The Role of Glutamate in Anxiety and Related Disorders

By Bernadette M. Cortese, PhD, and K. Luan Phan, MD

Needs Assessment

Recent preclinical and clinical evidence has implicated the glutamate system in animal models of anxious behavior and in human anxiety disorders, including clinical trials showing efficacy of glutamatergic agents in anxiety disorders. This review provides a framework for synthesizing the current findings for clinicians, researchers, and patients who stand to benefit from greater clarity as to their scientific and therapeutic significance.

Learning Objectives

At the end of this activity, the participant should be able to:

- Recognize the different mechanisms of action of various pharmacologic agents can have on the glutamate neurotransmitter system.
- Describe the effects of glutamatergic agents on different animal models of anxiety.
- Identify which pharmacologic agents with effects on the glutamate system have been shown to decrease the clinical symptoms of anxiety.
- Understand the potential that basic and translational neuroscience studies on the glutamate system hold for testing theories on the pathophysiology and developing novel pharmacotherapy of anxiety disorders.

Target Audience Neurologists and psychiatrists

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This activity has been peer-reviewed and approved by Eric Hollander, MD, professor of psychiatry, Mount Sinai School of Medicine. Review Date: August 22, 2005.

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Read this article, and the two CME-designated accompanying articles, reflect on the information presented, and then complete the CME quiz found on pages 840 and 841. To obtain credits, you should score 70% or better. Termination date: October 31, 2007. The estimated time to complete this activity is 3 hours.

ABSTRACT

Anxiety, stress, and trauma-related disorders are a major public health concern in the United States. Drugs that target the γ-aminobutyric acid or serotonergic system, such as benzodiazepines and selective serotonin reuptake inhibitors, respectively, are the most widely prescribed treatments for these disorders. However, the role of glutamate in anxiety disorders is becoming more recognized with the belief that drugs that modulate glutamatergic function through either ionotropic or metabotropic glutamate receptors have the potential to improve the current treatment of these severe and disabling illnesses. Animal models of fear and anxiety have provided a method to study the role of glutamate in anxiety. This research has demonstrated that drugs that alter glutamate transmission have potential anxiolytic action for many different paradigms including fear-potentiated startle, punished responding, and the elevated plus maze. Human clinical drug trials have demonstrated the efficacy of glutamatergic drugs for the treatment of obsessive-compulsive disorder, posttraumatic stress disorder, generalized anxiety disorder, and social phobia. Recent data from magnetic resonance imaging studies provide an additional link between the glutamate system and anxiety. Collectively, the data suggest that future studies on the mechanism of and clinical efficacy of glutamatergic agents in anxiety disorders are appropriately warranted.

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INTRODUCTION

Anxiety, stress, and trauma-related disorders are a major public health concern in the United States, with an estimated yearly burden of >$63 billion dollars in direct (eg, psychiatric and non-psychiatric care, hospitalization, emergency care and prescription drugs) and indirect (eg, reduced productivity and occupational absenteeism) costs.[1] Anxiety is commonly experienced and typically adaptive. However, for >15 million adults/year, this anxiety is excessive and dysfunctional, manifesting as an anxiety disorder; together, anxiety disorders are the most prevalent mental health problem in the US.[2] Furthermore, these individuals are more likely to be diagnosed with other medical conditions including irritable bowel syndrome and hypertension and are at an increased risk for other anxiety disorders and mood disorders, such as depression.[3]

Anxiety disorders, including generalized anxiety disorder (GAD), specific and social phobias, posttraumatic stress disorder (PTSD), obsessive-compulsive disorder (OCD), and panic disorder, are typically treated with medications that target the γ-aminobuytric acid (GABA) or serotonergic system. Benzodiazepines and selective serotonin reuptake inhibitors, are the most widely prescribed treatments for these disorders.[4] Some forms of anxiety are relatively resistant to treatment with these agents,[5,6] and both benzodiazepines and selective serotonin reuptake inhibitors can be associated with side effects, such as sedation, memory impairment, potential for substance abuse and withdrawal syndromes, sexual dysfunction, and weight gain. Noncompliance with these pharmacologic agents remains a problem, leading to increased risk for relapse.[7] Therefore, it has become increasingly apparent that alternative treatment strategies are needed.[8]

A novel avenue of neuroscience research involves the glutamate system, the major excitatory neurotransmitter in the mammalian brain. Given that many stress- and anxiety-related disorders are posited to stem from excessively responsive or hyperexcitable brain circuits, investigations of the role of glutamate in anxiety disorders and of drugs that modulate glutamatergic function have the potential to improve our understanding and treatment of these severe and disabling illnesses.

GLUTAMATE PHARMACOLOGY

Glutamate is ubiquitous within the central nervous system and has been shown to play important roles in many brain processes, including neurodevelopment (eg, differentiation, migration and survival),[9] learning (eg, long-term potentiation and depression),[10] acute neurodegeneration (eg, cerebral ischemia, traumatic brain injury),[11] chronic neurodegeneration (eg, Huntington's disease, Alzheimer's disease),[12] and, more recently, the stress response and anxiety disorders.[13] Exposure to severe stress has been associated with glutamate excitotoxicity, which, in turn, can cause neuronal damage and/or death.

Glutamate exerts its actions through ligand-gated ion channel (ionotropic) receptors, including the *N*-methyl-D-aspartate (NMDA), kainate, and α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) subtypes, and G protein-coupled metabotropic receptors (mGluR1-8).[14] The ionotropic glutamate receptors are distributed widely throughout the brain, although density is high in cortical and limbic regions.[15] The metabotropic receptors have a similar wide distribution, with a moderate to high expression in the hippocampus, prefrontal cortex, and amygdala regions associated with anxiety.[16] The mGluRs are further classified into three groups: group I receptors (mGluR1 and mGluR5) localized predominately on postsynaptic neurons are positively coupled to phospholipase C; group II receptors (mGluR2 and mGluR3), localized on pre- and post-synaptic neurons, and group III receptors (mGluR4, mGluR6, mGluR7, and mGluR8) are coupled in an inhibitory manner to adenylyl cyclase.[17] Limbic and associated paralimbic brain structures (amygdala, hippocampus, anterior cingulate cortex [ACC], orbitofrontal cortex, medial prefrontal cortex, insular cortex), regions extensively implicated in the mediation of fear, and anxiety in animals and humans[18-21] have been identified as being richly innervated by glutamatergic pyramidal cells.[22] Utilizing an immunohisochemistry tracing technique for localization of glutamate neurons, McDonald[22] reported that 85% to 95% of the neurons in the basolateral nucleus of the amygdala were both glutamate positive and projected to the prefrontal cortex and ventral striatum. Furthermore, glutamatergic pyramidal cells of the prefrontal cortex project back to numerous limbic regions, including the hippocampus and amygdala.[23]

Glutamate also exerts its actions in the brain by affecting the release of other neurotransmitters including monoamines and GABA. In vivo microdialysis experiments in awake, freely moving rats[24,25] have demonstrated that both mild stress (ie, handling) and intra-striatal infusion of AMPA/kainate agonists facilitate the presynaptic synthesis and release of dopamine in prefrontal cortex, while infusion of an NMDA agonist resulted in a trend toward a decrease in prefrontal cortical dopamine release.[24] GABA inhibitory interneurons have been impli-

cated to have a role in this latter effect.[24] Similar to dopamine, both in vitro and in vivo studies have demonstrated modulation of serotonin release by glutamate.[26,27] In addition, current treatments for anxiety disorders, including topiramate, lamotrigine, and phenytoin, have been shown to modulate monoamine release in the prefrontal cortex[28] and hippocampus[29] and enhance GABA in entorhinal cortex.[30] Results from these studies suggest the anxiolytic effects of glutamatergic drugs may be mediated in part by modulation of other neurotransmitter systems.

GLUTAMATE AND STRESS

Release of adrenal steroid hormones called glucocorticoids is a normal response to stress. However, chronic stress and release of glucocorticoids is associated with illness and specific neurotoxic events, including the excess release of glutamate in the hippocampus.[31] For example, chronic exposure to immobilization stress in rats is reported to increase glutamate release and uptake in the hippocampus and prefrontal cortex.[32-34] Stress caused by forced swimming has also been shown to increase glutamate in the hippocampus and prefrontal cortex.[35] Chronic stress may also alter glutamate gene expression, given that repeated immobilization in rats has been associated with increased hippocampal AMPA receptor messenger ribonucleic acid levels.[36]

The stress-related effects of glucocorticoids and subsequent excitotoxicity of glutamate in the hippocampus, make this brain region particularly susceptible to atrophy.[37] Animal studies have reported decreased dendritic branching, neuronal death, and decreased neuronal regeneration of hippocampal pyramidal cells in response to chronic immobilization stress.[38,39] In contrast to the degenerative effects demonstrated in the hippocampus, chronic immobilization stress produces hypertrophic effects in the amygdala, including enhanced dendritic arborization (ie, increase in dendritic length and branch points) of the pyramidal and stellate neurons of the basolateral complex of the amygdala[39] and bed nucleus of stria terminalis neurons of the extended amygdala.[40] The paradoxical stress-induced anatomical changes found in the hippocampus and amygdala appears consistent with their differential roles in the neural circuitry of stress. Specifically, the role of the hippocampus in the hypothalamic-pituitary-adrenal axis is inhibitory and in contrast to the excitatory regulation by the amygdala.[41]

Behavioral studies[42,43] have demonstrated this contradiction in that stress impairs hippocampal-dependent (ie, spatial) learning, but facilitates amygdala-dependent aversive learning. For example, chronic stress exposure has been shown to impair performance on a variety of hippocampal- and glutamate-dependent spatial learning tasks, including the radial arm maze[44] and Morris water maze.[45] On the other hand, excess glutamate release has been shown to facilitate other forms of learning, such as fear-related learning (ie, fear conditioning). For instance, restraint stress has been shown to increase freezing in a contextual, fear-conditioning paradigm.[46] Fear conditioning is a hippocampal- and amygdala-dependent type of learning in which emotional significance (ie, fear) develops and attaches to a neutral stimulus, for example, contextual cues, through the consistent pairing of the neutral stimulus with an aversive stimulus.[18] Pharmacologic studies have revealed the importance of both hippocampal[47] and amygdala[48] NMDA-type glutamate receptors in the acquisition and expression of contextual fear in rats. In addition to contextual fear conditioning, conditioned-fear paradigms, such as potentiated startle, have been instrumental in demonstrating the role of glutamate and glutamatergic receptors in the amygdala, in particular, in fear learning.[49]

Glutamatergic mechanisms are also hypothesized to have a role in certain behavioral manifestations common to PTSD, including dissociation and perceptual alterations.[50] More specifically, glutamatergic control of both hippocampal-dependent associative learning and amygdala-dependent emotional processing during and after a stressful event may be significant factors in these information processing distortions. Direct evidence for this includes reports[51] that NMDA receptor antagonism by ketamine can produce dissociative symptoms and perceptual alterations (ie, depersonalization, derealization, altered auditory, and visual acuity) akin to those observed with PTSD.

ANIMAL MODELS OF GLUTAMATE AND ANXIETY

Animal models of fear and anxiety have provided a method to study the neuroanatomy and neurochemistry of anxiety disorders.[52] Some of these animal models include punishment-induced (eg, Geller-Seifter) and ethological conflict paradigms (eg, elevated plus maze), aversive tests (eg, exposure to predator), conditioned-fear tests (eg, fear-potentiated startle), and pathophysiological models, including stress and trauma paradigms, such as chronic immobility and sleep deprivation. The elevated plus maze, constructed of two elevated closed arms and two elevated open arms, measures the conflict between an animal's natural tendency to explore and innate fear of heights and open spaces (ie, open arm avoidance).[53]

The Geller-Seifter test is a conflict procedure that measures an animal's acceptance of punishment (eg, foot shock) in order to obtain food reward.[54] The fear-potentiated startle paradigm of conditioned fear measures the increase in the amplitude of the acoustic startle reflex in the presence of a light stimulus that was previously paired with shock.[55]

In a recent study, Uhde and colleagues[56] utilized a sleep deprivation technique, a method shown to worsen symptoms of generalized anxiety disorder and induce panic attacks in humans with panic disorder, to study the effects of stress on brain chemistry in the rat. Compared with rats with normal sleep/wake cycles, 6 hours of sleep deprivation in rats produced significantly greater levels of glutamate and aspartate in the medial prefrontal cortex, as measured by high resolution magic-angle spinning proton magnetic resonance spectroscopy (^{1}H-MRS), a quantitative, ex vivo MR technique used to measure region-specific neurochemicals. In non-human primates, in vivo magnetic resonance spectroscopic imaging has also revealed significantly increased unresolved glutamate-glutamine-γ-aminobutyric acid (Glx) in response to stress.[57] Specifically, mother-infant macaque dyads were reared on a variable schedule for difficulty of food procurement, a method shown to have lasting stress-related behavioral and hormonal effects. At 10 years of age, the macaques that were exposed to this stressor during infancy had increased Glx/creatine ratios in the ACC compared with matched normal control subjects. Although the Glx resonance is a combined measure of glutamate, glutamine, and GABA, the literature consistently describes variations in Glx as changes in glutamate alone. This is based on several lines of evidence,[58-61] including reports that MRS-measured GABA levels are much lower than glutamate levels in human brain[58-60] and that glutamate is the most predominant individual component of the Glx resonance.[61] Nevertheless changes in Glx, measured at lower field strength (eg, 1.5 Tesla), warrant cautious interpretation.

Other anxiety-related behavioral paradigms have also been used to test drugs with potential anxiolytic action, including medications that alter glutamate transmission. The glutamate system has received much attention as a target for treatments of anxiety disorders due to both the preclinical animal studies and human drug trials that have provided good evidence of the efficacy of glutamatergic drugs in the treatment of anxiety (Table). The fear-potentiated startle paradigm has been instrumental in this respect.

Although fear conditioning and fear-potentiated startle paradigms have validated animal models of PTSD and revealed the involvement of other neurotransmitter systems,[62] there seems to be a specific role for glutamate. For example, administration of glutamate antagonists to rats has been used to effectively suppress trauma-enhanced acoustic startle response. In one study,[63] microinjections of DL-2-amino-5-phosphonopentanoic acid (AP5), a competitive NMDA receptor antagonist, into the caudal pontine reticular nucleus effectively suppressed fear-potentiated startle in rats. Others[49] have reported that NMDA glutamate receptors within the amygdala are particularly important to both the learning of fear-potentiated startle and the extinction of conditioned fear. The NMDA antagonist AP5 infused directly into the amygdala dose-dependently blocked the acquisition and expression of fear-potentiated startle.[64,65] Consistent with this, D-cycloserine, the NMDA receptor partial agonist acting at the glycine regulatory site, dose-dependently enhanced extinction of the startle response.[66] Other studies[67-69] have also revealed the enhanced extinction effects associated with D-cycloserine, including reports on the facilitatory effects of D-cycloserine on extinction of cue-conditioned freezing, a species-specific defense response.[67-69] In all, these data suggest a role for the NMDA system in both the acquisition and extinction of conditioned fear.

Medications that target non-NMDA glutamate receptors have also been shown to be an important component of fear behavior. Injections of the AMPA/kainate receptor agonists kainic acid into the dorsal region of the periaqueductal gray, a brain region critical to the expression of acoustic fear-potentiated startle, blocked this startle response, while injections with the AMPA/kainate antagonist 5R,10S)-(+)5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine enhanced this fear response.[70] Topiramate, an Food and Drug Administration-approved anticonvulsant with several mechanisms of action, including inhibition of the non-NMDA glutamate receptors AMPA/kainate,[71] has been shown to significantly reduce stress-induced increase in acoustic startle in rats.[72] Topiramate also acts in part to potentiate GABA by binding to the $GABA_A$ receptor,[73] an effect that could also explain its anxiolytic properties.

In addition, the animal data also implicated the metabotropic glutamate receptors as a potential site for anxiolytic action.[16] These receptors have shown to also be involved in fear conditioning and expression.[74] For example, rats given an injection of 2-methyl-6-(phenylthynyl)-pyridine, a highly potent group I metabotropic receptor antagonist (mGluR5), before fear conditioning, dose-dependently blocked the acquisition of fear and the expression of fear

TABLE. A SUMMARY OF THE ANIMAL AND HUMAN PHARMACOLOGIC EVIDENCE FOR THE ROLE OF GLUTAMATE IN ANXIETY

Preclinical Studies

Author(s) (Year)	Agent	Mechanism	Effect
Fendt et al (1996)[63]	AP5	NMDA receptor antagonist	▼ Fear-potentiated startle
Campeau et al (1992)[64]	AP5	NMDA receptor antagonist	▼ Fear-potentiated startle
Walker et al (2002)[66]	DCS	NMDA partial agonist	▲ Fear-potentiated startle (extinction)
Ho et al (2005)[75]	DCS	NMDA partial agonist	▼ EPM (time in open arms)
Karcz-Kubicha et al (1997)[76]	DCS	NMDA partial agonist	▲ EPM (time in open arms)
Anthony and Nevins (1993)[65]	DCS	NMDA partial agonist	▼ Fear-potentiated startle
Ledgerwood et al (2004)[69]	DCS	NMDA partial agonist	▼ Cue-conditioned freezing (reinstatement)
Ledgerwood et al (2003)[68]	DCS	NMDA partial agonist	▲ Cue-conditioned freezing (extinction)
Parnas et al (2005)[67]	DCS	NMDA partial agonist	▲ Cue-conditioned freezing (extinction)
Klodzinska and Chojnacka-Wojcik (2000)[77]	DCS	NMDA partial agonist	▲ Punished drinking
Xie et al (1995)[78]	MK-801	NMDA receptor antagonist	▲ Punished drinking
Fendt (2000)[70]	Kainic acid	AMPA/kainate receptor agonist	▼ Fear-potentiated startle
Fendt (2000)[70]	NBQX	AMPA/kainate receptor antagonist	▲ Fear-potentiated startle
Kotlinksa and Liljequist (1998)[79]	LY326325	AMPA/kainate receptor antagonist	▲ Punished drinking ▲ EPM (time in open arms)
Khan and Liberzon (2004)[72]	Topiramate	AMPA/kainate receptor agonist	▼ Stress-induced startle
Schulz et al (2001)[80]	MPEP	mGluR5 antagonist	▼ Fear-potentiated startle
Ballard et al (2005)[81]	MPEP	mGluR5 antagonist	▲ Punished responding
Linden et al (2004)[82]	LY354740	mGluR2/3 agonist	▲ EPM (time in open arms)
Shekhar and Keim (2000)[83]	LY354740	mGluR2/3 agonist	▼ Lactate-induced panic
Helton et al (1998)[84]	LY354740	mGluR2/3 agonist	▼ Fear-potentiated startle
Walker et al (2002)[49]	LY354740	mGluR2/3 agonist	▼ Fear-potentiated startle
Johnson et al (2005)[85]	3-pyridyl-methyl-sufonamides	mGlurR2R receptor agonist-potentiators	▼ Fear-potentiated startle ▼ Stress-induced hyperthermia
Steckler et al (2005)[86]	JNJ16259685	mGluR1 antagonist	▲ Punished drinking
Mirza et al (2005)[87]	Lamotrigine	Sodium channel blocker	▲ Conditioned emotional response
Mirza et al (2005)[87]	Riluzole	Glutamate release inhibitor	▲ Conditioned emotional response

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(ie, fear-potentiated startle), without causing sedative or analgetic effects.[88] Similarly, acute systemic and oral administration of the group II mGluR 2/3 agonist LY354740 produced a significant reduction in fear-potentiated startle, without any central nervous system impairments.[84,89] A recent study[84] suggests that mGluR2 potentiators, which act by increasing the affinity and apparent potency of glutamate agonists, also demonstrate efficacy in the fear-potentiated startle paradigm and other rodent models of anxiety, including stress-induced hyperthermia.

The punished responding paradigms and the elevated plus maze anxiety models have also been helpful in describing the role of glutamate in anxiety. For example, in a conditioned emotional response task (ie, lever pressing for food in the presence of a light that was previously paired with shock), drugs that inhibit glutamate release, such as lamotrigine and riluzole, demonstrate anxiolytic properties with increased conditioned emotional response rates during the presentation of the light.[87] Treatment with 2-methyl-6-(phenylthynyl)-pyridine also dose-dependently increased punished responding in several conflict paradigms with rats but did not significantly affect working memory or spatial learning at these anxiolytic doses.[81]

With respect to punished drinking, the studies are mixed as to whether the compound dizocilpine, a noncompetitive NMDA antagonist, has anxiolytic properties. Xie and colleagues[78] suggested that these discrepant findings may be due to the variable pretreatment intervals used in the previous studies, since a variety of pretreatment times were assessed and an optimal time range for administration was reported to significantly increase punished responding.[78] Similar to the anxiolytic effects on conditioned startle and freezing behaviors, administration of D-cycloserine in rats produced a significant increase in both punished drinking and time spent in the open arms of the elevated plus maze.[76,77] However, this latter effect was recently shown to be reversed (ie, an anxiogenic effect was measured by a decrease in time spent in the open arms) with the administration of much lower doses of the partial NMDA agonist D-cycloserine.[75] Acute and chronic administration of the mGluR1 antagonist JNJ16259685 and administration of the AMPA/kainate receptor antagonist LY326325 also produces a dose-dependent significant increase in punished

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TABLE. A SUMMARY OF THE ANIMAL AND HUMAN PHARMACOLOGIC EVIDENCE FOR THE ROLE OF GLUTAMATE IN ANXIETY

Clinical Studies

Author(s) (Year)	Agent	Mechanism	Effect
Grillon et al (2003)[89]	LY354740	mGluR2/3 agonist	▼ Fear-potentiated startle
Levine et al (2001)[90]	LY354740	mGluR2/3 agonist	▼ CO_2 challenge panic
Kellner et al (2005)[91]	LY544344	mGluR2/3 agonist	▼ CCK-4 challenge panic
Bremner et al (2004)[92]	Phenytoin	Inhibits glutamate transmission	▼ Symptoms of PTSD
Heresco-Levy et al (2002)[93]	DCS	NMDA partial agonist	▼ Symptoms of PTSD
Ressler et al (2004)[94]	DCS	NMDA partial agonist	▼ Symptoms of specific phobia
Berlant and van Kammen (2002)[95]	Topiramate	AMPA/kainate receptor agonist	▼ Symptoms of PTSD
Berlant (2004)[96]	Topiramate	AMPA/kainate receptor agonist	▼ Symptoms of PTSD
van Ameringen et al (2004)[97]	Topiramate	AMPA/kainate receptor agonist	▼ Symptoms of social phobia
Coric et al (2005)[98]	Riluzole	Glutamate release inhibitor	▼ Symptoms of OCD
Mathew et al (In press)[99]	Riluzole	Glutamate release inhibitor	▼ Symptoms of GAD

AP5=DL-2-amino-5-phosphonopentanoic acid; NMDA=*N*-methyl-D-aspartate; ▼=decreased; ▲=in creased; DCS=D-cycloserine; EPM=elevated plus maze; MK-801=dizocilpine; AMPA=α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid; NBQX=(5R,10S)-(+)5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine; MPEP=2-methyl-6-(phenylethynyl)-pyridine; mGluR=metabotropic glutamate receptor; CO_2=carbon dioxide; CCK-4=cholecystokinin tetrapeptide; PTSD=posttraumatic stress disorder; OCD=obsessive-compulsive disorder; GAD=general anxiety disorder.

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drinking.[79,86] This study[79] reported that treatment with LY326325 produced a significant increase in the time spent in the open arms of the elevated plus maze. Anxiolytic effects of LY354740 were also found for the elevated plus maze,[82] an effect prevented by pretreatment with an mGluR selective antagonist and not present in mGluR2 or mGluR3 receptor knockout mice.[100] The mGluR agonists have also been found to have anxiolytic properties in other animal models of anxiety. One study[83] demonstrated a similar efficacy of both LY354740 and alprazolam, a benzodiazepine proven to be a clinically effective anti-panic drug, in preventing lactate-induced panic-like attacks in rats.[83]

THE HUMAN PSYCHOPHARMACOLOGY AND CLINICAL STUDIES OF GLUTAMATE AND ANXIETY

Although relatively few in number, human genetic, physiological, and behavioral studies also present preliminary evidence for the involvement of glutamate in fear and anxiety (Table). Arnold and colleagues,[101] reported a significant association between a glutamate system gene and OCD by measuring the relationship between variants of the glutamate NMDA receptor subtype 2B (GRIN2B) and familial incidence and severity of OCD. Specific findings from this study included a significant positive association between 5072T/G, a single nucleotide polymorphism located in the 3' untranslated region of GRIN2B, and both OCD diagnosis and lifetime symptom severity. The authors concluded that GRIN2B could be associated with increased risk for OCD, a finding in support of glutamate system changes in the pathophysiology of anxiety. In a more recent study,[102] glutamate levels in cerebrospinal fluid (CSF) of psychotropic drug-naïve OCD patients were measured and found to be significantly higher when compared with the CSF glutamate levels of normal control subjects.[102] A significant relationship between OCD symptom severity and CSF glutamate levels was not reported. This could be due to the fact that increased glutamate in CSF has not been confirmed to directly reflect increased glutamate in the brain. However, it could indicate a number of brain processes, including abnormal activity of the glutamate/glutamine cycle. Nevertheless, the result of increased glutamate in CSF adds to the growing evidence in support of the role of glutamate in OCD and anxiety in general.

The fear-potentiated startle paradigm has also been utilized in humans as a model of conditioned fear. Similar to the animal studies, LY354740 has been assessed as a potential anxiolytic with the fear-potentiated startle paradigm in humans. Grillon and colleagues[89] reported both a reduction in fear-potentiated startle to shock anticipation and a lower self-reported level of state anxiety after treatment with LY354740 in healthy volunteers. LY354740 has also been shown to be efficacious in an experimentally induced anxiety model of panic attacks that utilized 35% carbon dioxide inhalation in patients suffering from panic disorder.[90] Furthermore LY544344, the peptidyl prodrug of LY354740 developed for better absorption and bioavailability, produced a significant decrease in cholecystokinin tetrapeptide-induced subjective anxiety ratings and panic symptoms in healthy humans who also demonstrated reduced cholecystokinin tetrapeptide-elicited adrenocorticotropin release.[91]

Clinical drug trials[92-99] provide convincing evidence for a role of the glutamate system in several of the anxiety disorders. Specifically, there are various reports on clinical drug trials for the treatment of anxiety disorders using compounds that have direct actions on glutamate receptors. Phenytoin, an anticonvulsant with several mechanisms of action that include both decreasing glutamate and increase GABA neurotransmission,[103] has recently been used to treat early abuse-, combat-, and car-accident-related PTSD.[92] In this small open-label trial, 3 months of phenytoin treatment resulted in a significant decrease in PTSD symptoms, including intrusions, avoidance, and arousal. In another group of patients with chronic PTSD,[93] treatment with D-cycloserine resulted in a significant improvement in anxiety-associated symptoms. Preclinical studies investigating the effects of D-cycloserine on the extinction of conditioned-fear responses[104] have led some to suggest a therapeutic role for D-cycloserine in the extinction of fear and anxiety associated with phobia, PTSD, OCD, and panic disorder in humans. This is especially true given that current behavioral treatments for fear and anxiety, including behavioral exposure therapy, is based on extinction.[94] In a recent randomized, double-blind, placebo-controlled study,[94] patients with acrophobia (ie, fear of heights) were given combination therapy of behavioral exposure to heights within a virtual reality glass elevator and oral administration of either D-cycloserine or placebo. The D-cycloserine-treated patients compared with the placebo controls demonstrated significantly larger reductions in acrophobia symptoms, both in the virtual environment and in real-world experiences. These effects were maintained for a 3-month period, demonstrating both the robust and lasting effect of D-cycloserine on the extinction of fear in humans.[94]

Non-NMDA glutamate receptors also have a role in anxiety in humans. Similar to the preclinical

studies with topiramate, human open-label studies of topiramate as a monotherapy or adjunctive treatment in adults with chronic PTSD[95,96] report significant decreases in nightmares and flashbacks, and a reduction in PTSD Checklist-Civilian Version score. Topiramate has also been assessed as a treatment for social phobia in a study by van Ameringen and colleagues[97] who reported a significant drop in the Liebowitz Social Anxiety Scale score in adult *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition*[105]-diagnosed social phobics (generalized type) after 16 weeks of open-label drug therapy. Again, it is important to note that like phenytoin, topiramate modulates both excitatory (ie, glutamate) and inhibitory (ie, GABA) neurotransmission, mechanisms that could both contribute to the anxiolytic effects demonstrated by these drugs.

Mathew and colleagues[99] reported the preliminary results of an 8-week, open-label study for treatment effectiveness of the anti-glutamatergic agent riluzole 100 mg/day in 18 medically healthy, adult patients with *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition*[105]-diagnosed GAD. After 8 weeks, the response rate in trial completers (n=15) was 80%, while the remission rate (Hamilton Anxiety Scale score <7) was 53%. Although larger, placebo-controlled studies are needed, the authors propose that riluzole may be an effective and well-tolerated anxiolytic medication with a relatively quick onset of therapeutic efficacy for the treatment of GAD. Coric and colleagues[98] recently demonstrated that augmentation of existing pharmacotherapy with riluzole was well tolerated and efficacious in treatment-resistant OCD. In an open-label trial, the authors reported that of the 13 patients studied, seven had a >35% reduction in Yale-Brown Obsessive-Compulsive Scale scores, and five were classified as treatment responders. These two studies prompt randomized placebo-controlled clinical trials of riluzole, a glutamate release inhibitor, in anxiety disorders.

HUMAN BRAIN IMAGING STUDIES OF GLUTAMATE AND ANXIETY

Magnetic resonance imaging (MRI) has been used to demonstrate the close, albeit indirect, relationship between glutamate and anxiety. Volumetric (ie, structural) MRI and functional magnetic resonance imaging (fMRI) studies show that glutamate-rich brain regions that have been implicated in the expression of fear and anxiety, such as the hippocampus, amygdala, and anterior cingulate cortex, are either structurally altered or functionally hyperactive in patients diagnosed with anxiety disorders. For example, studies[106,107] have reported reduced anterior cingulate gray matter volume in trauma survivors who developed PTSD. Hippocampal volume is also reduced in Vietnam veterans diagnosed with combat-related PTSD[108] and women diagnosed with PTSD associated with childhood abuse.[109] Although there have been other reports of no difference in hippocampal volume in patients diagnosed with PTSD,[110,111] a meta-analysis of nine studies published between the years of 1995 and 2003[112] revealed significantly smaller left and right hippocampal volume in traumatized adults diagnosed with PTSD compared with healthy and traumatized controls.

Interestingly, the human MRI studies that describe the effects of stress and trauma on the volume of hippocampus and amygdala are comparable with the animal studies of hippocampal and amygdalar dendritic arborization that were previously described. Consistent with the stress-induced hypertrophic effects described in animals, recent reports[113-115] show MRI-measured increases in the volume of amygdala in children and adolescents diagnosed with anxiety disorders, such as GAD and OCD.[113-115]

Functional brain imaging techniques, such as positron emissions tomography (PET) and fMRI, have revealed altered brain activity in the same neuroanatomical regions that have been linked with anxiety disorders through volumetric MRI. For example, panic disorder patients show altered PET-assessed brain metabolism in the hippocampus and ACC,[116] while combat veterans diagnosed with PTSD demonstrate differential blood flow patterns in the amygdala.[117] Other studies using fMRI have effectively linked a deficit in amygdala functioning during a social cue task in patients diagnosed with social anxiety disorder. Stein and colleagues[118] and Birbaumer and colleagues[119] have both shown hyper-responsive limbic areas (ie, amygdala) as a reaction to negatively biased social cues (eg, harsh/unaccepting faces) in subjects diagnosed with social anxiety disorder. Again, these imaging studies do not provide a direct relationship between glutamate functioning and the pathophysiology of anxiety. They only suggest that glutamate-rich brain regions are altered and/or dysfunctional in people diagnosed with anxiety disorders.

^{1}H-MRS is an in vivo imaging technique that enables the quantification of specific neurochemicals and some neurotransmitters including glutamate. While most studies focus on *N*-acetylaspartate and anxiety disorders,[120-125] several studies provide particularly strong evidence for regional-specific alterations in brain levels of glutamate in people diagnosed with anxiety disorders. Grachev and Apkarian[126] reported

an increase in ^{1}H-MRS-detected glutamate in the frontal cortex of healthy subjects with high versus low state-trait anxiety,[126] and changes in glutamate in humans diagnosed with specific anxiety disorders, such as social anxiety and OCD.[127,128] One of these studies[127] compared glutamate in the ACC and occipital cortices of humans diagnosed with generalized social anxiety disorder to age- and sex-matched controls. Social anxiety disorder patients had a 13.2% higher Glu/creatine ratio in the ACC than did their matched comparison subjects, while there were no differences between the groups in Glu/creatine in the control voxel in the occipital cortex. Furthermore, intensity of social anxiety symptoms, as measured by the Liebowitz Social Anxiety Scale, was correlated with the ratio of Glu/creatine, demonstrating the functional significance of glutamate in generalized social anxiety disorder. Interestingly, in this same group, Glu/creatine levels in the ACC were correlated with activation of the rostral ACC in response to harsh, aversive faces.[129] Rosenberg and colleagues[130] also examined the relationship between glutamate and anxiety by measuring the concentrations of the Glx (ie, the ^{1}H-MRS-assessed glutamate, glutamine and GABA complex) resonance in the frontal cortex of psychotropically naïve pediatric patients diagnosed with OCD. This study reported significantly reduced Glx concentrations in the ACC of the OCD group compared with healthy controls. A previous study by the same research group[128] demonstrated significantly increased glutamatergic concentrations (ie, Glx) in the caudate of treatment-naïve pediatric OCD patients compared with controls. Furthermore, this elevated Glx signal in caudate, along with OCD symptomology, was reduced to control levels after 12 weeks of pharmacologic treatment. Keshavan and colleagues[131] hypothesized that a possible tonic-phasic dysregulation of the glutamate system in prefrontal neural circuits may explain the apparent conflicting Glx levels reported in these OCD patients. In other words, reduced tonic Glx in ACC may lead to phasic Glx overactivity in the caudate and explain the reported decrease in ACC Glx and increase in caudate Glx. In addition, this opposed neurochemical finding is also consistent with previous neuroanatomical studies[132,133] that have reported inverse correlations between ACC and basal ganglia volume in OCD patients. Although few in number, the MRS neurochemical studies discussed in this review provide evidence that anxiety disorders are associated with alterations in the glutamate system.

CONCLUSION

The glutamate system function of regulating neuronal excitability in limbic/paralimbic brain structures is important in fear and anxiety responses. An emerging body of evidence supports the role of glutamate in mediating the physiological and behavioral sequelae associated with stress and anxiety in animals. Moreover, compounds that act on glutamate receptors have been shown to alleviate anxiety symptoms. The precise mechanism of anxiolytic action in humans has yet to be elucidated, though brain imaging studies suggest that abnormalities in glutamatergic function and regulation may underlie the pathophysiology of anxiety disorders. The emerging preclinical and clinical evidence suggests that future studies on the mechanism of and efficacy of glutamatergic agents in anxiety disorders are appropriately warranted. CNS

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