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Glutamate Receptor Genes

Susceptibility Factors in Schizophrenia and Depressive Disorders?

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Abstract

Schizophrenia, depression, and bipolar disorder are three major neuropsychiatric disorders that are among the leading causes of disability and have enormous economic impacts on our society. Although several neurotransmitter systems have been suggested to play a role in their etiology, we still have not identified any gene or molecular mechanism that might lead to genetic susceptibility for or protection against these neuropsychiatric disorders. The glutamatergic receptor system, and in particular the N-methyl-*D*-aspartate (NMDA) receptor complex, has long been implicated in their etiology. I review the current molecular evidence that supports a critical role for the glutamatergic receptor system in schizophrenia and the potential involvement of this receptor system in depression and bipolar disorder. It is likely that mutations in glutamate receptor genes might alter the risk of developing one of these disorders. Potential future research directions designed to identify these mutations and to elucidate their effect on mental health will be discussed.

Index Entries: Glutamate receptor; schizophrenia; bipolar disorder; major depression; NMDA; AMPA; kainate; ionotropic; metabotropic; genetic susceptibility; dopamine; glutamate.

Introduction

Schizophrenia, bipolar disorder, and depression are complex disorders with multiple genetic and environmental factors that contribute to the risk of manifestation of these disorders *(1)*. In the last decade, molecular neurobiology has uncovered many secrets about the human brain. Nevertheless, we still cannot answer two fundamental questions: Which genes increase the susceptibility to these neuropsychiatric disorders? And, what molecular events promote schizophrenia, depression, and bipolar disorder? Neuropsychiatric disorders are currently analyzed at the molecular, physiological, and behavioral levels with a diverse repertoire of methods. Historically, these stud-

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ies have been dominated by a focus on neurotransmitters and their receptors. Consequently, almost every neurotransmitter system has been studied intensively in these disorders. A great deal of attention has focused on the dysfunction of the monoamine receptor systems, particularly those for the neurotransmitters norepinephrine, serotonin, and dopamine, which have been consistently found to be altered in depressive disorders *(2).* Additionally, neuropeptide systems (e.g., corticotrophin-releasing factor, CRF) have been found to be involved in the pathophysiology of depression. The main disease hypothesis of schizophrenia is based on results that support a dysfunction of the dopamine receptor system; however, this hypothesis recently has been expanded to also include a dysfunction of the glutamatergic receptor system *(3).* The current hypothesis is based on the concept that neurotransmitter systems dynamically interact with one another in neuronal circuits, and that these interactions could be severely imbalanced in the disease state *(4,5).* This concept has important implications for the future design of experimental approaches that address the pathology of schizophrenia. It also alters our thinking about the strategies to develop new drugs that effectively treat schizophrenia and other neuropsychiatric disorders.

In this review, I focus on the discussion of molecular evidence that support a critical role of the glutamatergic system in schizophrenia and a potential role in depression and bipolar disorder. It is likely that some susceptibility genes for these disorders will be identified among the family of glutamate receptor subunit genes and that we will identify mutations in these genes that affect glutamate receptor function and contribute to an altered risk to develop these disorders.

The Glutamatergic Receptor System

The neurotransmitter L-glutamate is the major mediator of excitatory neurotransmission in the mammalian central nervous system (CNS). Glutamate acts through the activation of ionotropic ligand-gated ion-channels (iGluRs) and metabotropic G protein-coupled receptors (mGluRs) *(6–9).* Glutamate receptors are widely expressed throughout the CNS, localized pre-, post-, or extra-synaptically on neurons or on non-neuronal glial cells. Glutamate receptors are also expressed outside the CNS in peripheral tissues like the heart, skin, or bone *(10).* However, the role of glutamate receptors in glial cells or peripheral tissues is less studied and understood. Glutamate receptors mediate neurotransmission at central synapses and are significantly involved in the structural and functional plasticity of the synapse, including learning and memory processes *(11).*

Molecular studies have identified and cloned 17 cDNAs encoding ionotropic GluR subunits and 8 cDNAs encoding metabotropic GluRs *(6–9).* The ionotropic GluR subunits are grouped into three families based on their pharmacological properties, in particular the selectivity for the agonists N-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methyl-isoxazole-propionate (AMPA), and kainate *(6–9).* Six cDNAs have been cloned that encode NMDA receptor subunits: NR1, NR2A-D, and NR3A. Four cloned cDNAs encode AMPA receptor subunits: GluR1-4 and five cloned cDNAs encode the kainate receptor subunits GluR5-7, KA-1, and KA-2. Additionally, 2 cDNAs have been cloned that encode putative ionotropic GluR subunits, named delta-1 and delta-2. The topology of ionotropic glutamate receptors has been studied intensively: a receptor subunit contains an extracellular domain, 4 membrane spanning domains, and an intracellular carboxy-terminal domain of variable length *(12,13).* Amino acids in the second membrane domain determine the ion selectivity of ionotropic glutamate receptors *(14,15).* In contrast to membrane domain one, three, and four, membrane domain two is not a transmembrane domain, but instead forms a reentrant membrane pore-lining loop (P-loop) with both ends facing the cytoplasm *(12,13).* The glutamate-binding site is formed by two

regions that are localized to the extracellular amino-terminal domain and the extracellular region between membrane domains three and four *(16).* Ionotropic GluRs possess either a tetrameric or pentameric stoichiometry built by homomeric or heteromeric subunit assembly *(17,18).* The dogma that functionally assembled iGluRs contain only subunits from one of the subfamilies appears to be still valid.

Ionotropic glutamate receptors transduce the glutamate signal by changing their permeability for monovalent (Na^+, K^+) and divalent ions (Ca^{2+}) *(6–9).* Recent advances indicate that these receptors also transduce signals via protein-protein interaction *(19–21).* A large number of functionally diverse proteins have been identified that bind to the intracellular domain of glutamate receptors. These proteins either modulate receptor function or transduce a receptor signal *(19–21).* Beside the neurotransmitter glutamate a wide variety of endogenous molecules, ions, and cellular conditions also modulate ionotropic glutamate receptor function *(6–9).* The NMDA receptor does not conduct ions efficiently when activated by glutamate unless the voltage-sensitive magnesium blockade is removed through depolarization of the cell. Additionally, NMDA receptor activation requires the presence of the endogenous co-agonist glycine *(6–9).*

Calcium acting as a second messenger plays a central role in GluR mediated processes *(22).* Excessive calcium influx through iGluR activation, primarily NMDA receptors, contributes to excitotoxic mechanisms that lead to neuronal injury in hypoxia-ischemia, hypoglycemia, sustained seizures, physical brain trauma, and chronic neurodegenerative disorders (e.g., Huntington's disease and Alzheimer's disease) *(22–26).* Interestingly, the calcium permeability of several AMPA and kainate receptors is regulated by RNA editing *(27).* Several double-strand RNA dependent adenosine deaminases have been identified, which catalyze the conversion of specific adenosine residues to inosine in GluR2, 5, and 6 pre-RNAs *(28).* Inosine is recognized as guanosine during translation, which changes a glutamine to an arginine in the pore-forming membrane domain 2 of these receptors, reducing their calcium permeability *(14,15).* These RNA editing processes are intron-dependent and regulated in a developmental and cell type-specific manner *(29,30).*

The 8 known metabotropic glutamate receptors (mGluR1-8) have been classified into the superfamily of 7 transmembrane (7TM) G-protein-coupled receptors *(6–9,31,32).* They are divided in 3 subgroups based on downstream effector systems and their agonist selectivity *(6–9,31).* Metabotropic glutamate receptors are widely distributed in the CNS. Their activation modulates synaptic plasticity, is involved in learning and memory, but also plays a significant role in various CNS disorders (e.g., pain and analgesia, epilepsy, anxiety, and neurodegenerative disorders) *(6–9,31–33).*

Today, five excitatory amino acid transporters (EAAT1-5) have been identified in mammals *(34).* Glutamate transporter may regulate extracellular glutamate concentrations and influence excitatory neurotransmission *(34).* They are expressed in neurons or glia and transport L-glutamate with high affinity, but show also properties similar to ionotropic glutamate receptors *(34,35).* Glutamate uptake is coupled to influx of sodium (Na^+) and to the efflux of potassium (K^+), and can directly influence neuronal excitability *(34,36).* Dysfunctional glutamate transporter might cause an increase in extracellular glutamate, which is thought to be involved in acute CNS ischemia conditions and the pathology of neurodegenerative disorders like Alzheimer's disease (AD) and amyotrophic lateral sklerosis (ALS) *(37,38).*

We are just at the beginning of understanding which role some glutamate receptor subunits are playing in specific brain circuits or systems. The progress is hampered by the enormous diversity of native glutamate receptors in vivo generated through subunit assembly, RNA editing, and RNA splicing events *(6–9,31,32).* These molecular mechanisms create new receptor isoforms with different functional properties. However, rapid advances in

the field will reveal more and more of their functions, which will facilitate our understanding of the glutamatergic receptor system in the CNS and related disorders.

Pharmacological Evidence for the Involvement of the Glutamatergic Receptor System in Schizophrenia

The neuropsychiatric disorder schizophrenia is characterized by positive psychotic symptoms (hallucinations, delusions, paranoia), cognitive dysfunction, and negative symptoms (e.g., anhedonia, asociality). Our current understanding of the glutamatergic receptor system in schizophrenia has mainly been shaped by pharmacological research. Kim et al. initially proposed a glutamatergic hypothesis of schizophrenia *(39).* They detected reduced levels of the excitatory amino acid glutamate in the cerebrospinal fluid (CSF) of schizophrenic patients. Since then, strong support for the glutamatergic hypothesis of schizophrenia has come through observations that the noncompetitive NMDA receptor antagonists phencyclidine (PCP) and ketamine can induce psychotomimetic symptoms in healthy human individuals *(40–48).* These symptoms appear very similar to the positive symptoms and negative symptoms described in schizophrenia. Additionally, PCP and ketamine exacerbate these symptoms in schizophrenics at doses that do not affect healthy individuals, suggesting sensitization of NMDA receptor to these psychoactive compounds *(49,50).* Consequently, the NMDA receptor has received significant attention as a candidate receptor complex involved in schizophrenia, triggering an intensive search for evidence that the NMDA receptor is dysfunctional in schizophrenia.

Today a variety of NMDA receptor antagonists (e.g., MK801) have been developed, which are used as psychostimulants to produce animal models of schizophrenia *(6,8,51,52).* They produce cognitive impairments (learning and memory deficits) in rodents accompanied by altered social behavior and locomotion, and the induction of stereotypic behavior *(53–59).* These rodent models are currently the best available pharmacologically induced schizophrenia models that mimic a hypo-function of NMDA receptor *(60–62).*

NMDA receptor antagonists also increase presynaptic glutamate release in the cerebral cortex, which could act on AMPA, kainate, or metabotropic glutamate receptors. Thus, it is possible that the effect of NMDA receptor antagonists on rodents is mediated by multiple glutamate receptors *(60,63).* If the extracellular release of glutamate involves glial cells or a postsynaptic mechanism is presently unknown. However, Moghaddam and Adams found that pretreatment of rodents with the mGluR agonist LY354740 abolished PCP-induced glutamate release and reduced the PCP effect on animal behavior (locomotion, stereotypic behavior) *(60).* LY354740 is a selective agonist for group II metabotropic glutamate receptors. Other reports have shown that AMPA/kainate receptor antagonists and glutamate release inhibitors can reduce the behavioral effects of PCP *(63,64).* These results show that metabotropic glutamate receptors and AMPA/kainate can affect NMDA-receptor mediated signal transduction, and indicate the importance of considering other glutamate receptor subunits as target for the development of new antipsychotics to treat psychosis.

In contrast, other psychotomimetic drugs that induce schizophrenia like symptoms in humans target the dopamine receptor system. Amphetamine induces symptoms in humans that resemble the positive symptoms seen in schizophrenia *(50,65,66).* Amphetamine and amphetamine-like drugs have been shown to interact with monoamine transporters, in particular the dopamine transporter (DAT) *(67–69).* The result of these interactions is an increase in dopamine release and hyperactivity of the dopaminergic system *(70),* which is reflected by an increase in locomotor activity in rodents *(71–73).* Hyperactivity of the dopamine system in mesolimbic circuits appears to play a role in generating the posi-

tive signals of schizophrenia (e.g., psychosis) *(74–77)* and builds the basis of the dopamine hypothesis to explain psychosis *(77).* Most classical antipsychotics that are in clinical use act as antagonists at dopamine receptors *(78,79).* These drugs are effective in reducing amphetamine and PCP-induced hyperlocomotion and stereotypic behavior in rodents *(80–82).* These studies demonstrate that the dopamine receptor system can somehow affect the NMDA receptor system.

The glutamate and dopamine hypothesis in schizophrenia is now changing into one model integrating a dynamic interaction between dopamine, glutamate, but also serotonin, epinephrine, acetylcholine, and GABA in complex neural circuits *(4,5).* This model explains cognitive and behavioral symptoms in schizophrenia through increased neurotransmitter release that overstimulates postsynaptic neurons. The excitatory neurotransmitter glutamate might modulate inhibitory activity of GABAergic, noradrenergic, and serotonergic interneurons by acting on their NMDA receptors. These inhibitory interneurons could control multiple excitatory pathways in the limbic and cerebrocortical brain regions. NMDA receptor antagonists could cause an inhibition of these interneurons, which would lead to an increase in monoamine release and overstimulation of excitatory pathways. The dopamine system might act as modulator of the network by modulating presynaptic glutamate release on NMDA receptors. This could explain the beneficial effect of antipsychotic drugs acting on dopamine receptors or transporters in the treatment of schizophrenia. It also could explain the impact of antipsychotic drugs like olanzapine or clozapine in preventing NMDA receptor antagonist induced neurotoxcicity in the developing brain *(83).*

An important physiological connection between the dopamine and the glutamate receptor system has been identified in the neural circuits assumed to be involved in schizophrenia. Dopaminoceptive neurons express a potent and selective protein-phosphatase-1-inhibitor: the 32 kDa dopamine and cAMP-regulated phosphoprotein (DARPP-32) *(84–86).* Dopamine or the psychostimulant amphetamine strongly increases the protein kinase A dependent phosphorylation of serine 831 of the AMPA receptor subunit GluR1 in the neostriatum *(87).* A previous study by Snyder et al. revealed that the phosphorylation state of NMDA receptor in the nucleus accumbens is also modulated by the same pathway *(88).* Significantly, the modification of both the AMPA and NMDA receptor were attenuated in DARPP-32 knockout mice *(89).* These studies indicate that the dopamine release induced by psychostimulants can affects AMPA and NMDA receptor activity by altering receptor phosphorylation.

The NMDA receptor does not conduct ions efficiently when activated by glutamate unless the voltage-sensitive magnesium blockade is removed through depolarization of the cell. In many neurons, AMPA receptor activation facilitates depolarization of the cell. Ampakines, a new class of drugs, have been developed that positively modulate AMPA receptors and improve cognitive function (e.g., learning and memory) in rodent model *(90).* Ampakines have been shown to reduce amphetamine-induced locomotor activity in rats *(91).* Recent results provided the first evidence that ampakine action can enhance the activity of antipsychotics. Amphetamine induced locomotor activity was blocked by co-administration of ampakines with subthreshold doses of the antipsychotics haloperidol or clozapine *(91).* These studies indicate that AMPA receptor could be involved in the etiology of schizophrenia or other neuropsychiatric disorders.

The pharmacological and behavioral consequences of NMDA receptor antagonists suggest that NMDA receptors are hypo-functional in schizophrenia. This hypothesis stimulated the initial testing of potential therapeutic agents that act as agonists at the "glycine-site" of NMDA receptors. Small-scale clinical trials assessed the effectiveness of glycine, an endogenous NMDA receptor agonist, and D-cycloserine a partial agonist, in the treatment of schizophrenia *(92).* The reported studies

found conflicting results. The compounds reduced the negative symptoms and cognitive deficits of schizophrenia in only a subpopulation of all patients *(93–97).* However, the usefulness of NMDA receptor agonists in the treatment of schizophrenia has been criticized due to the high risk of inducing excitotoxic side effects. Despite all the evidence for a hypofunction of NMDA receptor in schizophrenia, none of the molecular studies in humans has identified a genetic factor causing hypofunction of NMDA receptor.

Pharmacological Evidence for the Involvement of the Glutamatergic Receptor System in Depressive Disorders

NMDA receptors have also been recognized as potential drug targets in the disorders depression and bipolar disorder (reviewed in detail *[98]*). Tricyclic antidepressants (TCA) (e.g., desipramine) affect NMDA receptor activity in hippocampal neurons, acting as weak open-channel blockers *(99).* Other studies showed that TCAs enhanced MK801-induced locomotor hyperactivity in mice. Three-week chronic administration of desipramine to mice produced a decrease in ^{3}(H)-MK801 binding in cortical membranes *(100).* NMDA receptor antagonists were also tested in animal behavior models for antidepressant-like activity. The behavioral responses of the animals in the enforced swimming test *(101),* the tail suspension test *(102),* the learned helplessness test *(103),* and the chronic mild stress test *(104)* were affected by the application of NMDA receptor antagonists (reviewed in ref. *[98]*). These pharmacological data suggest that NMDA receptors might be involved in neural circuits that are affected by antidepressant treatment.

A placebo-controlled, double-blind study performed on seven subjects with major depression tested whether intravenous treatment with a single dose of the NMDA receptor antagonist ketamine has antidepressant effects *(105).* The ketamine-treated subjects showed a significant improvement in depressive symptoms compared to the placebo group. This result supports the hypothesis that NMDA receptor antagonists without psychotomimetic properties might be useful for the development of antidepressants. However, it is not clear if NMDA receptors are directly involved in the etiology of depressive disorders. No genetic studies that tested for a relationship between NMDA receptors and depressive disorders have been reported.

A newly developed class of compounds (e.g., LY392098), which potentiate AMPA receptor function show antidepressant-like effects in animal models *(106,107).* Interestingly, LY392098 induces an AMPA-receptor mediated increase in brain-derived neurotrophic factor (BDNF) mRNA in neurons *(108).* BDNF itself shows antidepressant-like effects in animal models *(109).* These findings imply that AMPA receptors might play a role in depressive disorders by modulating BDNF expression. A relationship between stress, neurotrophic factors, and depression has been recognized and builds the basis of the neurotrophic model of depression *(110).*

Recent reports described a new potent and selective mGluR5 receptor antagonist with antidepressant-like activity in the tail suspension test *(111).* 2-methy1-6-(phenylethynyl)-pyridine (MPEP) exhibit no cross-reactivity with mGluR1 or other mGluRs, or against representative NMDA, AMPA, and kainate receptors *(112).* If MPEP or related compounds are suitable to treat depressive disorders awaits further assessments.

Lithium was recognized as a mood-stabilizing element in the 1950s and revolutionized the treatment of manic-depressive disorder *(113).* Despite intensive research, it is still unknown how lithium affects the etiology of bipolar disorder. Several studies have indicated that lithium affects the biophysical properties of ionotropic glutamate receptors *(114,115),* however, other proteins are also targets of lithium action *(116,117).* The potential significance of these findings for the function

of ionotropic GluRs in bipolar disorder has not been explored.

Pharmacological evidence supports the idea that NMDA receptors are involved in schizophrenia. However, we are far away from knowing the detailed expression pattern of glutamate receptor subunits in specific neuronal cell populations or circuits, which makes it difficult to correlate antipsychotic drug action with specific NMDA receptor subtypes. Increasing evidence suggests that also AMPA and metabotropic glutamate receptors may be involved in this disease, but these results need further evaluation. The analysis of the complex interactions between these receptors is an important prerequisite to develop new concepts about the role of glutamate receptors in schizophrenia. Additionally, also other proteins that affect the glutamatergic receptor system have to be included (e.g., glutamate transporter). There is only weak pharmacological evidence for a role of glutamate receptors in depression or bipolar disorder, but it appears intriguing to consider them as potential candidates. It seems reasonable to assume that mutation might exist in humans that cause a dysfunctional glutamate receptor system and alter the risk to develop these disorders.

Genetically Engineered Mouse Models

The major neuropsychiatric disorders schizophrenia, depression, and bipolar disorder show characteristic symptoms (e.g., psychosis, manic or depressive episodes) in humans. How can we generate animal models to study the molecular basis of schizophrenia, depression, or bipolar disorder? So far, the best approach for linking the neurobehavioral symptoms of neuropsychiatric disorders to animal behavior is correlating pharmacology with behavior. Drugs used in clinical therapy that affect neurotransmitter systems can be tested directly for effects on the behavior of wild-type or genetically altered mice. Good examples of this approach are PCP and ketamine. They induce psychotic symptoms in healthy individuals and relevant behavioral responses in rodents *(40–42).*

Mohn et al. (1999) recently reported the generation of a genetically engineered mouse that displays schizophrenia-like behavior *(55).* They created a mouse that expressed only 5% of the normal level of the NMDA receptor subunit NR1. NR1 is an obligate subunit for all NMDA receptors in the CNS and mice lacking the NR1 subunit die perinatally. Mohn et al. found in their mice an increase in locomotion and stereotypic behavior compared to wild-type mice *(55).* The NMDA receptor antagonists PCP and MK801 failed to affect locomotion or stereotypic behavior in these mice. These results indicate that the PCP or MK801 induced mouse behavior is mediated by NMDA receptors. In contrast, the antipsychotics haloperidol and clozapine attenuated the abnormal behavior of the mutant mice at doses that had no effect on wild-type mice. The mutant mice were 40 times more sensitive to the antipsychotic clozapine. The alterations in hyperlocomotion and stereotypy were independent of the dopaminergic system, as analyzed by microdialysis after application of MK801. Interestingly, the mice also showed social deficits (e.g., social avoidance of an intruder mouse) and abnormal sexual behavior. Social withdrawal was suppressed and sexual activity increased after application of clozapine. Social withdrawal in mice has been related to the negative symptoms of schizophrenia *(61,118).*

A previous study analyzed knockout mice that lacked the NMDA receptor subunit NR2A *(119).* A recent study with these mice detected a hyperlocomotion phenotype, similar to that derived from NMDA antagonist treated rodents *(54).* Hyperlocomotion was reduced by treatment with the antipsychotic drugs haloperidol and risperidone. Interestingly, neurotransmitter release assays detected a hyperfunction of the dopaminergic system in the striatum accompanied by a reduced activity of the inhibitory GABAergic system *(54).* The results link a hypo-

functional NMDA receptor system and a hyperfunctional dopamine receptor system, as it has been postulated in the disease model of schizophrenia *(4,5).*

All of the NMDA receptor subunits and a large number of AMPA, kainate, and metabotropic glutamate receptors have been knocked out in mice *(119–134).* The analysis of these mouse models revealed defects in various CNS systems, reflected by impairments in learning and memory, motor behavior, or synaptic plasticity. Except for the NR2A knockout mouse, none of the GluR knockout mice showed obvious behavioral abnormalities that have been linked to neuropsychiatric disorders. Experiments that test the effectiveness of psychostimulants, antipsychotics, and antidepressants on the behavior of these mice has not been included in their initial characterization.

Expression of Glutamate Receptors in Schizophrenia, Major Depression, and Bipolar Disorder Brains

The regulation of GluR subunit expression is an important mechanism modulating the functional properties of heteromeric ionotropic GluRs *(6–8).* Each GluR transcript shows cell type-specific and developmentally regulated expression pattern, shaping the particular receptor responsiveness of each cell *(6–8,135).* For example, the NMDA receptor subunit NR1 is essential for the assembly of functional NMDA receptors, therefore the expression level of this subunit determines NMDA receptor-dependent signaling at the synapse *(51,120).* Differential expression of the NR2A-D and NR3A receptor subunits affects gating and biophysical properties of NMDA receptor *(51).*

In situ hybridization, immunocytochemistry, and radio-ligand binding studies have been used to study the expression of glutamate receptor subunit mRNAs and proteins in brains from healthy individuals and patients with neuropsychiatric disorders. However, the hope that these methods would lead to the identification of specific GluR subunits expressed abnormally in neuropsychiatric disorders has not been completely satisfied. Several studies have reported abnormal expression of GluR subunits in schizophrenia. However, other studies could not reproduce these results *(136).*

Several *in situ* hybridization and ligand-binding studies consistently indicate that AMPA receptors are expressed at lower levels in the medial temporal lobe, hippocampal regions, and thalamic nuclei of schizophrenics probably as a result of decreased GluR1, 2, and 3 subunit expression *(136,137).* A small number of studies analyzed kainate receptor subunit expression in schizophrenic brains *(136).* A reduction in kainate receptor mRNA expression was detected in hippocampal regions, in the superior frontal gyrus, and in thalamic nuclei, probably a reflection of altered GluR6, GluR7, KA-1, and KA-2 subunit expression *(138–140).* Elevated [^{3}H]-kainate binding has been identified in multiple cortical areas, but it is not known which subunit is responsible for this observation *(136).* NMDA receptor subunit expression has also been studied to some degree in brains of schizophrenics *(136).* Results of these studies are also inconclusive. Lower expression of the NMDA receptor subunit NR1 has been found in several hippocampal regions, in superior temporal and frontal cortex from schizophrenia brains *(136).* In contrast, a 53% increase of NR2D mRNA expression was found in the prefrontal cortex of schizophrenics *(141).* The expression pattern and level of metabotropic glutamate receptors has not been intensively investigated in schizophrenic brains *(136).* In one single report so far, mGluR5 mRNA expression was increased in the orbitofrontal cortex of schizophrenic brains *(142).*

The expression of specific glutamate receptor subunits has not been compared between brains from normal individuals and brains from individuals with depressive disorders. This is surprising, given that expression of glutamate receptors in rodent brains is affected by chronic treatment with antidepressants *(98,143).* In particular, it has been reported that

radio-ligand binding of [^{3}H]-MK801 or other NMDA receptor antagonists is decreased in the cerebral cortex in these animal models *(98,100).* Alterations in NMDA receptor binding sites have been found in postmortem brains of depressed suicide victims *(144),* but not confirmed in other studies *(144,145).*

Why is glutamate receptor subunits expression altered in brains from schizophrenics? It is not known if either the altered expression pattern is caused by a disease mechanism or if the environment causes this alteration. An individual diagnosed with a neuropsychiatric disorder is likely to receive drug treatment and to undergo changes in life style. In most cases it is not possible to separate between these causes. Therefore, the significance of detecting altered expression of a glutamate receptor subunit in disease brains has to be confirmed by independent methods. The generation of mouse models simulating altered receptor expression would open up the possibility to test for a possible connection of this feature to the pathology of the disorder.

Due to the complexity of interactions between various circuits and neurotransmitter systems in the brain, it is plausible to assume that several different mechanisms might cause the same disease phenotype. For example, a recent report revived the viral infection hypothesis of schizophrenia by detecting retroviral genes more frequently in the genome of schizophrenics *(146).* We cannot expect to obtain consistent results by studying schizophrenia and depressive disorders before we can subdivide these disorders based on molecular criteria. To reach this goal we need to identify the susceptibility genes and associated mutations that might be involved in their etiology.

Genetic Association and Linkage Studies

Two types of genetic strategies are commonly used to identify genes involved in complex human diseases. Genome wide scans try to detect linkage disequilibria (LD) between molecular markers (e.g., single nucleotide polymorphisms [SNPs]) and the segregation of a disease phenotype. This approach can lead to the identification of an association between a chromosomal locus in the human genome and the investigated disease phenotype. Further studies are required to microdissect the disease locus and to identify the specific genes in this region. The candidate gene approach selects a gene based on a disease hypothesis or model as a target in linkage and association studies. This approach is the most frequently applied strategy in neuropsychiatric disorders because of its small scale and low cost.

The genetic studies that have analyzed a possible association or linkage of GluR genes to a neuropsychiatric disorder have focused mainly on schizophrenia. Pharmacological evidence suggests the NMDA receptor could be a likely candidate *(4,147).* However, no mutation has yet been identified in NMDA receptor subunit genes that both affects NMDA receptor function and increases susceptibility to or protection against schizophrenia.

Genetic polymorphisms identified in the NMDA receptor subunit genes NR1 and NR2B have been used in linkage and association studies to look for genetic evidence for the NMDA receptors role in schizophrenia *(148–151).* None of these studies detected evidence that support a role for the NMDA receptor in schizophrenia *(148–151).* It has been shown that the metabotropic glutamate receptor alters NMDA receptor responses in vivo *(152).* Therefore, it is possible that in schizophrenia, functionally altered metabotropic glutamate receptors could affect normal NMDA receptor function.

Genetic polymorphisms identified in the genes encoding the metabotropic receptor subunits mGluR2, 5, 7, and 8 were used to investigate a role of the metabotropic glutamate receptor system in schizophrenia *(153–155).* Only one of these studies identified a weak association of the mGluR5 gene with schizophrenia in a Scottish population *(154).* The results of this experiment have not yet been independently replicated. However, additional

evidence was found by Semple et al. during a project studying a translocation between the long arms of chromosomes 1 and 11, which was previously linked to schizophrenia *(156–158).* They identified the mGluR5 gene close to the translocation breakpoint on chromosome 11 *(158).* Alagarsamy et al. demonstrated a positive feedback interaction between mGluR5 and NMDA receptors *(159).* Based on these results, it might be interesting to consider the possibility that NMDA receptor function is altered in schizophrenia by a dysfunctional mGluR5 receptor. The translocation breakpoint identified by Semple et al. contains two additional genes that are involved in glutamatergic neurotransmission *(158).* These genes encode the N-acetyl-alpha-linked acidic dipeptidase II (NAALADaseII) and a homologue. NAALADaseII cleaves the neuropeptide N-acetylaspartylglutamate (NAAG) to glutamate and N-acetylaspartate. NAAG itself binds as selective agonist at the metabotropic glutamate receptor mGluR3 and shows a low potency to activate NMDA receptors (reviewed in ref. [*160*]). Tsai et al. found alterations in levels of aspartate, glutamate, and NAAG and also in NAALADase activity in brains from schizophrenics *(161).*

Other genetic studies have found no evidence for a role of the ionotropic kainate receptor subunit genes GluR5 and GluR6 in schizophrenia *(162,163).* Although recent pharmacological evidence also indicates AMPA receptor subunits as potential candidates in neuropsychiatric disorders *(91,164,165),* they have not been a focus of linkage and association studies.

Only two studies have tested for an association between glutamate receptor genes and depressive disorders. These studies tested whether the GluR3 AMPA receptor gene on chromosome 21 or the mGluR5 gene is related to bipolar disorder *(154,166).* The first study identified a translocation between chromosome 12 and the X chromosome in one patient diagnosed with bipolar disorder (BP) and X-linked mental retardation (MRX) *(166).* The breakpoint on chromosome X was fine mapped within the *GRIA3* gene that encodes the ionotropic glutamate receptor subunit GluR3. However, the analysis of only four additional patients: two BP and two MRX individuals did not detect abnormalities in the *GRIA* gene. The second study did not detect linkage of the mGluR5 gene to bipolar disorder *(154).*

None of the performed genetic studies could convincingly associate or link a glutamate receptor to schizophrenia or depressive disorders. What is the reason for the lack of success in detecting a genetic linkage of NMDA receptor subunit genes to schizophrenia? A major cause is probably the heterogeneity of the analyzed schizophrenia cases. We have insufficient knowledge about the molecular pathology of schizophrenia and are restricted to using a diagnosis that is based on a phenotypic description. Are all schizophrenia cases caused by the same mechanism? Is schizophrenia an assembly of different disorders with similar phenotype? We do not yet know the answers to these questions.

Although pharmacological evidence suggests that NMDA receptor could be hypofunctional in schizophrenia, none of the NMDA receptor subunit genes have been mapped to one of the identified schizophrenia loci (Table 1). It appears likely that mutations exist in NMDA receptor subunit genes, which affect the function of NMDA receptors and might increase the risk to develop schizophrenia. However, these mutations might be rare and difficult to identify in NR1 and NR2B subunit genes. Mice models have been generated, which demonstrate that the NMDAR subunits NR1 and NR2B are essential for viability *(120,121).*

It is now generally accepted that schizophrenia and depressive disorders are complex, probably caused by an unknown number of susceptibility genes, each might contribute only a minor amount to the etiology of the disorder. Individual humans might have a unique number and combination of susceptibility genes that interact with the environment and determine the risk of developing one of these disorders. These mutations might have equal, opposite, or synergistic effects on the manifestation of the disease phenotype. Susceptibility

Table 1
A Comparison Between the Chromosomal Location of Human Glutamate Receptor Genes, Imprinted Genes, and Disease loci[a]

Glutamate receptor	Gene	Chromosome region	Imprinted gene	Chromosome region	Disorder	Chromosome region	References
GluR1	*GRIA1*	5q33	*U2AFBPL*	5q22-q3	SCH	5q22-31	*(183–185)*
GluR2	*GRIA2*	4q32-q33			SCH	4q31	*(185,186)*
GluR3	*GRIA3*	Xq25-q26			BP	Xq24-26	*(186)*
					BP	Xq27-28	
GluR4	*GRIA4*	11q22-q23			BP	11q21-25	*(186)*
Delta 1	*GRID1*	10*					
Delta 2	*GRID2*	4q22					*(187)*
GluR5	*GRIK1*	21q22					*(188,189)*
GluR6	*GRIK2*	6q21	*HYMAI*	6q24	SCH	6q21-22	*(190,191)*
			ZAC/LOT1	6q24			
GluR7	*GRIK3*	1p34-p33	*NOEY2 (ARHI)*	1p31			*(192)*
			p73	1p36			
KA-1	*GRIK4*	11q22.3			BP	11q21-25	*(193)*
KA-2	*GRIK5*	19q13.2	*PEG3*	19q13.4			*(193)*
NR1	*GRIN1*	9q34.3					*(194–197)*
NR2A	*GRIN2A*	16p13.2					*(197,198)*
NR2B	*GRIN2B*	12p12					*(199)*
NR2C	*GRIN2C*	17q25					*(197,198)*
NR2D	*GRIN2D*	19q13.1-qter	*PEG3*	19q13.4			*(198)*
NR3A	n.d.	9*					
NR3B	n.d	19p*					
mGluR1	*GRM1*	6q24	*HYMAI*	6q24			*(200,201)*
			ZAC/LOT1	6q24			
			M6P/IGF2R	6q26-q27			
mGluR2	*GRM2*	3p12-p11					*(155,202)*
mGluR3	*GRM3*	7q21.1-q21.2	*GAMMA2-COP*	7q32			*(203)*
mGluR4	*GRM4*	6p21.3			SCH	6p22-24	*(204)*
mGluR5	*GRM5*	11q14.3					*(158,205)*
mGluR6	*GRM6*	5q35					*(206)*
mGluR7	*GRM7*	3p26.1-p25.2					*(207)*
mGluR8	*GRM8*	7q31.3-q32.1					*(203)*

[a] 17 genes encoding ionotropic glutamate receptor sunbunits and 8 genes encoding metabotropic glutamate receptor have been cloned and mapped on human chromosomes. Their chromosomal location is compared to the location of nearby imprinted genes and the current information about potential disease loci identified for schizophrenia (SCH), bipolar disorder (BP), and depression (D). The disease loci are taken from recent reviews discussing the current genetic findings for each disease *(169,171,182)*. The list of imprinted genes was derived from an on line database (http://www.geneimprint.com/databases/) and *(179,180)*. The putative NR3A homologue receptor subunit, here named NR3B, has not yet been cloned and characterized. However full-length sequence and gene structure are available in public DNA sequence databases. Chromosomal locations marked with * indicate putative locations based exclusively on analyzing the draft of the human genome.

genes probably contain mutations affecting protein function or gene expression. The frequency of these mutations can vary between ethnic groups or geographically separated populations. This diversity is another factor that can make it extremely difficult to identify the susceptibility genes in genetic studies. The detailed description of disease phenotypes is still critical for the outcome of genomic studies. To overcome the problem of studying a molecularly diverse schizophrenic population, it is necessary to identify specific endophenotypes in schizophrenics that can be correlated to individual or specific combinations of genetic markers *(167).* Additionally, it is widely underestimated what the impact of epigenetic modifications (e.g., genomic imprinting) is on the inheritance of complex disorders.

The genetic sequence information from the human genome project the Refseq database and the public initiative of the SNP consortium will facilitate genomic studies of human glutamate receptor genes and will lead to the discovery of hundreds of novel single nucleotide polymorphisms. 1.42 million single nucleotide polymorphisms (SNPs) in the human genome are already publically available so it is now possible to perform candidate gene linkage studies with individual markers or haplotypes for almost any glutamate receptor gene *(168).* The ability to perform these studies is significantly enhancing our efforts to discover new correlations between glutamate receptor genes and neuropsychiatric disorders. The miniaturization of the techniques in the form of genotyping chips and expression micro-arrays now allows us to analyze thousands of individuals or thousands of genes at the same time.

Chromosomal Localization of Human Glutamate Receptor Genes and Neuropsychiatric Disorder Loci

Most of the known 23 human glutamate receptor genes have been mapped in the human genome (Table 1). The sequence draft of the human genome reveals gene structures and sequence for these genes, which can span several hundred kilo-base pairs (unpublished observation). This information is invaluable for genetic studies that try to identify susceptibility genes for schizophrenia or depressive disorders. Positional cloning experiments will soon be replaced by direct utilization of the human genome sequence. Candidate gene-based and genomewide genetic linkage and association studies have identified several chromosomal loci linked to schizophrenia and depressive disorders (bipolar disorder, major depression) *(169–172).* We have compiled a list showing the glutamate receptor gene localizations on human chromosomes and their correlation to nearby loci for neuropsychiatric disorders (Table 1). Interestingly, none of the NMDA receptor subunit genes is close to a putative schizophrenia disease locus. In contrast several AMPA receptor subunits are mapped near disease loci for schizophrenia and bipolar disorder.

Studies that investigated the genetics of neuropsychiatric disorders detected parent-of-origin effects appear in schizophrenia, bipolar disorder, and major depression. Genomic imprinting, genetic anticipation, or mitochondrial inheritance of genes involved in these disorders, are mechanisms that could explain these parent of origin effects *(173,174).* Genomic imprinting is a molecular mechanism where the expression of one of the two parental allelels is silenced dependent on the origin of inheritance *(175,176).* This epigenetic modification can be regulated on a developmental and cell-type specific level, and can also be influenced by genetic background *(175,177,178).* The relevance of genomic imprinting for the genetics of a disease gene is obvious. A disease allele could be transmitted through generations without causing the disease due to silencing. An understanding of how this mechanism affect the genetics of disease target genes is important for the design and the interpretation of genetic studies of complex neuropsychiatric disorders.

About 43 human genes been have identified that are genomically imprinted *(179,180).* Table 1 also correlates the chromosomal loci of human GluR genes with known imprinting loci. In some cases it has been found that imprinted genes are clustered in a chromosomal region *(181).* Before starting a genetic study it should be common practice to analyze each candidate gene for the possibility that genomic imprinting modifies it. Several glutamate receptor genes are relatively close to genes that are genomically imprinted. In particular the genes encoding kainate receptor KA-2, the NMDA receptor subunit NR2D, and the metabotropic glutamate receptor mGluR1 are mapped in regions that contain imprinted genes. It will be interesting to evaluate if these genes are affected by genomic imprinting, which would be important for understanding their role in the CNS and disorders.

Conclusion

Genetic studies indicate that both genetic and environmental factors are important in the development of schizophrenia and depressive disorders. Although no mutation has yet been identified that makes a human individual more susceptible to develop schizophrenia, depression, or bipolar disorder, it is expected that such mutations will be identified in the near future. The current understanding of the neuropathology and pharmacology of schizophrenia suggests that the dopaminergic, the glutamatergic, and other neurotransmitter systems might be involved in their etiology. Genes from all these neurotransmitter systems are candidates to carry disease mutations. The glutamatergic receptor system consists of 25 receptor subunit proteins and numerous other proteins that modulate the system. Based on strong pharmacological evidence, NMDA receptor subunit genes, but also AMPA and metabotropic GluRs subunit genes should be considered for further investigations to discover susceptibility mutations for schizophrenia. The draft sequence of the human genome will facilitate the search for these mutations. Pharmacology also suggests that the glutamatergic system might be involved in depressive disorders. However, further studies are needed that identify individual GluR subunit genes as disease-related genes. The study of glutamate receptor genes as potential susceptibility genes in schizophrenia and depressive disorders might lead to new concepts about their disease etiologies and might stimulate the development of new therapeutics.

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