REVIEW

Cortical and subcortical gamma amino acid butyric acid deficits in anxiety and stress disorders: Clinical implications

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Abstract

Anxiety and stress disorders are a major public health issue. However, their pathophysiology is still unclear. The gamma amino acid butyric acid (GABA) neurochemical system has been strongly implicated in their pathogenesis and treatment by numerous preclinical and clinical studies, the most recent of which have been highlighted and critical review in this paper. Changes in cortical GABA appear related to normal personality styles and responses to stress. While there is accumulating animal and human neuroimaging evidence of cortical and subcortical GABA deficits across a number of anxiety conditions, a clear pattern of findings in specific brain regions for a given disorder is yet to emerge. Neuropsychiatric conditions with anxiety as a clinical feature may have GABA deficits as an underlying feature. Different classes of anxiolytic therapies support GABA function, and this may be an area in which newer GABA neuroimaging techniques could soon offer more personalized therapy. Novel GABAergic pharmacotherapies in development offer potential improvements over current therapies in reducing sedative and physiologic dependency effects, while offering rapid anxiolysis.

Key words: Brain imaging; Anxiogenesis; Gamma amino acid butyric acid; Anxiety disorders; Anxiolysis

Core tip: Preclinical and clinical studies strongly support the notion that impairments in gamma amino acid butyric acid (GABA) neurotransmission underpin human stress and anxiety disorders. Measurement of *in-vivo* brain GABA function with modern neuroimaging tools, such as proton magnetic resonance spectroscopy, in healthy and disease populations, has contributed greatly to this literature, and also offers the possibility of monitoring GABAergic anxiolytic therapy.

INTRODUCTION

Anxiety and stress disorders are a major public health problem. They are the most common mental health conditions in the United States with a 12-mo prevalence rate of 18%[1]. Moreover, in their lifetime, over 25% of the United States population is expected to suffer from at least one anxiety disorder[1]. Anxiety disorders are responsible for long-term morbidity, and are now thought to be even more chronic than either substance use or mood disorders[2]. Similar observations have been reported from surveys conducted around the globe[3-5]. Across these studies, another consistent finding was the disproportionate impact of clinical anxiety on women. Finally, the societal and economic impact of anxiety syndromes is remarkable. In 1990, for instance, the direct and indirect cost to the United States economy due to these disorders was $42.3 billion[6].

Over the last three decades, diagnostic assessment and treatment options for morbid anxiety have improved considerably. Despite many theories, however, the pathogenesis of these conditions remains unclear. With a deeper understanding of fear and stress neurocircuitry, and the availability of more sophisticated imaging and genetic analytic tools, progress is being made. Within the field, there has been an emerging emphasis on the role of amino-acid neurochemical systems, such as the amino-butyric acid [The gamma amino acid butyric gamma acid (GABA), the major inbibitory neurotransmitter in the central nervous system (CNS)] and its excitatory counterpart, glutamate, in anxiogenesis and anxiolysis. This review will examine the evidence implicating abnormalities in GABA neurotransmission in the genesis of stress and anxiety in health and disease. Key anxiety and stress disorders such as panic disorder (PD), generalized anxiety disorder (GAD), and posttraumatic stress disorder (PTSD) will be reviewed through the lens of relevant animal models and human imaging studies implicating GABA deficits in anxiogenesis. The potential role of GABA in developmental anxiety will be mentioned, as will the evidence for GABA deficits in other neuropsychiatric syndromes in which anxiety is prominent. Finally, an overview will be provided of anxiolytic agents, which directly or indirectly support GABA neurotransmission, and which can address deficits in GABA functioning in the clinical disorders.

RESEARCH

A literature search was conducted using the PubMed and Thomson Web of Science v5.15 search engines. References were identified that directly related to the search terms, "GABA and clinical anxiety", including several review papers. Preference for inclusion in the current paper was given to articles published after 2009. However, some key/landmark papers published prior to 2009 were also included.

GABA NEUROTRANSMISSION AND NORMAL ANXIETY

Several rodent models have highlighted the role of the GABA synthetic isoenzymes glutamic acid decarboxylase 65 (GAD65) and GAD67 in the expression of normal mammalian fear. For example, knockdown of GAD67 protein in the mouse amygdala impaired normal fear extinction and decreased sensitivity to the benzodiazepine anxiolytic, diazepam[7]. In another experiment, a genetic impairment in GAD65 expression was linked to decreased GABAergic transmission and plasticity in the lateral amygdala (LA), which, in turn, was associated with generalization of conditioned fear responses[8]. A study of male rats additionally demonstrated the importance of the sex hormone, 17-estradiol, as a promoter of GAD65 expression, with pharmacologic inhibition of 17-resulting in increasing expression of anxious behaviors (decreased open field exploration)[9].

Improvements in proton magnetic resonance spectroscopy (^{1}H-MRS) technology and editing have led to the ability to quantify regional brain GABA concentrations, and other related amino acid metabolites, non-invasively. As a result, over the last 5 years, a number of studies applying these techniques in healthy humans have been published. These investigations have begun to define the relationships between normal human stress responses, personality type, and cortical GABA changes. For example, harm avoidance, as a normal human temperament trait, was observed to correlate positively with anterior cingulate cortex (ACC) GABA concentrations, and negatively with glutamate levels[10]. Evaluating extraversion/introversion and neuroticism in healthy subjects, another group reported a negative correlation between frontal GABA/creatine ratios (data acquired at 3 Tesla), and extraversion[11]. In another line of inquiry, acute psychological stress in healthy humans (threat of footshock) was associated with an acute decrease in prefrontal cortical GABA concentrations, similar to acute stress findings in the animal literature[12]. Investigating the impact of wellness interventions, such as yoga and walking, on cortical GABA, one group reported a relationship between improvements in stress level and mood, and increases in thalamic GABA levels, for yoga subjects only[13]. Other investigators using an fMRI/MRS assessment strategy, observed, in healthy humans, that lower insula cortex GABA levels and enhanced insula responses to interoceptive stimuli, together predicted higher levels of reported depressive affect[14]. However, studying female subjects, others did not observe a relationship between low insula GABA and inclination toward fearfulness[15].

Thus, cortical and subcortical GABA concentrations can be informative biological correlates of components of personality function and emotional processing, and appear to be change-sensitive markers of normal responses to acute stress and relaxation. It is foresee-

able that routine assessment of inhibitory brain function in this manner is likely to enhance the effectiveness of early-intervention and prevention protocols designed to interrupt the genesis of chronic anxiety or depressive states.

GABA DEFICITS IN PD

Although the neurobiological mechanisms underlying this common and disabling psychiatric syndrome remain unclear, a range of preclinical and clinical findings have implicated disturbances in GABA function in its pathophysiology. Animal modeling work[16] has demonstrated that biochemically-induced GABA deficits in the dorsomedial hypothalamus (DMH) of rats predispose to sodium lactate-induced panic, also an important clinical feature of human PD[17]. Follow-up work with this particular model has observed that lactate sensitivity and other anxious rodent behaviors could be driven by loss of GABAergic inhibition to a local DMH and perifornical population of peptidergic orexin (ORX) neurons[18]. Thus, impaired GABA function may facilitate ORX neuronal hyperactivity, thereby leading to increased sympathetic activation, and panicogenesis.

Other animal models of chronic anxiety/panic have focused on deficits in functioning of synaptic GABA_A receptors as a risk factor for anxiety-proneness. For example, genetically induced deficits (moderate reductions) in expression of GABA_A receptor 2 subunits (by heterozygous knockdown or knockout), were associated with neophobic behaviors, behavioral inhibition, or exaggerated defensive responses to mild threat[19]. More recently, the same group demonstrated that 2-containing GABA_A receptor subpopulations are also implicated in the defensive response to mild threat, in that mice lacking 2 subunits exhibited anxious phenotype[20]. The animal models above also have parallel human findings, which we will now mention.

Deficits of GABA neuronal functioning have been implicated in the pathophysiology of PD by recent ^{1}H-MRS[21-23], GABA_A-benzodiazepine receptor single photon emission computed tomography (SPECT)[24] and positron emission tomography (PET) studies[25,26]. Not unlike the lactate-sensitive animals referred to earlier, humans with PD have been reported to have cortical GABA deficits in occipital, ACC/medial prefrontal cortex (mPFC), and basal ganglia regions of interest, though one MRS-GABA study of the prefrontal cortex was negative[27] (Table 1 for additional details). Similar GABA deficits (also identified by MRS) have been reported in other human anxiety spectrum disorders, such as social anxiety disorder[28] and obsessive compulsive disorder (OCD) (thalamic and mPFC deficits respectively)[29]. If GABA deficits in humans with PD also extend to impairment of GABAergic inhibition of DMH ORX neurons, this could account for spontaneous or lactate-induced panic in PD patients and in other anxiety patients who experience panic. Other domains

of PD symptomatology, such as neophobia, anticipatory fear, and phobic avoidance, could conceivably be more related to the cortical deficits in GABA_A receptor status identified by the PET and SPECT investigations above.

Furthermore, low cortical GABA in PD might be a trait-like entity, since neither acute nor chronic administration of anxiolytic pharmacotherapy was associated with reversal of these deficits[30]. Thus, low cortical GABA could be an important ongoing vulnerability factor conferring panic-proneness[31]. Moreover, in a retrospective analysis of one data set, the presence of a mood or anxiety family history appeared related to the magnitude of cortical GABA deficits observed in PD[32]. Low cortical GABA therefore has potential as a biomarker for PD and related stress conditions.

Human genetics studies of GABA and PD

The familiality and heritability of PD have been well established. Based on a heritability estimate of 43%[33], genetic factors are a significant contributor to the pathogenesis of PD. However, despite extensive clinical investigations in a number of anxiety disorders, the question remains open as to which genes are critically involved in anxiogenesis[34]; this is likely due to the fact that the "genetic architecture" of PD, similar to other high-prevalence medical conditions, is complex and attributable to multiple genes of small effect. The GABA neuronal system, though, continues to be a logical candidate system for future genetic studies of PD because of the current clinical neurobiological data (reviewed above) implicating GABA abnormalities in this condition, as well as the effectiveness of established and promising GABAergic therapies (Table 2).

Despite the recent positive GABA_A receptor PET imaging findings in PD, work evaluating the potential role of GABA_A receptor genes (GABRA2, 3, 6, and GABRAG2) in anxiety spectrum disorders has thus far been negative[35]. Other studies have focused on the genes GAD1 and GAD2, which code for the GABA synthetic isoenzymes, GAD67 (found throughout the neuron), and GAD65 (found more in axon terminals, and related to regulation of short-term demands for GABA), respectively. In the investigation of Hettema et al[36] 2006, involving 589 patients and 539 controls, the data suggested an association of several SNPs of the GAD1 gene with the personality trait of neuroticism (N), a risk factor for both anxiety disorders and major depression (MDD). A more recent case-control study, in a cohort of $n = 268$ anxiety patients, $n = 542$ MDD patients, and $n = 541$ healthy subjects, identified an association between elevated levels of behavioral inhibition trait (BI) in the patient groups, and several GAD2 gene SNPs[37]. Finally, an association study in a cohort of $n = 238$ anxiety patients (84% with PD), and $n = 267$ healthy subjects recently linked several SNPs (rs2930152, rs2697153, and rs956053) of the GAT1 transporter gene, SLC6A1, with panic attacks. The odds ratio of the association increased in more severely

Table 1 Neuroimaging studies of gamma amino acid butyric acid function in anxiety disorders

Method	Finding	Design	Clinical significance	Ref.
[123]I-Iomazenil SPECT	Decreased L hippocampus and precuneus GABA_A R binding	Parallel gp, 13 PD patients vs 16 healthy control subjects (HCs)	Chronic stress due to active PD can result in impaired limbic processing via reduced GABA_A receptor density or function	Bremner et al[24]
^{1}H-MRS	Decreased OC GABA in PD	14 PD vs 14 matched HCs (historical)	Could be related to impaired production of GABA or of GABA-glutamine cycling in PD	Goddard et al[21]
GABA, 1.5T	No change in OC GABA pre and post-acute and chronic BZD Rx	10 PD vs 9 HCs	Suggests low GABA is a trait-like biomarker for PD	Goddard et al[30]
^{1}H-MRS GABA, 3T	Decreased ACC and BG GABA in patients	22 PD (medicated) vs 24 matched HCs, single voxel study	Impaired top-down inhibition of limbic activity in PD	Ham et al[23]
[11]C-flumazenil PET	Reduced GABA_A R binding in L and R insula Cx of PD	11 PD vs 21 HCs	Interoceptive/somaticsensitivity in PD likely could be insula-mediated	Cameron et al[25]
^{1}H-MRS-GABA, 4T	Reduced thalamic GABA in SAD	10 SAD patients vs 10 matched HCs	Impaired GABA function in thalamus in SAD could affect social cognition via amplification of external threat cues	Pollack et al[28]
[11]C-flumazenil PET	Reduced frontal, temporal, parietal Cx GABA_A binding pot. in PD	15 BZD-naïve PD vs 18 HCs	Generalized cortical impairment in GABA_A function in PD could be a cause or effect of PD. If endogenous BZD-like ligands overproduced could be evidence of a compensatory response to chronic stress	Hasler et al[26]
^{1}H-MRS-GABA, 3T	Normal PFC GABA in PD	Parallel gp 17 PD vs 17 sex-matched HCs	In contrast to previous + results in ACC and OC ROIs	Hasler et al[27]
^{1}H-MRS-GABA, 3T	Reduced mPFC GABA in OCD	24 OCD patients vs 22 matched HCs	Could contribute to cortical-striatal circuit dysfunction in OCD	Simpson et al[29]
^{1}H-MRS-GABA, 3T	Reduced ACC/mPFCx GABA in PD	Parallel gp 11 PD vs 8 matched HCs	Effect size greater in FHx+ PD ACC GABA negatively correlated with enhanced ACC-precuneus, connectivity, 2 DMN nodes	Long et al[22] Shin et al[70]
^{1}H-MRS-GABA, 4T	Low R A^r insula Cx GABA in PTSD	13 PTSD patients vs 13 matched HCs	Relationship btw low insula GABA and higher state-trait anxiety levels	Rosso et al[50]
^{1}H-MRS-GABA, 4T	Lower GABA in tempo-parietal Cx and occipital-parietal Cx in PTSD	27 PTSD patients vs 18 trauma-exposed controls	Low GABA finding mediated by high levels of insomnia	Meyerhoff et al[51]
^{1}H-MRS-GABA, 3T	Elevated DLPFC and ACC GABA and glutathione levels	12 PTSD patients vs 17 non-PTSD trauma controls	Oxidative stress implicated in the pathophysiology of PTSD, as well as elevated prefrontal inhibitory neurotransmission	Michel et al[52]

SAD: Social anxiety disorder; PTSD: Post-traumatic stress disorder; GABA: Gamma amino acid butyric acid; mPFC: Medial prefrontal cortex; PET: Positron emission tomography; FDG: Fluorodeoxyglucose; PGB: Pregabalin; ACC: Anterior cingulate cortex; BZD: Benzodiazepine full agonist; MRS: Magnetic resonance spectroscopy; CNS: China national standards.

ill patients (frequent panickers), reaching a value of 2.5[38]. However, none of these studies assessed cortical GABA as part their study design. One recent investigation, employing this strategy to explore 5-HT/GABA interactions as a risk factor for panic/anxiety disorder, reported an association between (higher) prefrontal GABA concentrations and presence of a tryptophan hydroxylase isoform 2 gene polymorphism, especially in female mood/anxiety patients. This polymorphism had been previously linked to decreased TPH2 mRNA expression in PD. Follow-up studies are needed to confirm this association due to the small subgroup size of patients studied; however, this line of inquiry is exciting given the high rate of women affected by PD. There is also preliminary evidence of *GAD1* gene hypomethylation as a potential epigenetic response to negative life stressors in PD[39]. This is of significance clinically due to the close association of life events and onset of PD illness episodes, and in view of the MRS

data suggesting that low cortical GABA is a risk factor for panic-proneness.

GAD/TRAIT ANXIETY AND GABA

Several animal models have linked perturbations in GABA function to elevated trait anxiety. For example, mice bred for high anxiety behaviors (HAB), compared to control animals, were found to have a complex pattern of intra-amygdala GABA neuronal changes[40]. The amygdala has been identified as a key fear-processing structure within the fear circuit. Levels of GAD65 and GAD67 mRNA and protein were elevated in the basolateral amygdala (BLA) in HAB animals vs controls. In addition, mRNA expression of GABA_A receptor subunits 1, 2, and 2 in the BLA was increased in HAB mice, while transcription of 5 and 1 subunits was reduced in the central and medial amygdala. Also, BLA levels of FosB, a marker for neuronal activation,

Drug	Effect on GABA system	Test paradigm	Comments	Ref.
Benzodiazepines	Agonists at GABA_A receptor complex	Alprazolam blocked ACC activation induced by CCK4 in controls.	fMRI study in 16 healthy male subjects	Leicht et al[71]
		Preclinical study of BZD effects on conditioned and unconditioned fear in mice	α2 subunit-containing GABA_A receptors sufficient for BZD anxiolysis of unconditioned fear. α1 and α2 subunits needed to suppress conditioned fear	Smith et al[72]
SSRIs	Increased cortical GABA levels	Clinical MRS-GABA in MDD patients		Sanacora et al[73]
	Increased neuroactive steroid levels in the CNS	CSF ALLO increases in SSRI treated MDD patients (n = 15)		Uzunova et al[74]
TCAs	Increased release of GABA	Preclinical; desipramine effects studied		Korf et al[75]
MAOIs	Increase total brain GABA	Preclinical study of phenelzine effects		Paslawski et al[76]
Gabapentin	Increased cortical GABA	MRS-GABA in seizure patients	Chronic medication admin	Petroff et al[77]
PGB	Increased release of GABA	PGB decreases activation of left insula and amygdala in response to emotional images	fMRI activation study in 16 healthy humans	Aupperle et al[78]
Tiagabine	Blocks GAT-1 and inhibits synaptic GABA reuptake	FDG-PET study pre and post a 6-week tiagabine trial for social phobia	15 social anxiety disorder patients and 10 controls. vmPFC metabolism increased with treatment	Evans K et al[79]
Vigabatrin	Blocks GABA transaminase	Blocks CCK4 panic in healthy humans	Not clinically available due to ocular AEs	Zwanger et al[80]

GABA: Gamma amino acid butyric acid; mPFC: Medial prefrontal cortex; PET: Positron emission tomography; FDG: Fluorodeoxyglucose; PGB: Pregabalin; ACC: Anterior cingulate cortex; BZD: Benzodiazepine full agonist; MRS: Magnetic resonance spectroscopy; CNS: China national standards.

were notably increased. This pattern of findings in HAB animals can be interpreted as evidence of excessive excitation in the BLA due to loss of inhibitory GABA tone from the central and medial nuclei, with compensatory upregulation of BLA GABA synthetic enzymes. In another study, liver X receptor knockout mice were noted to exhibit anxious behaviors and to have reduced expression of GAD65 and 67 enzymes in the ventro-mPFC[41]. Anxiety in this protocol could have been mediated by loss of ventromedial prefrontal inhibitory GABA tone to the amygdala. Chronic anxiety in rodents was induced by inhibition of GABA synthesis in the bed nucleus of the stria terminalis area of the extended amygdala, a model reminiscent of human GAD, since these animals not only had persistent anxiety, but were also lactate-insensitive[42].

Thus far, there have been no clinical studies of cortical GABA levels or GABA_A receptor binding in GAD, and, to date, genetic association studies of GABA_A receptor subtypes have been negative[35]. Of interest clinically are studies which provide indirect evidence of excessive amygalar excitability in GAD patients. In one fMRI study comparing GAD patients and healthy subjects, neutral and threat cues triggered excessive amygdalar activation responses in GAD subjects[43]. This result is consistent with of an excitation/inhibition imbalance (glutamate/GABA function imbalance) within the amygdala in GAD, and is also consistent with the animal model findings presented above.

PTSD AND GABA FUNCTION

Animal studies

An unpredictable stress paradigm (unpaired odor-shock administration) in neonatal rodents, was associated with anxiety phenotypic behavior in adulthood, together with amygdalar upregulation of genes related to synaptic transmission, such as serotonin (5-HT) and GABA genes[44]. In another rodent study focusing on inescapable stress (inescapable footshock), and examining morphological and neurochemical changes in the prefrontal cortex and hippocampus, post-stress hippocampus cell damage was observed and found to be related to a glutamate/GABA neurochemical imbalance[45]. One group has recently developed a PTSD mouse model by inducing a null mutation in the GAD65 gene. The GAD65 enzyme, as noted previously, is critically involved in activity-dependent regulation of GABA release, and is necessary for proper maturation of the GABA system in adolescence. Mutant mice had hyperexcitability of the amygdala and hippocampus, increased anxiety, and pathological fear memory, all features of human PTSD[46]. In contrast, a GAD65 haplo-deficiency animal model was associated with delayed elevations in cortical and limbic GABA levels, yet was found in these animals to confer resilience to the effects of stress[47]. In another animal model of chronic stress (immobilization/restraint stress), tonic (but not phasic) GABA_A receptor currents in the amygdala were observed to be persistently reduced following this type of stress, resulting in excessive amygdalar excitability[48]. Such enduring, stress-related changes could be relevant to the development of human anxiety and stress disorders. Furthermore, chronic restraint stress in high-anxiety animals in one study led to decreased brain (cortex and hippocampus) expression of 2 subunits of the GABA_A receptor, suggesting a role for these changes in HPA axis dysfunction (upregulation) and stress

symptomatology[49].

Several MRS-GABA studies have also documented abnormalities in cortical GABA in human PTSD (Table 1). For example, insula cortex GABA levels in PTSD patients were recently found to be decreased[50]. In another protocol, combat PTSD patients *vs* non-combat controls exhibited abnormally low levels of parieto-occipital and temporal cortical GABA, which were accounted for by insomnia severity[51]. Yet another group has reported abnormal increases in prefrontal cortical GABA and glutathione in PTSD, implicating oxidative stress and possibly increased frontal cortical inhibition in PTSD pathophysiology[52].

Clinical significance

From the available data it is unclear whether deficits in GABA neurotransmission are a risk factor for PTSD, or are mainly the result of chronic stress, and the associated symptoms of PTSD. It is also unclear whether GABA deficits/dysfunction in PTSD could be the result of compensatory responses to stress that have become depleted, or whether they are particularly involved in the perpetuation of components of chronic PTSD (*e.g.,* hyperarousal, cue-sensitivity, increased startle).

SEPARATION ANXIETY AND GABA

Animal work has implicated the GABA system in unexpected ways in the behavioral and neurochemical response to maternal separation (MS). In one investigation, MS, in addition to promoting anxiety behaviors, enhanced tonic GABA currents in cortical layer 5 pyramidal neurons (juvenile rats), and promoted subsequent neurogenesis (subventricular zone, cortical layer 1, and dentate gyrus), and differentiation into GABA neurons (adult rats)[53]. These persistent brain changes might, in turn, predispose to later-life behavioral disturbances. Some investigators have noted that MS during breast-feeding produced behavioral changes and GABA$_A$ receptor 1 subunit expression changes that were gender-dependent[54]. In this study, MS male rats *vs* controls tended to exhibit less exploratory behavior, and have less 1 subunit expression in the amygdala, mPFC, and paraventricular nucleus (PVN). Females, however, had more exploratory and head-dipping behaviors *vs* controls, and less subunit staining in the mPFC, PVN, preoptic area, and hippocampus. These results highlight the possibility of gender difference in mechanisms of anxiogenesis. This line of inquiry may well improve our understanding of gender differences in the expression of human anxiety syndromes. A recent review of biological underpinnings of critical periods in fear learning and memory encoding highlighted the potential role of the GABA system on neuroplasticity in the peri-adolescent period. Adolescence is a time when GABA neurotransmission is rapidly improving in efficiency, and disruption in these often nonlinear developmental processes could predispose to anxiety and mood patho-logy in the adolescence and beyond[55]. To date, there has been little work in humans directly exploring the impact of developmental events/stressors on GABA function, but this could be a fruitful area of investigation.

GABA DEFICITS AND ANXIETY IN OTHER NEUROPSYCHIATRIC DISORDERS

Studies of other pathological conditions with anxiety as a feature [traumatic brain injury (TBI), temporal lobe epilepsy (TLE), and depression (MDD), have also suggested a relationship between impaired GABA function and anxious behavior. Using a rodent model of mild TBI (mild controlled cortical impact), experimenters reported trauma-related increases in anxiety, and reduced BLA GABA function (decreased GABA cell numbers) leading to BLA hyper-excitability[56]. In TLE patients with mood or anxiety syndromes, together with several other GABA system changes, temporal lobe tissue levels of GABA were noted to be lower *vs* autopsy controls[57]. Finally, in a CSF assessment study of un-medicated MDD patients, those with anxious features tended to have abnormally low CSF GABA levels in contrast to patients without anxious features[58]. Thus, abnormal GABA function could be an important mediator of anxiety across multiple neuropsychiatric syndromes, and these preliminary data suggests the potential efficacy of GABAergic pharmacotherapies to stabilize this symptom cluster (Table 2).

DISCUSSION/TREATMENT IMPLICATIONS

Table 1 summarizes the human neuroimaging studies implicating GABA neuronal dysfunction in anxiety and stress disorders. The majority of the studies, utilizing ^{1}H-MRS techniques, have reported cortical or subcortical GABA deficits in structures relevant to the fear circuit across several different diagnoses. Most studies, however, have been conducted with relatively small samples. The PTSD findings should be interpreted with caution as other factors such as state anxiety, and insomnia appear to be mediating some of the GABA changes reported. In the case of PD, there were 3 positive findings (2 in the ACC/mPFC and 1 in the OC), and one negative finding (prefrontal cortex). One of the ACC studies (Ham *et al*[23]), however, was conducted in medicated PD patients, and hence it is impossible, in this instance, to attribute the GABA changes to the PD diagnosis. The ability to look at interrelated amino-acid metabolites (GABA, glutamate, glutamine) can add power to a study, as demonstrated in the OCD report of Simpson *et al*[29] where impaired mPFC GABA inhibition of subcortical structures was associated with elevated glutamate/glutamine levels in the thalamus. The majority of studies provided a baseline GABA assessment, and therefore, while suggestive of an

association with a specific disorder, at this stage of the research, the idea that the findings indicated effects of chronic stress cannot be ruled out totally. One exception was one of the PD studies in which acute and chronic benzodiazepine medication effects were prospectively measured, and which suggested loss of normal acute GABA counter-regulatory mechanisms, and tonically low GABA in PD[30]. A limitation of this study was the selection of the OC ROI, which is not directly related to fear-processing circuitry. While there is more consistency with the GABA_A receptor findings in PD, again cause-effect relationship cannot readily be disentangled with the study designs used. Future study designs are likely to benefit power-wise from a careful assessment of family history status[32], and the use of a combination of functional imaging techniques (*e.g.,* fMRI or fcMRI together with MRS-GABA assessments), as well as the use of more dynamic MRS approaches (*e.g.,* 13C-labelled glucose/MRS evaluations assessing neuronal and glial contributions to the total GABA pool). The use of more dimensional approaches to anxiety psychopathology classification, as proposed in the NIMH RDoC project, may improve consistency of results (*e.g.,* studying acute responses to fear *vs* anticipatory fear across a range of DSM-V anxiety conditions). Finally, there are important limitations with the GABA neuroimaging paradigms reviewed. MRS evaluations of GABA offer an integrated assessment of intra-neuronal GABA in a large ROI, while current SPECT and PET methodologies offer the ability to study post-synaptic GABA_A receptor status. However, the ability to adapt PET methodology to study the intra-synaptic fraction of GABA, as recently reported[59], now permits a more comprehensive evaluation of GABA neurotransmission across neuropsychiatric disorders.

GABA AND ANXIOLYTIC TREATMENT MECHANISMS

The GABA system has been implicated in the therapeutic mechanism of action of a number of psychotropic agents with anxiolytic activity (Table 2). Benzodiazepine full agonists (BZDs) are the prototypical class of agents in this respect, and their allosteric enhancement effect at the BZD site of GABA_A receptor complex is well known[60]. Preclinical work has further defined the role of discrete GABA_A receptor subunits in the separate clinical effects of the BZDs such as anxiolysis, sedation, muscle relaxation, and anticonvulsant effects. The 2 subunit for instance, is necessary to the anxiolytic action of BZDs[60]. In contrast, sedative, anticonvulsant, amnestic, and dependency effects in general require the presence of the 1 subunit[61]. Antidepressant agents also have the capacity to facilitate GABA function *via* augmentation of GABA levels, and neurosteroid levels. Also, newer-generation GABAergic anticonvulsant medications have begun to demonstrate anxiolytic effects, in parallel with localized physiological changes within the fear circuit.

From the overview provided in Table 2, enhancement of GABA neurotransmission might be viewed as a final common pathway of anxiolytics in general, or at least a key pathway for anxiolysis. If, as the literature currently suggests, GABA neuronal deficits/abnormalities are present in a range of clinical anxiety conditions, then GABA enhancers of different types might be expected to offset these deficits, thereby promoting anxiolysis, and restoration of function. The future prospect of more predictive and personalized anxiety treatment planning and monitoring is also attainable given the availability of imaging tools that can reliably measure cortical GABA, and other amino-acid metabolites. In the specific case of PD, where cortical GABA level deficits may be trait-like, it would be of interest to know if long-term GABA deficits persist, or whether they resolve at some point during maintenance treatment. "Normalization" of CNS GABA levels might be a more appropriate end point/cue to taper psychotropic treatment when combined with more traditional clinical indices of remission.

NOVEL ANXIOLYTICS TARGETING GABA

The GABA system provides a rich array of molecular targets for ongoing drug development initiatives, offering hope for stress/anxiety conditions in which GABA impairments are implicated. Considerable effort has been devoted to the enterprise of generating non-sedative anxiolytics, based on targeting selective GABA_A receptor subunits. While, in this regard, 2/3 subunit-selective compounds have shown much promise in the lab[62], translation to the clinical has been limited by adverse events (*e.g.,* liver toxicity)[63]. More recently, attention has focused on subunit-selective compound development with both preclinical[64] and clinical progress being made[65]. The latter agent, etifoxine, exhibits a dual mechanism, 2/3 subunit selective agonism and neurosteroidogenic stimulation, to enhance GABA neurotransmission. A novel molecular target for ligand development, a mitochondrial Translocator Protein (18 kD), regulates the initial and rate-limiting step in neurosteroidogenesis[66]. Neuroactive steroids synthesized from this pathway, such as allopregneno-lone, act as positive allosteric modulators at a specific neurosteroid site within the GABA_A receptor complex. Positive synthetic ligands at this site include the agent XBD173, which showed preliminary clinical benefit for panic anxiety, and YL-IPA08, which displayed anxiolysis in a PTSD animal model[67]. In addition, a synthetic neurosteroid analog, ganaxolone, has shown therapeutic potential in a mouse model of PTSD[68]. Within the glutamate system, bilateral intra-amygdala (BLA) administration of the GluK1 kainate receptor agonist ATPA, facilitated GABA neurotransmission to promote anxiolysis in one animal model of stress[69].

CONCLUSION

Anxiety and stress disorders are a major public hea-

lth concern. However, their pathophysiology is still unclear, and requires ongoing investigation. The GABA neurochemical system has been strongly implicated in their pathogenesis and treatment by numerous preclinical and clinical studies, the most recent of which have been highlighted in this review. Changes in cortical GABA appear related to normal personality styles and normal responses to stress. While there is accumulating *in-vivo*, neuroimaging evidence of cortical and subcortical GABA deficits across a number of anxiety conditions, a consistent pattern of findings in specific brain regions for a given disorder is yet to emerge. Neuropsychiatric conditions with anxiety as a clinical feature may have GABA deficits as an underlying feature. Different classes of anxiolytic therapies appear to support GABA function, and this may be an area in which newer GABA neuroimaging techniques could soon offer more personalized therapy. Novel GABAergic pharmacotherapies in development offer potential improvements over current therapies in reducing sedative and physiologic dependency effects, while offering rapid anxiolysis.

ACKNOWLEDGMENTS

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