rabbits tested with delay eyeblink classical conditioning. Behav Brain Res. 2003;143:159-167.
232. Stevens KE, Kem WR, Mahnir VM, Freedman R. Selective alpha7-nicotinic agonists normalize inhibition of auditory response in DBA mice. Psychopharmacology (Berlin). 1998;136:320-327.
233. Kitagawa H, Takenouchi T, Azuma R, et al. Safety, pharmacokinetics, and effects on cognitive function of multiple doses of GTS-21 in healthy, male volunteers. Neuropsychopharmacology. 2003;28:542-551.
234. Martin LF, Freedman R. Schizophrenia and the alpha7 nicotinic acetylcholine receptor. Int Rev Neurobiol. 2007;78:225-246.
235. Olincy A, Harris JG, Johnson LL, et al. Proof-of-concept trial of an alpha7 nicotinic agonist in schizophrenia. Arch Gen Psychiatry. 2006;63:630-638.
236. Freedman R, Olincy A, Buchanan RW, et al. Initial phase 2 trial of a nicotinic agonist in schizophrenia. Am J Psychiatry. 2008;165:1040-1047.
237. Tregellas JR, Olincy A, Johnson L, et al. Functional magnetic resonance imaging of effects of a nicotinic agonist in schizophrenia. Neuropsychopharmacology. 2010;35:938-942.
238. Rezvani AH, Kholdebarin E, Brucato FH, Callahan PM, Lowe DA, Levin ED. Effect of R3487/MEM3454, a novel nicotinic alpha7 receptor partial agonist and 5-HT3 antagonist on sustained attention in rats. Pugl Neuropsychopharmacol Biol Psychiatry. 2009;33:269-275.
239. Biton B, Bergis OE, Galli F, et al. SSR180711, a novel selective alpha7 nicotinic receptor partial agonist: binding and functional profile. Neuropsychopharmacology. 2007;32:1-16.
240. Pichat P, Bergis OE, Terranova JP, et al. SSR180711, a novel selective alpha7 nicotinic receptor partial agonist: (II) efficacy in experimental models predictive of activity against cognitive symptoms of schizophrenia. Neuropsychopharmacology. 2007;32:17-34.
241. Barak S, Arad M, De Levie A, Black MD, Griebel G, Weiner I. Pro-cognitive and antipsychotic efficacy of the alpha7 nicotinic partial agonist SSR180711 in pharmacological and neurodevelopmental latent inhibition models of schizophrenia. Neuropsychopharmacology. 2009;34:1753-1763.
242. Bertrand D, Gopalakrishnan M. AllostERIC modulation of nicotinic acetylcholine receptors. Biochem Pharmacol. 2007;74:1155-1163.
243. Hurst RS, Hadas M, Ragonnass M, et al. A novel positive allostERIC modulator of the alpha7 neuronal nicotinic acetylcholine receptor: in vitro and in vivo characterization. J Neurosci. 2005;25:4396-4405.
244. Broad LM, Zwart R, Pearson KH, et al. Identification and pharmacological profile of a new class of selective nicotinic acetylcholine receptor potentiators. J Pharmacol Exp Ther. 2006;318:1108-1117.
245. Young GT, Zwart R, Walker AS, Sher E, Millar NS. Potentiation of alpha7 nicotinic acetylcholine receptors via an allostERIC transmembrane site. Proc Natl Acad Sci U S A. 2008;105:14686-14691.
246. Freedman R, Goldowitz D. Studies on the hippocampal formation: From basic development to clinical applications: Studies on schizophrenia. Prog Neurobiol. 2010;90:263-275.
247. Liu J, Pearlson G, Windemuth A, Ruano G, Perrone-Bizzozero NI, Calhoun V. Combining fMRI and SNP data to investigate connections between brain function and genetics using parallel ICA. Hum Brain Mapp. 2009;30:241-255.

231. Woodruff-Pak DS. Mecamylamine reversal by nicotine and by a partial alpha7 nicotinic acetylcholine receptor agonist (GTS-21) in rabbits tested with delay eyeblink classical conditioning. Behav Brain Res. 2003;143:159-167.
232. Stevens KE, Kem WR, Mahnir VM, Freedman R. Selective alpha7-nicotinic agonists normalize inhibition of auditory response in DBA mice. Psychopharmacology (Berlin). 1998;136:320-327.
233. Kitagawa H, Takenouchi T, Azuma R, et al. Safety, pharmacokinetics, and effects on cognitive function of multiple doses of GTS-21 in healthy, male volunteers. Neuropsychopharmacology. 2003;28:542-551.
234. Martin LF, Freedman R. Schizophrenia and the alpha7 nicotinic acetylcholine receptor. Int Rev Neurobiol. 2007;78:225-246.
235. Olincy A, Harris JG, Johnson LL, et al. Proof-of-concept trial of an alpha7 nicotinic agonist in schizophrenia. Arch Gen Psychiatry. 2006;63:630-638.
236. Freedman R, Olincy A, Buchanan RW, et al. Initial phase 2 trial of a nicotinic agonist in schizophrenia. Am J Psychiatry. 2008;165:1040-1047.
237. Tregellas JR, Olincy A, Johnson L, et al. Functional magnetic resonance imaging of effects of a nicotinic agonist in schizophrenia. Neuropsychopharmacology. 2010;35:938-942.
238. Rezvani AH, Kholdebarin E, Brucato FH, Callahan PM, Lowe DA, Levin ED. Effect of R3487/MEM3454, a novel nicotinic alpha7 receptor partial agonist and 5-HT3 antagonist on sustained attention in rats. Pugl Neuropsychopharmacol Biol Psychiatry. 2009;33:269-275.
239. Biton B, Bergis OE, Galli F, et al. SSR180711, a novel selective alpha7 nicotinic receptor partial agonist: binding and functional profile. Neuropsychopharmacology. 2007;32:1-16.
240. Pichat P, Bergis OE, Terranova JP, et al. SSR180711, a novel selective alpha7 nicotinic receptor partial agonist: (II) efficacy in experimental models predictive of activity against cognitive symptoms of schizophrenia. Neuropsychopharmacology. 2007;32:17-34.
241. Barak S, Arad M, De Levie A, Black MD, Griebel G, Weiner I. Pro-cognitive and antipsychotic efficacy of the alpha7 nicotinic partial agonist SSR180711 in pharmacological and neurodevelopmental latent inhibition models of schizophrenia. Neuropsychopharmacology. 2009;34:1753-1763.
242. Bertrand D, Gopalakrishnan M. AllostERIC modulation of nicotinic acetylcholine receptors. Biochem Pharmacol. 2007;74:1155-1163.
243. Hurst RS, Hadas M, Ragonnass M, et al. A novel positive allostERIC modulator of the alpha7 neuronal nicotinic acetylcholine receptor: in vitro and in vivo characterization. J Neurosci. 2005;25:4396-4405.
244. Broad LM, Zwart R, Pearson KH, et al. Identification and pharmacological profile of a new class of selective nicotinic acetylcholine receptor potentiators. J Pharmacol Exp Ther. 2006;318:1108-1117.
245. Young GT, Zwart R, Walker AS, Sher E, Millar NS. Potentiation of alpha7 nicotinic acetylcholine receptors via an allostERIC transmembrane site. Proc Natl Acad Sci U S A. 2008;105:14686-14691.
246. Freedman R, Goldowitz D. Studies on the hippocampal formation: From basic development to clinical applications: Studies on schizophrenia. Prog Neurobiol. 2010;90:263-275.
247. Liu J, Pearlson G, Windemuth A, Ruano G, Perrone-Bizzozero NI, Calhoun V. Combining fMRI and SNP data to investigate connections between brain function and genetics using parallel ICA. Hum Brain Mapp. 2009;30:241-255.