Excitatory Amino Acid Receptors in Schizophrenia

by Jolanta Utas and Carl W. Cotman

Abstract

Growing evidence suggests an involvement of excitatory amino acid (EAA) systems in schizophrenia. Precedent exists for changes in binding to kainate, \( \alpha \)-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid, \( N \)-methyl-\( \beta \)-aspartate subtypes of EAA receptors. Current evidence indicates that in schizophrenia, EAA receptor levels can be decreased, unchanged, or even increased in certain brain regions and certain cases. It is likely that variability may arise from different drug histories of patients, other coexistent and undetected disease states, and the inherent heterogeneity of schizophrenia. On the other hand, it is possible that schizophrenia reflects a pattern of imbalances, not a simple unidirectional change. If so, even subtle changes may contribute significantly to the overall status of ongoing circuitry function in key brain areas implicated in schizophrenia. Together with other neurotransmitter systems, for example, dopaminergic, the net effect of EAA receptor imbalances may be greater than changes in the individual receptors and their neurotransmitters.

Recent in vivo imaging data such as magnetic resonance imaging (MRI) and computed tomography (CT) (Shelton and Weinberger 1986; Suddath et al. 1989, 1990), and postmortem neuropathological studies (Roberts and Bruton 1990) suggest that there are multiple structural abnormalities in the brains of schizophrenic patients. In addition to significant ventricular enlargement, widespread cerebral gray matter volume deficits, and reduced size of schizophrenic brain, specific aberrant anatomical changes have been found in the temporal lobe that affect primarily the hippocampus, adjacent parahippocampal gyrus, and amygdala (Kovelman and Scheibel 1984; Bogerts et al. 1985, 1990a, 1990b; Altshuler et al. 1987; Falkai et al. 1988; Jeste and Lohr 1989).

These brain regions are thought to be crucial for the integration of emotional responses and intellect, functions that are known to be compromised in schizophrenia. Both integrative cortical association and efferent pathways, projections from cerebral cortex to the striatum, and corticothalamic projections, as well as the perforant pathway that projects from the entorhinal cortex to the hippocampal formation use excitatory amino acids (EAAs; see Rao et al. 1991). L-Glutamate is the major excitatory neurotransmitter. L-Glutamate is also a neurotransmitter of excitatory intrinsic connections within the hippocampus (Cotman and Monaghan 1987). The entorhinal cortex, which in primates is known as Brodmann's area 28 and constitutes the anterior portion of the parahippocampal gyrus (Van Hoesen et al. 1991), appears to be a key site of interneuronal communications between various cortical and subcortical regions and the hippocampal formation. The entorhinal cortex conveys the cortical inputs to the hippocampus and also conveys hippocampal outputs back to the cortex (Swanson and Kohler 1986). Thus, damage to glutamatergic transmission may be an important factor in the develop-

Reprint requests should be sent to Dr. C.W. Cotman, Irvine Research Unit in Brain Aging, University of California, Irvine, CA 92717-4550.
ment of some symptoms of schizophrenia.

The cortex, hippocampus, and parahippocampal gyrus also have a high level of EAA receptors (Geddes and Cotman 1986; Cotman and Monaghan 1987; Monaghan et al. 1987; Jansen et al. 1989; Geddes et al. 1992b; Ulas et al. 1992a). These receptors are known to be involved in a variety of processes, ranging from learning and memory, synaptic and developmental plasticity, sensory information and coordinated movement patterns to, if excessively activated, seizures and excitotoxicity (Cotman and Iversen 1987; Cotman and Monaghan 1987, 1988; Muller et al. 1988; Cotman et al. 1989; Flood et al. 1990; McDonald and Johnston 1990). Thus, their altered function or density may also be of significance in the pathophysiology of schizophrenia.

The synaptic responses elicited by EAAs are mediated by at least three different receptor subtypes (table 1), named for the agonists by which they are selectively activated: N-methyl-D-aspartate (NMDA), kainate (KA), and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA). The NMDA receptor-ion channel complex contains several binding sites (figure 1). The NMDA-sensitive neurotransmitter recognition site can be selectively labeled with L-glutamate when KA and AMPA sites are blocked by specific compounds. A phencyclidine (PCP) receptor site is located within the ion channel and requires agonist activation. This binding site interacts with drugs such as PCP, ketamine, (+)-5-methyl-10,11-dihydro-5H-dibenz[a,d]cyclohepten-5,10-imine maleate (MK-801), and 1-(1-thienylcyclohexyl)piperidine (TCP). In addition, the NMDA receptor com-

<table>
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<th>Table 1. Subtypes of excitatory amino acid receptors</th>
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<tr>
<td><strong>Receptor</strong></td>
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<tr>
<td><strong>NMDA receptor complex</strong></td>
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<tr>
<td>NMDA-sensitive site</td>
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<tr>
<td>Ibotenate</td>
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<td>L-Glutamate</td>
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<td>L-Aspartate</td>
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<td>Kainate</td>
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<td>L-Glutamate</td>
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<tr>
<td>Quisqualate</td>
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<tr>
<td>AMPA</td>
</tr>
<tr>
<td>Quisqualate</td>
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<td>L-Glutamate</td>
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</table>

*Note.—AMPA = α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; AP5 = 2-amino-5-phosphonovalerate; AP7 = 2-amino-7-phosphonoheptanoate; CNQX = 6-cyano-7-nitroquinoxaline-2,3-dione; CPP = 3,3-(2-carboxypiperazine-4-yl)propyl-1-phosphate; DNQX = 6,7-dinitroquinoxaline-2,3-dione; NMDA = N-methyl-D-aspartate.*

**Figure 1. The N-methyl-D-aspartate (NMDA) receptor complex**

The transmitter recognition site binds agonists such as NMDA, L-glutamate, and L-aspartate. This binding opens the ion channel allowing Na⁺ and Ca²⁺ ions to flow inside the neuron and K⁺ ions to flow out. The strychnine-insensitive glycine site allosterically modulates receptor function, probably by increasing the frequency of agonist-induced channel opening. The NMDA receptor complex is also regulated by polyamines and Zn²⁺ at two other modulatory sites. At resting membrane potential Mg²⁺ blocks the ion channel in a voltage-dependent manner and this block is relieved during membrane depolarization. The ion channel also contains a phencyclidine (PCP) recognition site which can bind PCP, ketamine, (+)-5-methyl-10,11-dihydro-5H-dibenz[a,d]cyclohepten-5,10-imine maleate (MK-801), and 1-(1-thienylcyclohexyl)piperidine (TCP), serving to block the open channel and interfere with ionic conductance. Recent data suggest that haloperidol may modulate the functions of the NMDA receptor complex by interaction with this PCP receptor binding site.
plex has at least four other recognition sites, including a strychnine-sensitive modulatory glycine site, a polyamine recognizing site, and Mg$^{2+}$ and Zn$^{2+}$ binding sites (Cotman et al. 1989; Wood et al. 1990).

The involvement of EAA receptors in schizophrenia is supported by observations that PCP, the non-competitive NMDA receptor antagonist, reproduces (Allen and Young 1978; Petersen and Stillman 1978) or exacerbates (Luisada and Brown 1976) psychotomimetic symptoms in humans that resemble schizophrenia, while in experimental animals the effects of PCP or related drugs such as MK-801 mimic the action of the dopaminergic agent amphetamine (Freed et al. 1980; Clineschmidt et al. 1982; Koek et al. 1989; Javitt and Zukin 1991).

Further support for the role of the EAA system in schizophrenia comes from postmortem studies of EAA levels in the diseased brain that reported abnormal levels of amino acids in the cerebrospinal fluid (CSF) or blood of schizophrenic patients (Kim et al. 1980; Macciardi et al. 1990). It should be pointed out, however, that the findings of these studies were highly variable and controversial. For example, Kim and colleagues (1980) observed markedly reduced (about 50%) levels of free glutamate in the CSF of schizophrenic subjects, while other researchers failed to reveal any changes between normal and schizophrenic patients (Korpi et al. 1987). The latter study sampled patients who had been treated with haloperidol or were in a drug-free period.

This study also suggests that there are no significant differences in amino acid concentrations (glutamate, glycine) between paranoid and undifferentiated schizophrenic patients. In apparent contrast with these data, some studies have described an effect of neuroleptic treatment on the level of EAAs in CSF. Gattaz and colleagues (1985) demonstrated a lack of change in glutamate levels in the CSF of neuroleptic-free, paranoid schizophrenic patients but significantly elevated (33%) levels of glutamate in patients on neuroleptic drugs (butyrophenones and phenothiazines). Highly increased concentrations of glutamate, glycine, and serine were also found in the blood of paranoid, disorganized, or undifferentiated schizophrenic patients (Macciardi et al. 1990).

The idea that EAAs have a role in schizophrenia emerged also from recent studies indicating that there are interactions between dopaminergic and glutamatergic systems in the CNS. It has been shown that glutamate stimulates dopamine release in the striatum (Krebs et al. 1991; Rao et al. 1991). On the other hand, the release of glutamate from the striatal terminals is inhibited by dopamine (Mitchell and Doggett 1980; Ronalds and Roberts 1980; see Carlsson and Carlsson 1990). These findings suggest that overactivity of the dopaminergic system observed in schizophrenia may be due to a primary abnormality in glutamatergic transmission or that the "hyperdopaminergic" state of schizophrenia could be responsible for a disturbance of the EAA system.

In this article we will focus on an evaluation of the EAA hypothesis of schizophrenia. The status of EAA receptors in schizophrenia and their possible relation to anatomical abnormalities, as well as possible therapeutic approaches, will be discussed.

**EAA Receptor Dysfunction in Schizophrenia**

In spite of the interest in the role of excitatory neurotransmitters in schizophrenia, there are few studies describing the regional pattern of changes in EAA receptor density and their properties in schizophrenic brains (Nishikawa et al. 1983; Kerwin et al. 1988, 1990; Toru et al. 1988, 1992; Deakin et al. 1989; Kornhuber et al. 1989). Furthermore, these studies are inconclusive and have not produced a simple conclusion. Using postmortem brain tissue from different brain regions, these studies employed either binding to EAA receptors in membrane preparations or quantitative receptor autoradiography. Since analysis of receptors in schizophrenia involves the use of brain tissue derived from patients whose diagnosis was inherently somewhat uncertain and whose disease may not have been homogeneous, it is important to have well-characterized and preferably prospectively diagnosed cases. Only in this way is it possible to relate findings to possible heterogeneity or complications of the disorder. Our previous work with Alzheimer's disease patients and age-matched controls has underscored the notion that individual differences in pathological changes and levels of EAA receptors do exist and that it is important to take these differences into account while examining for the mechanism(s) underlying the disease (Ulás et al. 1992).

**The Medial Temporal Lobe.**

Recently we have undertaken a detailed investigation of the tissue from well-characterized schizophrenic patients, focusing on EAA receptor changes in the hippo-
An in vitro quantitative autoradiography technique was employed to examine NMDA and non-NMDA (KA, AMPA) receptors in four schizophrenic (table 2) and four control subjects. Care was taken to use tissue samples from comparable anatomical levels of the hippocampus and parahippocampal gyrus and to use specimens matched for age, sex, and postmortem interval. The tissue from the right brain hemisphere was used. Because of the advanced age of the individuals used in our study (60-79 years) and recent data by Soustek (1989) indicating a high incidence (about 40%) of Alzheimer's disease pathology in the older age (61-90 years) population of schizophrenic patients, all cases were analyzed for the presence of Alzheimer's pathology in the structures examined.

Analysis of NMDA receptors, using the agonist L-[3H]glutamate, did not reveal any statistically significant changes in receptor binding levels in the hippocampus and parahippocampal gyrus in the schizophrenic versus the control group. [3H]KA binding levels were also largely maintained in schizophrenia patients, although a small (16%, \( p < 0.05 \)) decrease in binding was found in the molecular layer of the subiculum (figure 2). Similarly, [3H]AMPA binding remained unchanged, with the exception of a 19 percent (\( p < 0.05 \)) decrease in the cornu Ammonis (CA)I stratum pyramidale. Thus, the analysis of binding did not reveal striking changes in the overall pattern of binding to NMDA and non-NMDA receptors between schizophrenic and control groups.

When the same schizophrenic subjects were compared with control group data augmented by the data from eight additional control cases that also matched schizophrenic cases according to age and postmortem delay, all the previously statistically significant changes in binding to KA and AMPA receptors in the schizophrenic group were no longer significant. Slightly, but not significantly, elevated (15%-20%) binding to NMDA receptors was detected in the strata oriens and pyramidale of the CA1 region and in the parahippocampal gyrus. [3H]KA binding levels did not differ significantly from those in the control group, and [3H]AMPA binding was mostly unchanged, although a small (20%) decrease in binding in the strata pyramidale and oriens of CA1 and CA3 subfields, and outer and inner layers of the parahippocampal gyrus was observed. Since the accuracy of our binding assays is not an issue (in rodent tissue, the standard error for any investigated EAA receptor does not exceed 10%), these findings clearly show the impact of intersubject differences within the control group. A similar situation may be true for schizophrenic cases.

Closer analysis of binding to non-NMDA receptors in individual schizophrenic patients revealed quite different patterns of receptor changes. For example, in one schizophrenic subject (patient No. 1679, table 2), a pronounced increase (44% vs. group of 4 control subjects and 37% vs. group of 12 controls) in [3H]KA binding was found in the outer two-thirds of the dentate gyrus molecular layer (figure 3C). The increase in [3H]KA binding was expressed as a widening of the narrow zone (inner one-third of the molecular layer) normally occupied by KA.

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Diagnosis</th>
<th>Age</th>
<th>Sex</th>
<th>PMD (hr)</th>
<th>Treatment</th>
<th>Cause of death</th>
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<tr>
<td>1679</td>
<td>Paranoid schizophrenic</td>
<td>79</td>
<td>M</td>
<td>5.5</td>
<td>ECT, haloperidol, thioridazine,</td>
<td>Heart disease</td>
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<td></td>
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<td></td>
<td></td>
<td>trihexyphenidyl hydrochloride</td>
<td></td>
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<tr>
<td>2291</td>
<td>Paranoid schizophrenic</td>
<td>60</td>
<td>M</td>
<td>24</td>
<td>ECT, trihexyphenidyl hydrochloride,</td>
<td>Heart disease</td>
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<td></td>
<td></td>
<td></td>
<td>chlorpromazine hydrochloride</td>
<td></td>
</tr>
<tr>
<td>1620*</td>
<td>Paranoid schizophrenic</td>
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<td>F</td>
<td>11</td>
<td>ECT(?), theophylline</td>
<td>Respiratory failure</td>
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<td>1541</td>
<td>Schizophrenic (chronic</td>
<td>72</td>
<td>M</td>
<td>9</td>
<td>Haloperidol, trihexyphenidyl</td>
<td>Respiratory failure</td>
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<tr>
<td></td>
<td>undifferentiated)</td>
<td></td>
<td></td>
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<td>hydrochloride, thioridazine,</td>
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<td>fluphenazine hydrochloride</td>
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Note.—PMD = postmortem delay; ECT = electroconvulsive therapy.

*According to the records this patient refused to undergo neuroleptic treatment.
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Figure 2. [3H]Kainate (KA) binding (50 nM) in select regions of the hippocampal formation and parahippocampal gyrus in control (solid bars) and schizophrenic (open bars) individuals.

Open circles = individual controls; solid circles = individual schizophrenia cases; solid lines (error bars) = standard deviation; oml = outer two-thirds of the dentate gyrus molecular layer; iml = inner one-third of the molecular layer; cornu Ammonis (CA)3 pyr = stratum pyramidale of CA3; CA1 pyr = stratum pyramidale of CA1; sub mol = molecular layer of the subiculum; phg in = inner layers of the parahippocampal gyrus; phg out = outer layers of the parahippocampal gyrus.

*p < 0.05 vs. control (one-way analysis of variance, Scheffe F test).

Receptors in the molecular layer of control subjects (figure 3A). In addition, the same individual exhibited a substantial increase (40% vs. 4 control subjects and 34% vs. 12 controls) in [3H]KA binding in the infragranular layer, which was paralleled by a similar elevation (39% vs. 4 controls and 51% vs. 12 controls) of [3H]AMPA binding. In contrast, another schizophrenic individual (patient No. 1541) demonstrated levels of binding to KA receptors similar to those in controls, with the exception of the inner one-third of the dentate gyrus molecular layer, where [3H]KA binding was also elevated (28% vs. 4 control subjects, and 22% vs. 12 controls [figure 3B]).

In view of the marked alterations in binding to EAA receptors in patient No. 1679, it is of interest to present the clinical history and neuropathological findings of this individual.

The patient was a 79-year-old cachectic male who died of heart failure. He was first diagnosed with paranoid schizophrenia at age 28. His psychiatric symptoms involved visual and auditory hallucinations, delusions, unusual thought content, and blunted affect. Neuropathological studies did not reveal the plaques or tangles indicative of Alzheimer's disease in the hippocampus and parahippocampal gyrus. However, Cresyl violet stain revealed a disarray of pyramidal cells in the hippocampus similar to that previously reported for schizophrenic patients (figure 3E). His pharmacological management consisted of haloperidol, thioridazine, and trihexyphenidyl hydrochloride. He also received temporal lobe electroconvulsive therapy (ECT), possibly bilateral.

It should be noted that not all schizophrenic subjects described in our study exhibited marked pyramidal cell disorientation (compare figure 3D with 3E), at least at the level of the hippocampus we investigated. There was one schizophrenic patient (No. 2291) whose hippocampus did not demonstrate obvious cell disorganization. However, since disarray of the pyramidal cells has been reported to be more pronounced in the anterior parts of the hippocampus and to diminish toward the posterior end (Kovelman and Scheibel 1984), we cannot exclude the possibility that the other segments of this individual's hippocampus do show neuronal disorientation.

Recently, responses of KA and AMPA receptors similar to that observed in the hippocampus of schizophrenic patient No. 1679 were observed in the molecular layer of the dentate gyrus of some Alzheimer’s patients (Geddes et al. 1985, 1992b). The redistribution and increased density of [3H]KA binding sites has also been re-
Figure 3. Distribution of $[^3]H$kainate (KA) binding sites in the hippocampus and parahippocampal gyrus of a control, elderly individual (A) and two schizophrenic patients (B, C).

Areas enriched in KA receptors appear as bright regions. B. $[^3]H$KA binding in schizophrenia case No. 1541 (table 2). In this case the distribution and levels of KA receptors were similar to those in controls, with highest density of binding in stratum lucidum of cornu Ammonis (CA)3 and moderate binding in inner one-third of dentate gyrus molecular layer. C. $[^3]H$KA binding in schizophrenia case No. 1679 (table 2). Note increase in binding accompanied by widening of zone occupied by KA receptors in the dentate gyrus molecular layer (black arrowheads) and significant increase in binding in the infragranular layer (white arrowheads). D. Normal pyramidal cell orientation in hippocampal CA2/CA3 area of control subject. E. Disarrayed cells in CA2/CA3 region of schizophrenic (No. 1679, table 2) hippocampus. F. Expansion of $[^3]H$KA binding zone in denervated dentate gyrus molecular layer of rat ipsilateral hippocampus 30 days after lesions of the entorhinal cortex (figure 3F). This receptor induction seems to reflect anatomical reorganization taking place in the molecular layer after damage to its glutamatergic input from the entorhinal cortex (Ulás et al. 1990). The induction occurs over approximately 30 days and appears to persist for the life of the animal. The expansion of KA receptors appears to be related to the sprouting of commissural/associational fibers into the denervated zone normally occupied by entorhinal terminals. The induction of KA receptors has also been observed in animal models with different manipulations. For example, following fimbria-fornix lesions, KA receptors show a similar pattern of expansion (Geddes et al. 1992a). The cause of this is hypothesized to be a loss of input from cholinergic and/or other systems passing through the fimbria-fornix.

This similarity of EAA receptor responses suggests that denervation of the hippocampus and consequent anatomical reorganization may play a role in several disease states, including schizophrenia. Plasticity of KA sites in the dentate gyrus is of interest in light of data suggesting anatomical abnormalities in the entorhinal cortex of schizophrenic patients. It appears that pre-α cells, which form clusters in layer II of the entorhinal cortex, do not show the slight separation as “doublets” seen in normal brains and lie closer to the surface of the cortex (see Roberts and Bruton 1990). In addition, the number of neuronal cells in the entorhinal cortex appears to be significantly reduced (Falkai et al. 1988). Those changes may arise early in development, be a conse-
quence of late degeneration and rearrangement, or result from a combination of these factors.

In light of these data and the pattern of changes in binding to KA receptors in the dentate gyrus molecular layer of schizophrenic patients, we speculate that changes in KA binding sites in patient No. 1679 may be a result of entorhinal cell loss. Although this patient also underwent ECT, which might be expected to cause changes in KA sites (see Geddes et al. 1991), our study indicates that ECT alone does not cause KA receptor changes in schizophrenic subjects. No similar changes in binding to KA receptors were found in two other ECT schizophrenic patients (No. 1620 and No. 2291). Further work is continuing on additional cases.

Another postmortem receptor autoradiography study of schizophrenic brains also reported changes in KA receptors but not in NMDA receptors (Kerwin et al. 1990). Data obtained from eight control and seven schizophrenic brains indicated a significant loss in the density of KA receptors in the hippocampus (34%-73%) and parahippocampal gyrus (39%-45%) in schizophrenia. The reduction of KA receptors was bilateral in the dentate gyrus, CA4/CA3 regions, and in the parahippocampal gyrus, whereas in the CA2 and CA1 regions, decreased binding was noted only in the left hippocampus (Kerwin et al. 1990). Similarly, [3H]6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) binding to AMPA receptors was more reduced in the left hippocampus; the CA4 region was affected bilaterally while the CA3 region was affected only on the left side. Decreased levels of non-NMDA receptors in schizophrenia were also confirmed at the level of gene expression: a striking loss (70%) of the messenger ribonucleic acid (RNA) that encodes a non-NMDA (KA/AMPA type) receptor was detected in the CA3 region of hippocampal tissue of six schizophrenic patients (Harrison et al. 1991). Hybridization signal in other regions of the hippocampus (dentate gyrus, CA1, CA4, subiculum) was reduced to a lesser degree.

The differences in the response of KA receptors observed by Kerwin and colleagues (1988, 1990) and our group may be only partially explained by an asymmetric loss of glutamate receptors in the hippocampus of schizophrenic subjects, thus implicating higher involvement of the left hippocampus. Kornhuber and colleagues (1989) recently demonstrated an involvement of the right side of the brain in glutamatergic abnormality in schizophrenia through a study of tissue samples, mostly from the right side of brain. This study demonstrated an elevated binding to NMDA receptor channel-associated PCP sites ([3H]MK-801 binding) in the hippocampus (43%) and entorhinal cortex (18%) of schizophrenic subjects. Thus, biochemical evidence contradicts the contention that schizophrenia is predominantly a disorder of the left temporal lobe (Newlin et al. 1981; Reynolds 1983; Reveley et al. 1987; Kerwin et al. 1988; Deakin et al. 1989; Bogerts et al. 1990a; Reynolds et al. 1990). The cause of increased [3H]MK-801 binding in the schizophrenic hippocampal formation is unclear, especially since binding of L-[^3H]glutamate to the agonist site of the NMDA receptor complex appears relatively preserved in schizophrenic hippocampus, both in the left and right hemispheres (Kerwin et al. 1990; Ulas et al. 1992b). It might be that PCP receptor sites within the NMDA receptor-ion channel complex are regulated in a different way than NMDA-sensitive L-[3H]glutamate binding sites. Our group has recently observed different sensitivity of various domains of the NMDA receptor complex in Alzheimer's disease (Ulas et al. 1992a).

**Other Brain Regions.** Altered levels of EAA receptors have also been described in other brain structures. Significantly enhanced (44%) [3H]MK-801 binding to PCP sites of the NMDA receptor complex was found in the putamen of 10 schizophrenic patients (Kornhuber et al. 1989). Although this brain membrane study did not present saturation analysis of the binding, the authors speculate that an upregulation of receptors may occur. In contrast, diminished [3H]PCP binding was reported in occipital (25%) and parietal (26%) cortices of schizophrenic subjects (Weissman et al. 1988).

Increased levels of binding to non-NMDA EAA receptors were found in several cortical regions. For example, Nishikawa and colleagues (1983) reported a 48 percent increase in the density of KA receptors with no changes in receptor affinity in the medial frontal (Brodmann's areas 9, 10, and 46) and eye movement areas (25% increase) of 12 schizophrenic patients. This effect appeared to be unrelated to neuroleptic treatment, since the increased [3H]KA binding was found both in patients who had discontinued neuroleptic treatment (at least 40 days before death) and in those patients who were treated with neuroleptics throughout their lives. Nishikawa and colleagues (1983) conclude that
increased binding to KA receptors may represent supersensitivity of KA receptors in response to reduced glutamatergic transmission. This increased binding may also account for the impairment of prefrontal cortical functions, including some cognitive functions, alterations in affective and social behavior, and abnormal eye movement. In fact, later studies from this group suggest a negative correlation between glutamate levels in several subcortical areas and \(^{3}H\)KA binding in the medial frontal cortex (Toru et al. 1988, 1992) and, therefore, support the notion that elevated density of KA receptors can be due to a denervation supersensitivity following presynaptic glutamatergic hypofunction. Increase in \(^{3}H\)KA binding was also observed in both the left (13%) and right (23%) orbital frontal cortex of schizophrenic brains (Deakin et al. 1989). The same study showed that an increase in KA receptor binding is accompanied by a bilateral increase in D-\(^{3}H\)aspartate binding to glutamate uptake sites (a marker for glutamatergic neurons). Since no changes in \(^{3}H\)KA and D-\(^{3}H\)aspartate binding to membranes from other brain regions (amygdala, hippocampus, superior gyrus, middle gyrus, and Brodmann's area 10) were found, the authors postulate that alterations of binding to uptake sites and KA receptors were specific and not due to antemortem neuroleptic treatment. They therefore suggest that elevated uptake in the orbital frontal cortex might reduce synaptic levels of glutamate to an extent that eventually induces a compensatory increase in postsynaptic \(^{3}H\)KA binding. Alternatively, increased D-\(^{3}H\)aspartate binding may reflect disturbances in the normal development of cortical glutamatergic neurons resulting, for example, in an abnormally dense glutamatergic innervation of the orbital frontal cortex.

**EAA Receptor Regulation by Neuroleptics**

As mentioned above, abnormalities in EAA receptor binding in schizophrenic brain may be related to antemortem chronic neuroleptic treatment. Haloperidol is one of the most frequently used antipsychotics in the treatment of schizophrenia and is primarily associated with the blockade of dopamine D\(_2\) binding sites (LaHoste et al. 1991). Several studies suggest that neuroleptics interact with the NMDA receptor-ion channel complex. It has been shown, for example, that in the rat brain, chronic haloperidol treatment (0.24 mg/kg per day for 12 days) upregulates binding of \(^{3}H\)TCP (an analog of PCP and noncompetitive NMDA receptor antagonist) to the PCP receptor located in the NMDA receptor-ion channel (Byrd et al. 1987). The increase in \(^{3}H\)TCP binding levels appears to be due to a striking (50%) increase in the maximal density of PCP receptors and a smaller (26%) decrease in receptor affinity. These findings suggest that TCP binding to PCP receptors is regulated by the dopaminergic system and may have some implications for the treatment of schizophrenia-like PCP psychosis with haloperidol. Since treatment with haloperidol increases the number of PCP receptors, there is a possibility that long-term treatment with haloperidol can exacerbate the PCP patient's symptoms by increasing receptor availability for PCP. The elevated density of PCP receptors after chronic haloperidol treatment contrasts with observations by Kornhuber and coworkers (1989) suggesting that haloperidol does not interact directly with \(^{3}H\)MK-801 binding to NMDA receptor-ion channel PCP sites in postmortem human brain.

Clozapine is another antipsychotic agent that interacts with the NMDA receptor-ion channel complex. Unlike haloperidol, this atypical neuroleptic preferentially modulates dopamine D\(_3\) receptors (Rupniak et al. 1985; see LaHoste et al. 1991) and is effective in the treatment of refractory schizophrenic patients. Alternatively, it also has been suggested that these two groups of neuroleptics may act on the same dopamine receptors but at different brain loci (see LaHoste et al. 1991; Janowsky et al. 1992). Clozapine has been shown to inhibit \(^{3}H\)MK-801 binding with K\(_i\) in nanomolar range, which is comparable to brain levels that were found after clozapine administration. Moreover, the regional distribution of specific \(^{3}H\)clozapine binding matches EAA innervation (Janowsky and Berger 1989). Clozapine has been found to increase glutamate levels in the rat medial prefrontal cortex as early as 2 hours after drug administration (20 mg/kg i.p.; Pehek et al. 1991). This may indicate that clozapine increases neuronal and metabolic glutamatergic activity in the frontal cortex and raises an important question as to whether different clinical profiles of haloperidol and clozapine reflect different mutual interactions of these drugs with both the glutamatergic and dopaminergic systems.

In contrast to the NMDA receptor complex, binding to other subclasses of EAA receptors, namely KA binding sites, does not seem
to be affected by chronic neuroleptic treatment (Nishikawa et al. 1983; Kerwin et al. 1990; Toru et al. 1992). However, more detailed studies on the effects of neuroleptics on the kinetics of binding to non-NMDA receptors are clearly needed, in particular since binding to KA and AMPA receptors seems to be altered in some schizophrenic subjects.

Glutamatergic Strategy in Schizophrenia Pharmacotherapy

Glycine Therapy. Interference of typical neuroleptics and clozapine with the glutamatergic system, as described in previous sections, suggests that EAA receptors, in particular the NMDA receptor-ion channel complex, may be a valid target for developing intervention strategies designed to overcome glutamatergic hypofunction, which may underlie schizophrenia. One way to facilitate glutamatergic transmission may be an activation of the NMDA receptor-ion channel with glycine, the potent allosteric modulator. In the presence of glutamate, glycine, acting through glycine-recognition site(s), which are distinct from the NMDA recognition site, stimulates binding of noncompetitive NMDA antagonists (e.g., PCP, TCP, MK-801), most probably by increasing the frequency of channel opening and by increasing the affinity of the receptor for the ligand (Johnson and Asher 1987; Wong et al. 1987). This action of glycine is strychnine insensitive and thus is distinct from interactions of glycine with inhibitory glycine receptors in the brainstem and spinal cord (White et al. 1989). Further, glycine may reverse some of the antagonistic effects of PCP and MK-801 on NMDA-mediated cationic conductance.

Although the role of the glycine site in schizophrenia has not been clearly defined, Toth and Lajtha (1986) have shown that exogenous administration of glycine counteracts PCP-induced hyperactivity in mice. Glycine in large doses (5-25 g/day, taken for several months) has also been reported to exert an antipsychotic action in 4 of 11 schizophrenic patients whose neuroleptic treatment was either rapidly or gradually decreased and then discontinued (Waziri 1988). In this study, two responders to glycine were completely withdrawn from neuroleptic treatment. It is interesting that one of the responders developed a florid psychosis 3 weeks after discontinuation of glycine treatment. In a separate open study by Costa and colleagues (1990), glycine was administered for 5 weeks (15 g/day) to six male schizophrenic patients as an adjunct to their regular doses of thiothixene or fluphenazine. In this study, two of six glycine-treated patients displayed a significant reduction in their Brief Psychiatric Rating Scale (BPRS; Overall and Gorham 1962) score. Also, a recent study by Potkin and colleagues (1992) on 11 glycine- and 7 placebo-treated schizophrenic patients demonstrated that those treated for 6 weeks with glycine (15 g/day in 3 doses) in addition to their standard neuroleptic treatment showed more improvement in emotional withdrawal, depression, hostility, and uncooperativeness, but less improvement in mannerisms in comparison with the placebo-treated group.

These studies raise the intriguing possibility that in spite of its glutamatergic action, glycine displays an additive or synergistic action to that exerted by neuroleptics. If this is the case, glycine treatment may allow the lowering of neuroleptic doses and protect against side effects of standard antipsychotic therapy, such as tardive dyskinesia and perioral tremor. However, these studies should be treated as preliminary. Replication of the glycine effects on larger populations of schizophrenic subjects and under more strictly controlled conditions is required before any definite conclusions can be drawn about the therapeutic value of glycine in schizophrenia.

Conclusion

Overall, it appears that changes in EAA receptors exist in the schizophrenic brain. However, the literature is inconsistent, leaving the precise nature of the changes unclear. Variability may arise from long postmortem intervals, different drug histories, other undetected disease states, and the inherent heterogeneity of schizophrenia. Variability may also result from combining data from subjects who were in fundamentally different stages of the disease and from lack of information on the pathological changes in the control brain tissue, for example, extent of neuron loss. Therefore, analysis of better characterized control and schizophrenic subjects is clearly needed. Nonetheless, it appears that imbalances do exist, albeit subtle, among EAA receptor types in schizophrenia. When these imbalances are combined with anatomical changes, they may influence synaptic events and the emergence of higher functions.

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**The Authors**

Jolanta Ulas, Ph.D., is Assistant Researcher, and Carl W. Cotman, Ph.D., is Professor of Psychobiology, Irvine Research Unit in Brain Aging, University of California, Irvine, CA.

**Announcement**

Pacific Clinics and the San Gabriel Valley Alliance for the Mentally Ill will sponsor a continuing education conference on *Working With Families of the Mentally Ill* to be held in Pasadena, California, March 26–27, 1993. Christopher S. Amenson, Ph.D., will review treatment outcome research technologies and curricula that form an effective basis for intervention with these families. He will teach professionals how to (1) conduct psychoeducational classes and groups, (2) consult with families in solving the many problems associated with having a mentally ill member, and (3) select when to use and how to modify traditional individual, marital, and family system therapies.

For registration information, please contact:

Georgette Shatford
Pacific Clinics
909 South Fair Oaks Avenue
Pasadena, CA 91105-2625