

# Regulation of Intermittent Oscillatory Activity of Pyramidal Cell Neurons by GABA Inhibitory Interneurons is Impaired in Schizophrenia: Rationale for Pharmacotherapeutic GABAergic Interventions

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## ABSTRACT

GABA, the major inhibitory neurotransmitter in the brain, is synthesized from L-glutamate and packaged within a family of highly differentiated inhibitory interneurons. Individual GABA inhibitory interneurons in the frontal cortex can make terminal synaptic connections with more than 200 distinct pyramidal neurons, the principal output neuron. Moreover, the sites of these synaptic connections include shafts of dendritic spines, soma, dendritic branches, and initial axon segments. The phasic activity of GABAergic neurons regulate intermittent oscillations of assemblies of pyramidal cell neurons, which are critical for many higher cortical functions such as working memory. Potentially, there are several viable pharmacotherapeutic strategies for facilitating GABAergic neurotransmission. A major research question is whether tonically-administered, selective GABAergic therapeutic interventions can mimic and correct disruptions of the intermittent oscillatory activity of assemblies of cortical pyramidal cell neurons.

Schizophrenia is a neurodevelopmental disorder of disturbed synaptic connectivity. Cognitive deficits correlate with poor functional outcomes, do not vary with state of illness or medication status, and can often be detected in milder form in unaffected closely-related biological relatives of patients with schizophrenia. Disturbances of GABAergic inhibitory influences in the dorsolateral prefrontal cortex contribute to cognitive symptoms and serve as therapeutic targets in schizophrenia (1-6). Importantly, a delicate balance must exist between GABAergic inhibitory influences and excitatory influences that are primarily mediated by L-glutamate (7).

## GABAergic DYSFUNCTION IN SCHIZOPHRENIA

GABA is the major inhibitory neurotransmitter in the brain, mediating fast synaptic neurotransmission on a less than 100-millisecond timeframe via its ability to gate a receptor-associated chloride ion channel, referred to as the GABA<sub>A</sub> receptor (8-11). GABA is a critical neurotransmitter involved in the control of cortical oscillatory rhythmic activity, during which fundamental cognitive processes occur; these GABA-regulated oscillatory network activities and their associated cognitive processes are often disturbed in schizophrenia (e.g., beta<sub>2</sub> and gamma oscillations) (1-5, 9, 12, 13).

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Abnormalities of GABAergic inhibitory tone in the cortex of patients with schizophrenia were suggested by postmortem studies reporting decreased activity of glutamic acid decarboxylase (GAD), decreased GABA reuptake and a “compensatory” increase of GABA receptor binding (8, 9, 14-18). These earlier studies were corroborated by later gene expression studies showing diminished cortical expression of mRNA transcripts expressed in GABAergic neurons: GAD<sub>67</sub>, the biosynthetic enzyme for GABA; reelin, a signaling-protein that is critical for the correct lamination of the developing brain and changes in dendritic spine density that serve as morphological correlates of neuronal plasticity; parvalbumin, a calcium ion binding protein; and the GABA membrane transporter (GAT1), which mediates presynaptic reuptake of GABA and termination of its synaptic actions (8, 9, 14-19).

Diminished expression of parvalbumin in GABAergic neurons (e.g., chandelier neurons) may be a compensatory response that facilitates presynaptic GABA release because, ordinarily, parvalbumin is responsible for decreasing the transient elevations of intraneuronal calcium ion concentrations that mediate depolarization-dependent GABA release from GABA nerve terminals (9). Similarly, reduced expression of GAT1 on the nerve terminals of chandelier cells, which would increase synaptic levels of GABA, and increased density of  $\alpha 2$ -containing GABA<sub>A</sub> receptors on the axon initial segment of pyramidal neurons may reflect a compensatory, but inadequate, “facilitation” of GABA signaling (9, 15, 16-18).

Isolation-induced rearing of rats after they are weaned leads to a quantifiable deficit in sensorimotor gating (i.e., impaired prepulse inhibition (PPI) of the acoustic startle response) that is also manifest by patients with schizophrenia and their closely-related biological relatives (20). Of possible interest to the GABAergic dysfunction of schizophrenia, which may be widespread throughout the cerebral cortex and hippocampus, is that the immunoreactive protein content of parvalbumin and calbindin, two calcium ion binding proteins expressed in GABA inhibitory interneurons, was significantly reduced in several hippocampal subfields (i.e., dentate gyrus, CA2/3, and CA1 [only calbindin]) of the isolation-reared rats (20). These data are important for several reasons: they suggest that an environmentally-relevant social stressor can selectively alter hippocampal expression of proteins in animals displaying deficits relevant to the pathophysiology of schizophrenia and can cause dysfunction of GABAergic

neurons. Presumably, this effect of isolation-rearing is mediated by epigenetic mechanisms that affect expression of parvalbumin and calbindin (20).

## PHARMACOLOGICAL ANATOMY OF THE GABA<sub>A</sub> RECEPTOR COMPLEX

The GABA<sub>A</sub> receptor complex is a member of a superfamily of ligand-gated ion channel receptors that includes nicotinic acetylcholine receptors; the receptor is a pentameric protein complex constructed from five constituent polypeptide subunits (9-11). The individual subunits are integral membrane proteins that share significant sequence homology with each other, as well as a common motif: a large extracellular N-terminal domain, three membrane-spanning hydrophobic domains (M1-M3), an intracellular cytoplasmic loop between M3 and M4, a final membrane-spanning domain (M4), and a C-terminal extracellular end. The M2 domain from each of the five constituent polypeptides align themselves in such a manner that they form a potential pore or channel, whose kinetics of opening is determined by the binding of GABA to the receptor. The intracellular loop has multiple phosphorylation sites; the state of phosphorylation may be regulated by the second messenger cascades of other neurotransmitter receptors, reflecting “cross-talk” between GABA and these other neurotransmitters. Based on the extent of their sequence homology, at least 16 individual polypeptide subunits, which are encoded by distinct genes, are grouped into families identified by Greek letters:  $\alpha 1$ -6,  $\beta 1$ -3,  $\gamma 1$ -3,  $\delta$ ,  $\epsilon$ ,  $\theta$ ,  $\pi$ , and  $\rho 1$ -3. Most commonly, functional receptors contain two  $\alpha$  subunits, two  $\beta$  subunits, and a  $\gamma$  subunit, the  $\gamma$  subunit is rarely replaced by a subunit from another family (9).

If the constraint of subunit combinations is restricted to receptors containing two  $\alpha$  and two  $\beta$  subunits, combinatorial diversity could result in more than 2,000 pharmacologically-distinctive GABA<sub>A</sub> receptors; however, fewer than 20 of the 2,000 possible combinations are commonly detected in the brain. The  $\alpha 1$ -containing receptors are most abundant, they are located synaptically on cell bodies and dendrites of principal neurons and extrasynaptically; they are frequently combined with  $\beta 2$  and  $\gamma 2$  subunits, and have a high-affinity for classical benzodiazepines, mediating their sedative, amnestic and anticonvulsant properties (10, 11, 21).  $\alpha 2$ -Containing receptors are expressed less commonly than  $\alpha 1$ -containing receptors, are usually combined with

$\beta 3$  and  $\gamma 2$  subunits, and have a high-affinity for classical benzodiazepines, mediating primarily their anxiolytic actions (9-11). The localization of  $\alpha 2$ -containing GABA<sub>A</sub> receptors on the initial segment of principal neuron axons in the cerebral cortex and hippocampus is consistent with their functional role in controlling the output of these neurons. Mice with a genetically-engineered knockout of the gene for the  $\alpha 3$  subunit show a profound deficit of prepulse inhibition of the acoustic startle reflex, reflecting a deficit in sensorimotor gating that models what is frequently seen in patients with schizophrenia (9, 21).  $\alpha 5$ -Containing GABA<sub>A</sub> receptors are located extrasynaptically in hippocampus and the cerebral cortex; their location on the dendritic spines of hippocampal pyramidal cells, a site of excitatory N-methyl-D-aspartate (NMDA) receptors, implicates their complementary involvement with NMDA receptors in regulating activity of this principal hippocampal efferent neuron (1, 9, 21). The  $\alpha 5$ -containing receptor is involved in a variety of hippocampal functions, including spatial and temporal associative memory, spatial learning, fear conditioning and prepulse inhibition. Although not sufficient by itself, the presence of the  $\gamma 2$  subunit contributes to benzodiazepine sensitivity; receptors containing either  $\gamma 1$  or  $\gamma 3$  subunits have reduced affinity for classical benzodiazepines (9, 21). Clearly, the inhibitory tone of the brain, including phasic inhibition that supports critical oscillatory rhythms, is highly regulated. However, the diversity of pharmacologically-distinctive GABA<sub>A</sub> receptors presents, at least theoretically, the opportunity for the development of highly-selective therapeutic interventions, such as  $\alpha 3$  and  $\alpha 5$ -selective GABA<sub>A</sub> receptor agonists (9, 21, 22).

### **GABA INHIBITORY INTERNEURONS REGULATE PRINCIPAL PYRAMIDAL CELL NEURON OUTPUT**

Pyramidal neurons are primarily glutamatergic and serve as the principal output neurons of the dorsolateral prefrontal cortex, integrating excitatory and inhibitory inputs and sending axonal projections to other cortical regions (layers 2 and 3), striatum and subcortical structures (layer 5), and thalamus (layer 6), among other possible projections (1-6). A possible morphological correlate of disturbed synaptic connectivity in schizophrenia is a lowered density of dendritic spines on pyramidal neurons in deep layer 3; also, there may be a reduction of somal area of these pyramidal cell neurons (4, 5, 9). The mediodorsal thalamus provides excitatory input to

the pyramidal neurons in layers 3 and 4 of the dorsolateral prefrontal cortex (5). Postmortem data show that the cortical neuropil, which is the “space” between neurons comprised of axon terminals, dendritic spines and glial processes, is reduced in the dorsolateral prefrontal cortex of patients with schizophrenia, which is consistent with disruption of synaptic connectivity.

GABAergic inhibitory neurons can be distinguished from each other by shape, laminar locations, projections to principal neurons (e.g., dendritic spines, soma, or initial axon segment) and other GABAergic cells, membrane firing properties, and expression of specific proteins and neuropeptides (e.g., parvalbumin and calcitonin, which are calcium ion binding proteins, and somatostatin and cholecystokinin, which are neuropeptides) (5, 9). Moreover, GABAergic interneurons show a domain-specific innervation of the principal cells to which they project (e.g., hippocampal pyramidal cells) affecting both the input that these principal cells receive as well as their output, which is regulated by axo-axonic GABAergic interneurons. Importantly, the response of the principal neuron is not only affected by the spatial distribution of GABAergic innervation and the morphological type of interneuron, but also by the type of synaptic and extrasynaptic GABA<sub>A</sub> receptor (5, 9). For example, there are two types of GABAergic “basket” cells that innervate the cell body of the hippocampal cell: so-called “fast spiking” parvalbumin-containing basket cells that synapse with  $\alpha 1$ -containing GABA<sub>A</sub> receptors and “regular spiking” cholecystokinin (CCK)-positive basket cells that synapse with  $\alpha 2$ -containing GABA<sub>A</sub> receptors (5, 9). Within the dorsolateral prefrontal cortex, parvalbumin-containing GABAergic neurons are of two types: chandelier neurons that form synapses on the axon initial segments and basket neurons that form synapses on the cell bodies and proximal dendrites of pyramidal neurons (5, 9).

Working memory is very sensitive to disruption of the normal activity of GABAergic inhibitory neurons in the dorsolateral prefrontal cortex (1, 5, 9, 12). Chandelier and basket GABAergic inhibitory neurons subserve the timing mechanism that synchronizes the activity of local populations of pyramidal neurons that they target, which underlie the gamma oscillations in the 30 to 80 Hz band. The power of the gamma oscillations in the dorsolateral prefrontal cortex are associated with working memory load; both working memory load and the power of gamma oscillatory activity in the dorsolateral prefrontal cortex and elsewhere are diminished in schizophrenia (5, 12).

The synchronous firing of assemblies of cortical pyra-

midal neurons in prefrontal cortex that is controlled by fast-spiking GABAergic interneurons is intermittent, which is consistent with a role in higher cognitive processes that are activated selectively and according to specific task demands. Cortical GABAergic inhibitory interneurons may establish synapses with more than 200 different pyramidal neurons, which serves as the anatomic substrate for orchestrating the intermittent synchronous firing of these assemblies of pyramidal neurons. As discussed, the output of the principal cortical pyramidal cell neurons (including locally-evoked graded membrane potentials, triggering of action potential that invades the axon, and depolarization-dependent and calcium ion-dependent release of glutamate) is multiterminated and depends on their laminar locations, efferent input of morphologically and biochemically-distinct GABAergic interneurons, and sites of synaptic connections (e.g., proximal dendrite or distal branch, dendritic spine or neck of dendritic spine, cell body, or initial axon segment). Thus, pyramidal cell bodies and proximal dendrites receive innervation from basket cells in cortical layers II, III, V, and VI, axon initial segments receive innervation from chandelier interneurons in middle cortical layers, and double-bouquet and horizontal cells innervate shafts of dendritic branches in layers II/III and layer I, respectively (21).

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### BIOCHEMICAL HETEROGENEITY OF GABA INHIBITORY INTERNEURONS

GABAergic inhibitory interneurons can be biochemically distinguished from each other by whether or not they constitutively express and release reelin, a “signaling” glycoprotein that shares structural analogies with extracellular matrix proteins (19, 23-25). In the developing fetal brain, reelin-signal transduction is critical for the correct positioning of post-mitotic neurons migrating from the ventricular zone, formation of appropriate synaptic connections and cortical lamination, whereas in the fully-developed and mature brain, reelin-signal transduction participates in the mediation of experience-dependent synaptic plasticity (23). A morphological correlate of reelin’s role in experience-dependent synaptic plasticity is the creation and reshaping of dendritic spines, which depend on the stable assembly of correctly-aligned microtubules. Two members of the family of low-density lipoprotein receptors that are expressed in brain (i.e., very low-density lipoprotein [VLDL] receptor and apolipoprotein E

[apoE] type 2 receptor) participate in the transduction of the reelin signal, a process that includes several significant “downstream” consequences (23). At least one of these important “downstream” consequences that may be related to changes in the size, shape and density of dendritic spines, a morphological correlate of experience-dependent learning, is the stabilization of microtubules by inhibiting hyper-phosphorylation of the tau protein, an important microtubule-associated protein. Therefore, it is of interest that GABAergic interneurons regulate both the synchronous intermittent firing of assemblies of cortical pyramidal neurons, which is part of the electrophysiological substrate for gamma oscillations that are necessary for cognitive processes such as working memory, and changes in size, shape and density of dendritic spines, a morphological correlate of experience-dependent learning (5, 12, 23).

There are emerging data suggesting that in at least some patients with schizophrenia, there is diminished expression of GAD<sub>67</sub>, the rate-limiting enzyme for GABA biosynthesis with a high-affinity for binding its pyridoxal phosphate cofactor, and reelin in a selective population of GABAergic neurons (9, 14, 19). Because of its high-affinity for pyridoxal phosphate and saturation of the binding site on the enzyme with ordinary tissue levels of this cofactor, the activity of GAD<sub>67</sub> is regulated by gene expression; increased activity is related to increased translation of new GAD<sub>67</sub> mRNA. Thus, if there is diminished expression of GAD<sub>67</sub>, there is shrinkage of the neurotransmitter pool of GABA.

Diminished expression of both GAD<sub>67</sub>, which leads to a presynaptic deficiency of GABA in selected neurons, and reelin, which leads to disruption of the morphological expression of synaptic plasticity (i.e., changes in size, shape and density of dendritic spines), can occur as a result of altered epigenetic regulation of the genetic expression of these two proteins in patients with schizophrenia (19).

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### GABAergic DYSFUNCTION UNDERLIE DEFICITS OF WORKING MEMORY IN SCHIZOPHRENIA: EFFECTS OF NON-SELECTIVE GABAergic INTERVENTIONS

The effects of lorazepam, a GABA<sub>A</sub>/benzodiazepine receptor agonist, and flumazenil, a GABA<sub>A</sub>/benzodiazepine antagonist or partial inverse agonist on working memory performance were studied while patients and control subjects underwent functional magnetic resonance imaging (fMRI) (13). The goal of the study was

to show differential effects of the two benzodiazepine receptor ligands on task performance and fMRI, as well as differences in the modulatory effects of the two ligands between patients and control subjects. Eleven right-handed, male patients with chronic schizophrenia (mean duration of illness  $\pm$ SD =  $17.0 \pm 8.3$ ) stably medicated with antipsychotic medications (10 of whom were receiving clozapine) and showing impairment of episodic memory and verbal fluency and moderate symptom severity were matched for gender, age, handedness and premorbid IQ with 11 control subjects (13). In this placebo-controlled, double-blind study, participants underwent four imaging sessions each of which was separated by two weeks: baseline and then, in counterbalanced, randomized fashion, assigned to lorazepam (2 mg oral capsule), flumazenil (IV bolus of 0.9 mg, followed by constant IV infusion at a rate of 0.0102 mg/min), or matching placebo condition. While the images were obtained, the participants performed an “N-back test of verbal working memory” at three levels of task difficulty. The task required the participants to indicate whether a current visually-presented letter was the same as the one immediately preceding it (N=1) or the same as the one presented two (N=2) or 3 (N=3) letters earlier. Across all participants, task performance worsened with increasing difficulty and cognitive demands of the task, as measured by a discrimination index derived from signal detection theory that corrects for false positive responses. There was also a significant interaction between drugs and task difficulty with the effects of lorazepam and flumazenil most marked at the N=1 and N=2 levels of difficulty. Among the patients with schizophrenia, lorazepam, the GABA<sub>A</sub>/benzodiazepine receptor agonist, significantly worsened working memory performance, whereas flumazenil, the GABA<sub>A</sub>/benzodiazepine receptor antagonist or partial inverse agonist actually improved performance (13). These data are somewhat contrary to what was expected in what may be our limited “linear” conceptualizations of effects of diminished GABAergic tone on, or phasic regulation of, integrated assemblies of principal pyramidal neurons by the parvalbumin-expressing, chandelier GABAergic cortical interneurons. Rather, the exacerbation of the already poorer performance of the patients with schizophrenia relative to controls by lorazepam, a positive allosteric modulator of GABA<sub>A</sub> receptors, and improvement of performance by flumazenil, which might be expected to dampen the endogenous GABAergic tone or be devoid of any effect suggests that mechanisms of

working memory impairment and disturbed pyramidal oscillatory activity in schizophrenia may be more complex, involving “overinhibition.” The findings were consistent with effects of GABA<sub>A</sub> receptor-modulating drugs on working memory performance, as opposed to nonspecific effects on response latency (13, 21).

In the control subjects performing the working memory task under the placebo condition, the fMRI data showed that there were areas of brain activation, including the bilateral prefrontal, premotor, parietal and anterior cingulate cortices, and areas of brain deactivation, including the bilateral temporal and posterior cingulate regions, in association with increasing levels of task difficulty (13). The magnitude of the coordinated changes of activation and deactivation was reduced in the patients with schizophrenia, compared to the control subjects. The data suggested that flumazenil had significantly different effects on the fMRI data in the patient group, enhancing deactivation generally and possibly increasing activation in the anterior cingulate cortex. The fMRI data highlight an important role for GABA in coordinating and assuring efficiency of diffuse brain regions while performing complex cognitive tasks. This efficiency appears to be impaired in schizophrenia and may be improved by selective interventions with GABA<sub>A</sub> receptor-modulating drugs.

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### EPIGENETIC REGULATION OF GABAERGIC FUNCTION: POTENTIAL OPPORTUNITIES FOR THERAPEUTIC INTERVENTION

An epigenetic mechanism that could suppress expression of both GAD<sub>67</sub> and reelin is the “hypermethylation” of the 5' position of the cytosine ring in so-called CpG islands in the promoter regions of the genes for these two proteins. An enzyme responsible for methylating cytosine rings in promoter regions is DNA-methyltransferase 1 (DNMT1), whose preferential expression may be increased in the same cortical GABAergic interneurons (i.e., there is increased mRNA and protein content for DNMT1 in these neurons) showing decreased expression of GAD<sub>67</sub> and reelin (19, 24, 25). If true, it is conceivable that epigenetic therapeutic strategies can be developed to “inhibit” DNMT1 and, thereby, promote expression of GAD<sub>67</sub> and reelin. Additionally, strategies to promote chromosomal remodeling that involve inhibiting the deacetylation of histone proteins can lead to promotion of gene expression (26). Thus, in terms of a potential mechanism of pathogenesis of schizophrenia, it is very provocative that both the mRNA and protein content of DNMT1 are increased

in cortical GABAergic interneurons of layers I, II, and IV in postmortem brains obtained from carefully-diagnosed patients with the disorder; interneurons in these same cortical layers show deficient expression of GAD<sub>67</sub> and reelin (25).

The hypermethylation hypothesis of specific cytosine residues in the promoter regions of the genes for reelin and GAD<sub>67</sub> is an appealing epigenetic mechanism that could account for GABAergic dysfunction. Unfortunately, when the DNA methylation status of the promoter region for the reelin gene (RELN) was studied in prefrontal cortices of 14 patients with schizophrenia and 13 controls using a different methodology (referred to as the pyrosequencing method), especially focusing on cytosines at positions -139 and -134 that were reported to be hypermethylated in an earlier study (24), the level of DNA methylation in both groups was below the level of detection (27). Thus, this independent study did not confirm that the RELN promoter is hypermethylated in the prefrontal cortex of patients with schizophrenia. There are several possible reasons for the discrepant findings, including differences in brain regions between the studies and the likely possibility that hypermethylation, if it occurs, would be confined to specific subpopulations of GABAergic interneurons (27). Clearly, in view of the reported increase of DNMT1 expression, the possible role of this proposed epigenetic mechanism must be studied further.

### NEUROTRANSMITTER RECEPTOR-MEDIATED REGULATION OF GABA INHIBITORY INTERNEURONS: TARGETS FOR THERAPEUTIC INTERVENTION

Alternative mechanisms for creating and addressing functional deficits of prefrontocortical “fast-spiking” GABAergic interneurons include receptor-mediated regulation of their firing and GABA release by both glutamate and acetylcholine (28-34). Glutamate would act primarily at the N-methyl-D-aspartate (NMDA) receptor, a type of glutamate-gated calcium ion channel receptor on the surface of these “fast-spiking” interneurons, while acetylcholine would act at cell-surface nicotinic acetylcholine receptors containing the  $\alpha_7$  subunit on these same interneurons. The relationship of the firing of the “fast-spiking,” probably parvalbumin-containing, GABAergic interneuron to the firing of the “regular-spiking” principal pyramidal output neuron was studied in the medial prefrontal cortex of freely-moving rats

(3). Essentially, inhibition of the firing rate of the “fast-spiking” interneurons was achieved with a systemic dose of MK-801 (dizocilpine; 0.1 mg/kg, ip), a use-dependent, open-channel, noncompetitive NMDA receptor antagonist that disrupts working memory and set-shifting in these animals, “symptoms” relevant to the cognitive deficits of schizophrenia. While MK-801 inhibited a majority of the “fast-spiking” interneurons, treatment with MK-801 increased the firing rate of the majority of the “regular-spiking” pyramidal neurons. The data show that NMDA receptor-mediated inhibition of GABAergic inhibitory interneurons leads to “disinhibition” of the firing of cortical pyramidal cell neurons. Importantly, there was a lag between the “start/plateau” of MK-801-induced inhibitions of the “fast-spiking” interneurons and its excitations of “regular-spiking” pyramidal neurons, consistent with both models and electrophysiological data of synchronized firing of cortical networks subserving higher-executive functions. In any event, tonic inhibition of NMDA receptors by MK-801 disrupted GABAergic inhibitory control of a synchronized network of “regular-spiking” pyramidal neurons (3).

#### A. NMDA RECEPTOR

There are several nonmutually exclusive and nonoverlapping ways of creating “NMDA receptor hypofunction” on the surface of GABAergic inhibitory interneurons, whose end-result would be disinhibition and disruption of the synchronous firing of assemblies of cortical pyramidal cell neurons. These mechanisms can include alterations of genetic expression of specific NMDA receptor polypeptide subunits, post-transcriptional changes in mRNA splice variants of specific receptor subunits, and post-translational modifications of the phosphorylation state of the intracellular loop of receptor subunits (2, 29, 32). The ability of glutamate to gate the opening of its associated ion channel successfully is also influenced by the extent of saturation of the strychnine-insensitive glycine co-agonist binding site, as well as by the availability of both locally produced and circulating allosteric modulators such as polyamines and neurosteroids (2). The receptor’s function is influenced by the local balance of oxidative and reducing equivalents. Very recently, interest has focused on the regulation of the presynaptic release of glutamate by specific G-protein-coupled metabotropic glutamate receptors in the area of the NMDA receptor synapse that, in turn, affects activation of the NMDA receptor; specifically, strategies for inhibiting enzymatic cleavage of N-acetylaspartate-glutamate, an acidic dipeptide

that may be the naturally-occurring agonist for specific metabotropic glutamate receptors have been proposed as a therapeutic intervention (35). Thus, NMDA receptor hypofunction can come about by a variety of mechanisms, which also suggest a variety of possible therapeutic interventions. These NMDA receptor-targeted interventions include administration of glycine complete or partial agonists such as D-serine and glycine reuptake inhibitors related to sarcosine; as mentioned, glycine is an obligatory co-agonist whose presence enhances and is necessary for glutamate to be effective in promoting channel opening. Ideally, this would address the GABAergic deficit that results from hypofunction of NMDA receptors on their cell-surface. A provocative therapeutic strategy also includes addressing the downstream consequences of the excessive excitation of pyramidal cell neurons and the possible pathological consequences of the excessive release of glutamate resulting from GABAergic dysfunction (2, 36). This strategy will be facilitated by the development and introduction of AMPA/kainate receptor antagonists into Clinical Medicine (37-39). There has been some preliminary investigation of the therapeutic efficacy of topiramate in schizophrenia, whose properties include kainate receptor antagonism.

#### B. $\alpha_7$ -CONTAINING NICOTINIC ACETYLCHOLINE RECEPTORS

In addition to the NMDA receptor, nicotinic acetylcholine receptors, which are also ligand-gated ion channel receptors that contain the  $\alpha_7$  subunit are expressed on the surface of GABAergic inhibitory interneurons (28, 30). The  $\alpha_7$  subunit confers unique electrophysiological and pharmacological properties, including relatively high permeability to calcium ions and rapid desensitization. Also, choline, which is the locally-generated hydrolytic split product of acetylcholine by the catalytically very efficient acetylcholinesterase, is a full agonist that mimics acetylcholine itself (31, 40). Although hydrolytic cleavage of acetylcholine is the major mechanism of its synaptic inactivation, the rapid local generation of choline, a full agonist, and the rapid desensitization kinetics of the receptor suggest that this may be an additional mechanism for locally and rapidly regulating synaptic neurotransmission (31, 40). There are compelling data supporting the regionally-selective diminished expression of the  $\alpha_7$ -containing nicotinic acetylcholine receptor in the brains of patients with schizophrenia, which may underlie the deficits in sensory impairment (i.e., the P50 auditory-evoked potential abnormality) and voluntary smooth pursuit eye-tracking mani-

festated by many patients with schizophrenia and their closely-related biological relatives (28, 41). There are provocative data showing that schizophrenia, P50 sensory gating abnormalities and the increased prevalence of smoking behavior in this disorder may be linked or associated with abnormal promoter variants for the  $\alpha_7$  subunit in the q13-q14 region of chromosome 15 (41). In any event, like NMDA receptor hypofunction, deficient expression of the  $\alpha_7$  subunit may result in GABAergic dysfunction and “downstream” disinhibition. The existence of a positive allosteric modulatory site on nicotinic acetylcholine receptors and the fact that galantamine is a positive allosteric modulator, in addition to an inhibitor of acetylcholinesterase, have stimulated thinking about the development of therapeutic interventions (30, 42). In addition to choline, there are anabaseine derivatives that serve as selective agonists for receptors containing the  $\alpha_7$  subunit; unfortunately, the receptor undergoes rapid desensitization on exposure to agonist. Nonetheless, targeted therapeutic interventions for the  $\alpha_7$ -containing nicotinic acetylcholine receptor are under consideration for the treatment of schizophrenia (30).

#### C. CB1-CANNABINOID RECEPTOR

The firing of subpopulations of GABAergic interneurons is regulated, at least in part, in a local “retrograde” manner by endogenous cannabinoids (endocannabinoids), such as anandamide, which are released from principal pyramidal cell neurons and bind to CB1-cannabinoid receptors present on pre-

**Table 1.** *Potential Therapeutic Strategies for Improving GABAergic Function*

##### PRESYNAPTIC

- a. Metabotropic Glutamate Receptor Agonist Intervention
- NAAG Peptidase Inhibition

##### GABA INHIBITORY INTERNEURON

- a. NMDA Receptor Agonist Intervention
  - Glycine Reuptake Inhibitor (e.g., Sarcosine)
  - Glycine Agonist (e.g., D-Serine)
  - Neurosteroids
  - Polyamines
- b.  $\alpha_7$  Nicotinic Acetylcholine Receptor Agonist Intervention
  - Positive Allosteric Modulator (e.g., Galantamine)
  - Selective Agonist (e.g., Choline, Anabaseine Derivative)
- c. CB1-Cannabinoid Receptor Antagonist
- d. Epigenetic Intervention
  - Histone Deacetylase Inhibitor
  - DNA Methyltransferase 1 Inhibitor

##### POSTSYNAPTIC

- a. GABA<sub>A</sub> Receptor Agonist Intervention
  - Selective Benzodiazepine Ligands
  - Neurosteroids
- b. Inhibition of Phosphorylation of Tau Protein (Preservation of Assembly, Stability and Alignment of Microtubules)

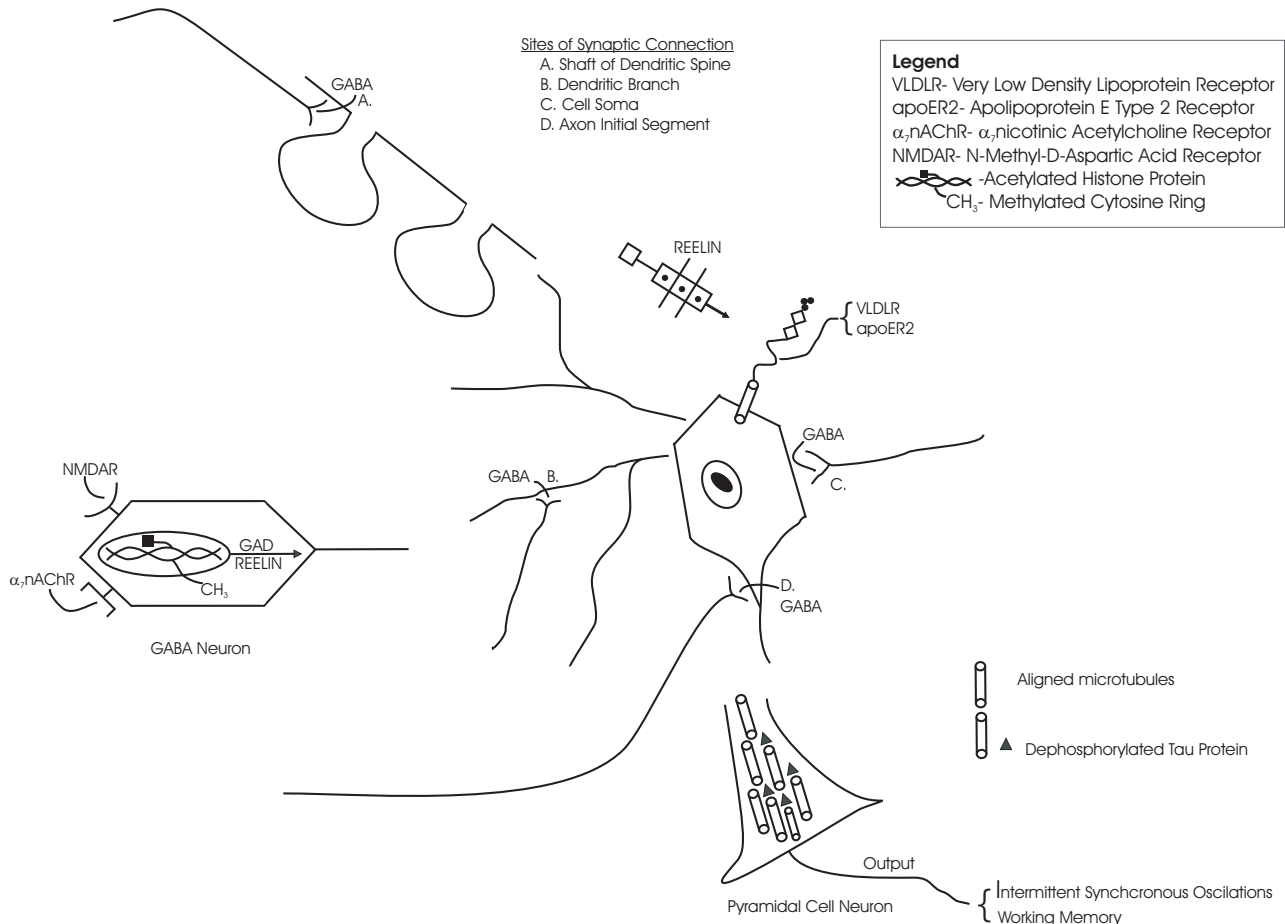
synaptic GABA inhibitory interneurons (9, 43). The synthesis and release of endocannabinoids is dependent on the activity of the pyramidal cell neurons; the consequence of the retrograde signal is the transient inhibition of GABA release from terminals containing the CB1-receptors, referred to as depolarization-induced suppression of inhibition. For example, CB1-cannabinoid receptors are present on the terminals of CCK-containing GABAergic basket cells in the hippocampus and amygdala (9).

## CONCLUSIONS

It is very clear that the phasic inhibitory regulation of the intermittent oscillatory activity of assemblies of pyramidal cell neurons is necessary for the performance of higher cortical functions and may be disturbed in schizophrenia. GABA-gated chloride ion conductance via GABA<sub>A</sub> receptors is a basic element of this regulatory process; disturbances of GABAergic function can come about by a variety of mechanisms, many of which can

**Figure 1.** This fictionalized cartoon depicts sites of potential synaptic innervation of principal pyramidal output neurons by GABA inhibitory interneurons. The output of fast-spiking GABA inhibitory interneurons is controlled, in part, by both NMDA and  $\alpha 7$ -containing nicotinic acetylcholine receptors on their surface. The firing of the pyramidal neuron will be determined by the site of the synaptic connection. In addition to controlling the firing of pyramidal cell neurons, GABAergic neurons may also regulate their morphological appearance, especially the density

of their dendritic spines. The figure also depicts some of the possible therapeutic targets for facilitating GABAergic function and neurotransmission, including the NMDA and  $\alpha 7$ -containing nicotinic acetylcholine receptors and epigenetic interventions. Ultimately, pyramidal cell function may depend on the correct alignment of microtubules, and the intermittent oscillatory output of assemblies of these neurons may be critical for higher executive functions, including working memory.



also serve as therapeutic targets. Moreover, because the function of individual GABA<sub>A</sub> receptors is determined, in large part, by the unique combinations of individual receptor subunits, it is possible to develop subtype selective interventions that target GABA<sub>A</sub> receptors containing specific subunits (e.g.,  $\alpha 3$  and  $\alpha 5$ ). Unfortunately, pharmacotherapeutic interventions are usually administered systemically and would facilitate GABAergic neurotransmission widely throughout the brain, whereas the precise pathophysiological disruption may be confined to specific GABA inhibitory interneurons within specific regions and cortical layers. Also, usual pharmacotherapeutic interventions lead to sustained “steady-state” levels of the active medication in blood and other biological compartments, which may not mimic phasic activity and release of GABA. Nonetheless, in view of the current limitations of the pharmacotherapy of schizophrenia that focuses on selective blockade of specific dopamine receptor subtypes with efficacy and effectiveness confined primarily to positive symptoms, the development of alternative and complementary strategies that target cognitive symptoms must be pursued.

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