Novel concepts and therapeutic options for the treatment of SCHIZOPHRENIA

Schizophrenia affects a large number of individuals irrespective of social status and education. Current treatments of schizophrenia represent the second largest central nervous system (CNS) pharmaceutical market, exceeding $12 billion per year. Schizophrenia is a complex mental disease affecting multiple neuronal systems, whose etiology and mechanisms are still not fully understood. However, new insights into the pathological molecular mechanisms have revealed entirely new opportunities for the discovery and development of next generation treatments. Hypofunction of glutamatergic neurotransmission is an emerging hypothesis supported by multiple lines of evidence that accounts for important mechanisms of schizophrenia. New approaches to enhance and restore the activity of glutamatergic neurotransmission may lead to the next generation of anti-psychotic drugs.

By Dr Karoly Nikolich and Dr Roman Urfer

Schizophrenia (Greek for 'split mind') is a severe mental disorder that affects about 1% of the population in industrialised countries. The estimated cost of the disease to the American society exceeds $32 billion annually. The disease is severely incapacitating for patients in managing their lives as masterfully portrayed in the book *A Beautiful Mind* and the 2002 Academy Award-winning movie with the same title[1]. The first symptoms of schizophrenia occur typically between the ages of 15 and 25. Some patients fully recover following treatment but most continue to have moderate or severe symptoms, particularly in response to stress. About 15% of patients return to a normal life after a single episode while 60% will have intermittent episodes throughout their lives and another 25% will never recover their ability to live as independent adults.

The first therapeutics for schizophrenia were discovered 50 years ago with Chlorpromazine as the first drug to be used for psychotic symptoms in the late 1940s. The agent became the main therapy during the 1950s and 60s. Since then a large number of agents similar to chlorpromazine have been

Figure 1
Leading therapy classes in 2003 global pharmaceutical sales

Rank	Therapy Class	2003 Sales ($bn)	% Global sales ($)	% Growth (constant $)
1	Cholesterol Reducers	26.1	6%	+14%
2	Anti-ulcerants	24.3	5	9
3	Antidepressants	19.5	4	10
4	Antirheumatic & NSAIDs	12.4	3	6
5	***Antipsychotics***	***12.2***	***3***	***20***
6	Calcium Antagonists, Plain	10.8	2	2
7	Erythropoietins	10.1	2	16
8	Anti-Epileptics	9.4	2	22
9	Oral Antidiabetics	9.0	2	10
10	Cephalosporins & Combinations	8.3	2	3
Total Leading 10 TCs		**$142.0bn**	**30%**	**+11%**

Source: IMS World Review 2004

developed and were used until the mid-1990s, when the 'atypical' antipsychotic agents were introduced. The atypical agents led to a breakthrough in managing patients as they have significantly improved efficacy and side-effect profiles.

Schizophrenia is currently the second largest CNS market behind depression and has surpassed epilepsy during the late 1990s based on the success of the atypical type agents. Anti-psychotics have been one of the fastest growing classes of therapeutic agents totalling more than $12 billion in sales in 2003 (**Figure 1**). The most successful agent to date, Zyprexa™ (Olanzapine) developed and marketed by Lilly, rapidly became a blockbuster in the management of schizophrenia and has created a global market that reached $4.8 billion annually in the past year. Zyprexa™ was the highest selling CNS drug and the third ranking drug of any class during the past year (**Figure 2**). The only other CNS drug among the top 10 was the anti-depressant Zoloft.

Schizophrenia is a chronic and debilitating psychiatric disorder characterised by severe disturbances in complex mental functions as perception, logical thinking and emotion. The complex symptoms vary significantly among individual patients. Experts define the observed symptoms in two broad categories: positive and negative symptoms. Positive symptoms generally include distortion or exaggeration of normal sensation and perception manifested as delusions, hallucinations, paranoia, and incoherent speech. Negative symptoms generally include the absence or diminution of normal cognitive, behavioural, motivational and affective

Figure 2
Leading products in 2003 global pharmaceutical sales

Rank	World product sales	2003 Sales ($bn)	% Growth (constant $)
1	Lipitor	10.3	+14%
2	Zocor	6.1	-4
3	***Zyprexa***	***4.8***	***+13***
4	Norvasc	4.5	+7
5	Erypo (Eprex/Procrit)	4.0	+13
6	Ogastro/Prevacid	4.0	0
7	Nexium	3.8	+62
8	Plavix	3.7	+40
9	Seretide/Advair	3.7	+40
10	Zoloft	3.4	+11
Total 10 Leading Products		**$48.3bn**	**+14%**

Source: IMS World Review 2004

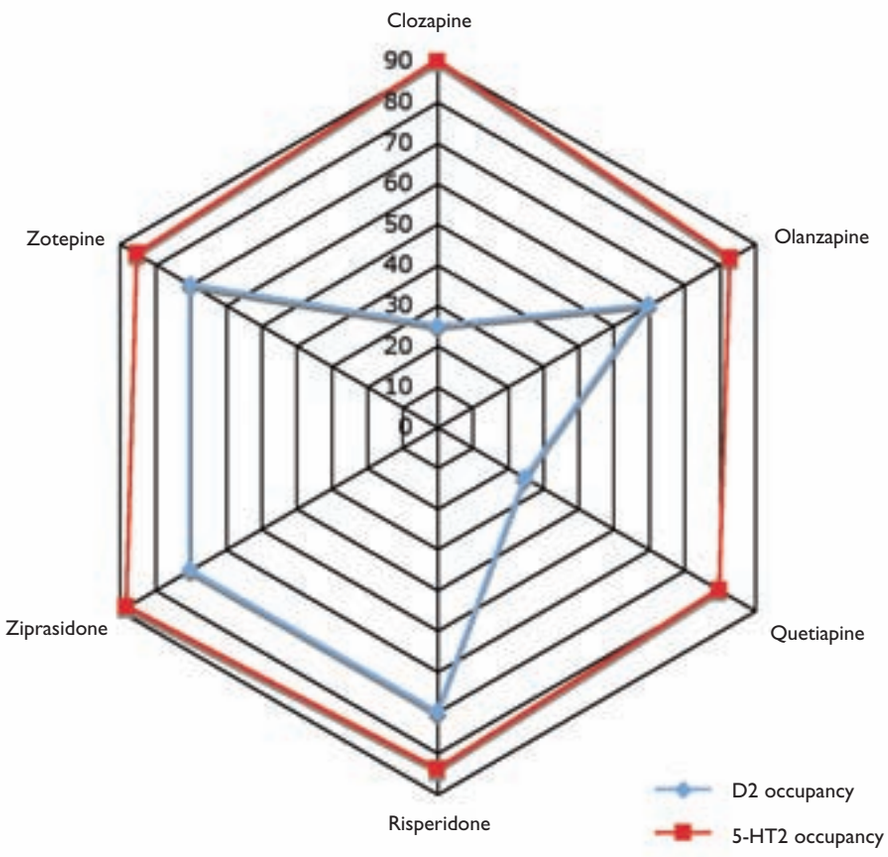
functioning, often characterised as apathy. Most patients experience both positive and negative symptoms. In addition, these problems are often accompanied by depression and anxiety. This broad failure and distortion of normal mental and cognitive facilities involves cortical systems, the limbic system, basal ganglia and the thalamus. Given the complexity of the neural networks involved in cognition, perception and emotion, it is not surprising that the neurobiological basis of the disease pathology is still not clearly understood[2,3].

Current anti-psychotics modulate dopaminergic and serotonergic mechanisms

A leading view of the underlying pathomechanism of schizophrenia is that there is excessive dopamine neurotransmission in the limbic system, which correlates with the manifestation of positive symptoms. Both baseline dopamine neurotransmission and stimulated release of dopamine are abnormally high in mesolimbic systems of the schizophrenic brain. Earlier pharmacologic data have now been supported by neuroimaging techniques. Increased subcortical dopamine turnover has been demonstrated in drug-naïve schizophrenic patients using positron emission tomography (PET). In addition, amphetamine-induced dopamine release is significantly greater in drug-naïve schizophrenic patients than in control subjects and this elevation correlates with the onset of positive symptoms[4].

In contrast to mesolimbic hyperdopaminergic activity, there is evidence that negative symptoms

Figure 3
Receptor occupancy of atypical antipsychotics



correlate with hypodopaminergic activity in cortical regions involved in communication and cognition. PET imaging of the brains of schizophrenic patients experiencing negative symptoms has revealed reduced blood flow and decreased metabolic activity in the prefrontal cortex, an area innervated by the mesocortical system.

Dopamine is a major inhibitory neurotransmitter that binds to and acts via cell surface proteins belonging to the 7-transmembrane type G-protein coupled receptors. These fall into two main groups based on specificity and pharmacology: The D1 family (D1 and D5 receptors) and the D2 family (D2, D3, and D4 receptors). Most antipsychotics block D2 receptors, which correlates with their therapeutic efficacy. However, the relationship between D2 receptor occupancy and clinical effect is not straightforward and antagonism at D3, as well as D4 receptors has been found to provide beneficial activity (**Table 1**).

Most atypical anti-psychotics show dual occupancy of dopamine and serotonin (specifically 5-HT2A) receptors (**Table 1** and **Figure 3**). Partial blockade of serotonin receptors is thought to be beneficial for the reduction of extrapyramidal symptoms (EPS), one of the main side-effects of the typical agents. In addition, atypical agents show clear superiority over older anti-psychotics as they appear to reduce tardive dyskinesia and prolactin related side-effects. However, significant weight gain is commonly observed with the consequence of increased cardiovascular risks and the negative impact on patient compliance. In addition, atypical antipsychotics bind to the adrenergic receptors, which cause QTc prolongation, one of the most serious side-effects.

Molecular studies to understand disease mechanisms

In order to complement the current atypical therapeutics, pharmaceutical companies are exploring new types of agents based on new concepts and mechanisms. Desirable properties of new drugs include broader-based efficacy, better side-effect profile, faster action and anti-depressant effect. In particular, there is a major need for anti-psychotic therapies that more effectively relieve the negative symptoms of schizophrenia.

New insights from genetics, molecular and pharmacological studies

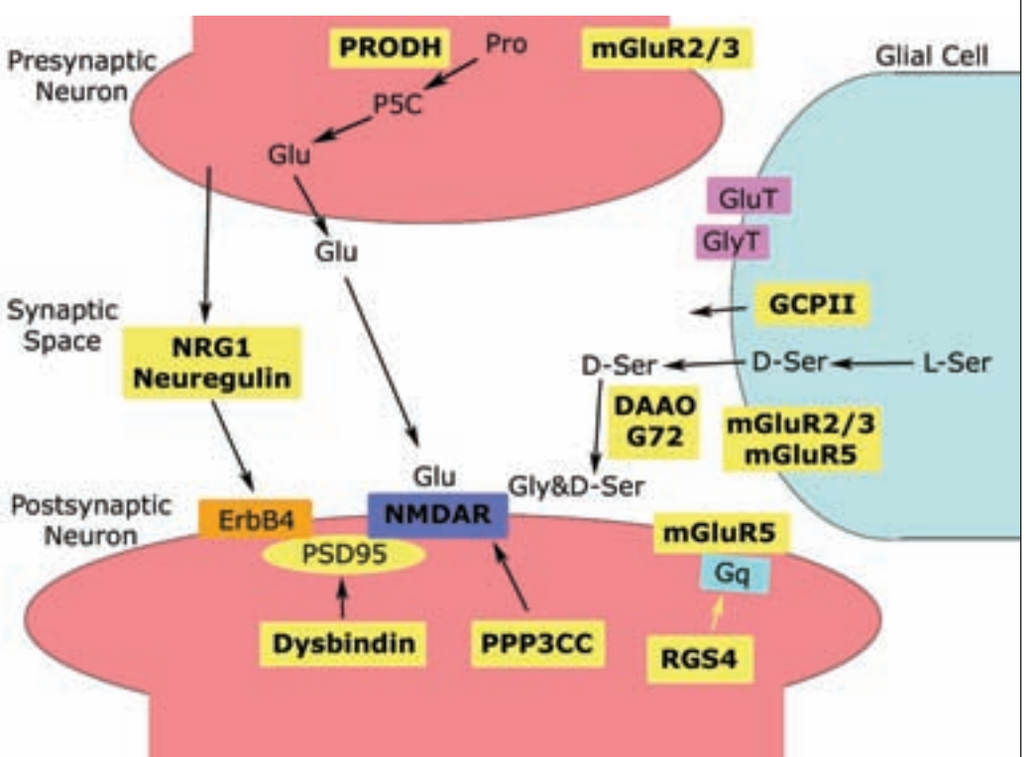
Schizophrenia is highly heritable, however, the search for chromosomal loci and specific genes has been challenging. There is no monogenic (Mendelian) form of the disease, which may be due to the complexity of the pathology. Multiple susceptibility genes may be contributing fractional effects, which in turn may act in concert with epigenetic processes and environmental factors. However, significant progress has been made in a) establishing linkages to several chromosomal regions and in refining the regions of linkage based on replicated experiments, and b) in the identification of single nucleotide polymorphisms (SNPs) associated with schizophrenia, and in the identification of candidate gene(s) containing the associated SNPs and haplotype (a combination of SNPs). These studies are pointing toward an essential role for glutamatergic transmission as a key pathway of the pathology as summarised in **Figure 4** (modified after 5 and 6). Mutations and/or levels of specific genes and proteins summarised in the figure lead to impaired glutamatergic transmission via the multiple mechanisms involved.

Glutamate is the major excitatory neurotransmitter in the mammalian CNS acting through both ligand gated ion channels, the ionotropic receptors and G-protein coupled receptors, referred to as metabotropic receptors. Activation of these receptors is responsible for basal excitatory synaptic transmission and many forms of synaptic plasticity such as long-term potentiation

RECEPTOR	CLOZAPINE	OLANZAPINE	QUETIAPINE	RISPERIDONE	ZIPRASIDONE	ZOTEPINE
D1	■	■	■			■
D2	■	■	■	■	■	■
D3	■	■			■	■
D4	■	■				■
D5						
5-HT1A	■		■	■	■	
5-HT1C				■		
5-HT1D				■	■	
5-HT2A	■	■	■	■	■	■
5-HT2C	■	■			■	■
5-HT3	■	■				
5-HT6	■	■	■			■
5-HT7	■		■	■	■	■
α1	■	■	■	■	■	■
α2	■		■	■		

Table 1: Receptor binding profiles of leading antipsychotic agents

Figure 4
Proteins associated with schizophrenia



Proteins/genes that have been associated with schizophrenia are shown in bold. PRODH: Proline dehydrogenase; mGluR2/3: metabotropic glutamate receptor 2/3; DAAO: D-amino acid oxidase; G72: Activator of DAAO; NRG1/Neuregulin; NMDAR: NMDA receptor (NR1 and NR2 subunits); RGS4 = regulator of G protein signalling 4; Gq = mGluR signalling G protein; PPP3CC: calcineurin subunit; GCP II: Glutamate decarboxylase II

(LTP) and long-term depression (LTD), which are believed to be cellular mechanisms that underlie learning and memory.

There are two main types of ionotropic glutamate receptors, which are ligand gated ion channels. The AMPA and kainate receptors mediate fast synaptic transmission. The NMDA receptors are specialised coincidence detectors. At resting membrane potentials, NMDA receptors are inactive due to a voltage-dependent block of the channel pore by magnesium. Sustained activation of AMPA receptors depolarises the post-synaptic cell, releasing the channel inhibition and thus allowing NMDA receptor activation. NMDA receptor activation leads to a calcium influx into the post-synaptic cells, a crucial signal for the induction of mechanisms underlying cognitive functions.

Metabotropic glutamate (mGluR) receptors are G-protein coupled receptors (GPCRs) that can be subdivided into three groups, based on sequence similarity, pharmacology and intracellular signalling mechanisms. Group I mGlu receptors are coupled to PLC and intracellular calcium signalling, while group II and group III receptors are negatively coupled to adenylyl cyclase.

The involvement of the glutamatergic system in schizophrenia has been suggested through earlier independent findings, as well. The potent psychotomimetic drug phencyclidine (PCP) is an antagonist of the NMDA receptor[7]. Phencyclidine was introduced in the 1950s as an anesthetic, but it was found to cause severe postoperative hallucinations and psychosis. In patients with schizophrenia, PCP led to profound exacerbation of pre-existing symptoms.

Recent clinical trials using various NMDA antagonists found transient psychosis and cognitive deficits that are similar to those observed in schizophrenia[8]. A single dose of the antagonists is sufficient to produce these behavioural effects, suggesting that transient changes in the functional state of the NMDA receptor may be sufficient for inducing schizophrenia-like symptoms. Animal studies using NMDA receptor antagonists also indicate a role for NMDA receptors in a wide spectrum of behaviours that are relevant to schizophrenia[9]. Similar behavioural patterns were observed in transgenic mice in which the NR1 subunit of the NMDA receptor was substantially reduced[10].

In fact, the converging discoveries of genetics, SNPs, transgenic and knockout mouse data, as well as pharmacological data pointing towards a glutamatergic component of schizophrenia are also in agreement with the anatomy of connections involved. Reciprocal connections between the cortex, the limbic system and the thalamus are glutamatergic: they use glutamate as their primary neurotransmitter. In addition, efferents from all cortical areas, as well as key corticolimbic regions, such as the hippocampus and amygdala, to motor effector sites are glutamatergic. Glutamatergic neurons are, therefore, the exclusive means by which aberrant information is transferred within and between these regions. Abnormal CSF levels of glutamate and postmortem glutamate receptor binding in schizophrenic individuals have been reported since the 1970s. However, the limitations of these crude measures prevented them from being taken seriously. The glutamate theory of schizophrenia was brought into the mainstream as a result of more sophisticated analyses of postmortem brains of schizophrenics[11].

Novel opportunities for therapeutic intervention based on the modulation of glutamatergic transmission

The option of glutamate receptor stimulation is very appealing. As discussed, NMDARs are the underlying signalling principles for processes involved in learning and memory. If their hypofunction is one of the causes of schizophrenic symptoms, the restoration of this function offers an ideal mechanism for therapeutics. The negative symptoms of schizophrenia are largely related to cognitive dysfunction, lack of motivation, apathy and depressed states. This could well correlate with a hypoglutamatergic mechanism as suggested by the emerging literature.

However, several pathologies have taught us that NMDARs can be overstimulated, and this mechanism can lead to a spreading damage in neuronal tissues. In fact, this phenomenon triggered by seizures, lack of energy supply, termed glutamate 'excitotoxicity', is believed to be involved in neuronal cell death following stroke, epilepsy, head trauma, spinal cord injury and other conditions. Major efforts have been invested in the development and testing of NMDAR antagonists as neuroprotective agents against a wide range of pathologies.

Fortunately, major new developments during the past couple of years have taught us that elevated NMDAR will not necessarily cause excitotoxicity. Transgenic mice overexpressing NMDAR do not show any signs of excitotoxicity demonstrating that elevated levels of NMDAR *per se* do not cause damage[12]. These mice showed enhanced cognitive functions supporting the idea that stimulation of NMDAR is beneficial. How can the danger of excitotoxicity be avoided and yet NMDAR stimulating or enhancing agents be developed into therapeutics? One possibility is identifying the correct dosing for direct or indirect stimulatory agents. A second possibility is to achieve only moderate stimulation through allosteric ligands. A third possibility is to stimulate downstream signalling and provide a moderate amplification of the intrinsic, activity-dependent signal without the danger of broad stimulation. Based on our newly emerging knowledge, several of these alternatives are being explored by the pharmaceutical industry. All of these agents represent promising new avenues towards novel therapeutics. Each of them is unique and promising, yet they all have certain limitations which are discussed below.

mGluR5 agonists and positive allosteric regulators

Stimulation of mGluR5 post-synaptically is expected to stimulate NMDARs. However, mGluR5 is expressed also pre-synaptically (**Figure 4**); this is expected to lead to stimulation of all postsynaptic glutamate receptors, including AMPARs. Excessive activation by mGluR5 agonists leads to epileptic activity as shown in knockout mice.

mGluR2/3 agonists and positive allosteric regulators

SNP analysis has implicated mGluR3 in schizophrenia (GRM3 gene). The presynaptic activation of mGluR2/3 decreases glutamate release and counters the activation of NMDAR signalling.

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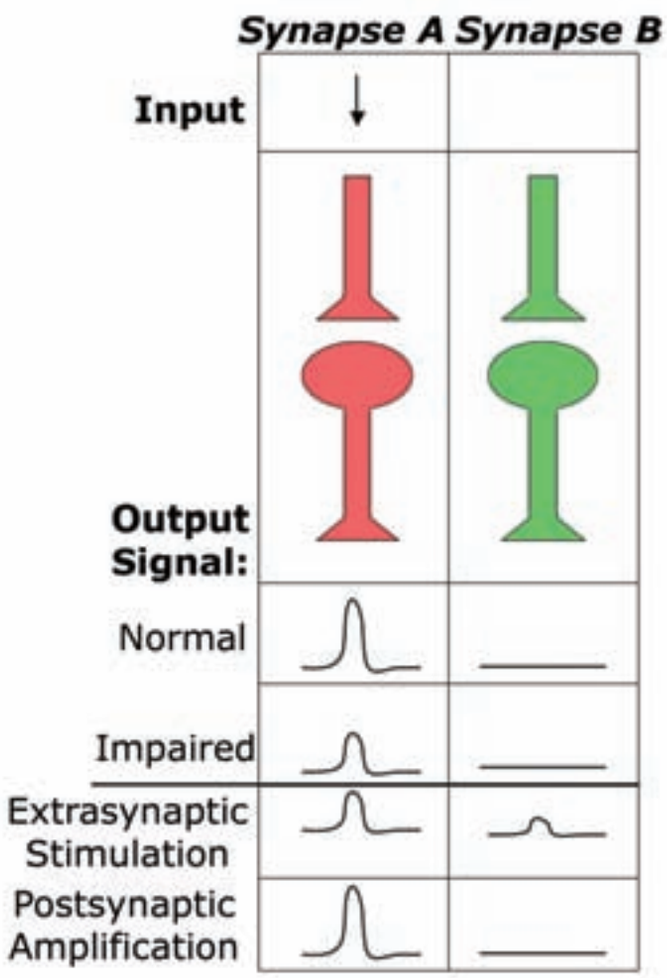
Figure 5
Options for restoring impaired synaptic signals



In this model, synapse A mediates the primary transmission of a neural circuit. Stimulation of synapse A leads to a normal output, and no output through synapse B. Impaired (eg schizophrenic or aged) output is weakened. Stimulation with an agent acting extrasynaptically leads to an elevation of the signal, however, with a higher background and a weak stimulation of synapse B, as well. An optimal and selective method of restoring the normal output transmission would be amplification of the postsynaptic signalling pathway

Post-synaptically, the cAMP-protein kinase A signaling pathway is negatively modulated by mGluR2/3 leading to an inhibition of the CREB pathway, which may turn out to be a problem, as the CREB pathway plays an important role in learning and memory processes.

Glycine site modulators

D-amino acid oxidase, DAAO, has been linked to schizophrenia. This enzyme processes D-amino acids, including D-serine that binds to the glycine modulatory site of NMDARs. Agonists including glycine, D-serine and D-cycloserine have been found to be beneficial in animal models of schizophrenia. They have also been used with success clinically in combination with other antipsychotics. However, glycine and D-cycloserine have limited blood-brain-barrier penetration properties and synthetic co-agonists have been difficult to develop.

Glycine transporter (GlyT1) inhibitors

Elevating glycine levels in the synaptic space can also be achieved by inhibiting the reuptake of this amino acid. However, this mechanism may be difficult to control. GlyT1 knockout mice show severe motor and respiratory deficits and die shortly after birth. Use of GlyT1 inhibitors in normal mice suppresses respiratory activity.

Future directions

Stimulating glutamatergic neurons can be achieved through multiple ways by the stimulation or inhibition of specific proteins presynaptically, postsynaptically, or synaptically (**Figure 4**). Several of these targets and their modulatory agents are being explored by the pharmaceutical industry. However, one of the most challenging aspects of all these efforts will be to achieve specific, selective enhancement of glutamatergic transmission that will depend on intrinsic activity. Disease states such as schizophrenia, ageing, and other causes lead to a weakened signal (**Figure 5**). Amplification of an impaired endogenous glutamate signal would be an ideal way to restore selective signalling. This could be achieved via the understanding of downstream, intraneuronal signalling pathways and modulation of the best targets involved. Phosphorylation of the subunits of the NMDA receptor is critical for proper activity, and therefore, hypoglutamatergic transmission by the unphosphorylated receptor may be involved in the schizophrenic disease condition. That this concept may have clinical relevance was recently demonstrated by the finding that schizophrenic patients had a large fraction of the NR1 subunits of their NMDA receptor unphosphorylated[13]. Restoration of phosphorylation by blocking phosphatase(s) may thus offer a promising option towards new, specific amplification of glutamatergic transmission. DDW

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