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

Andrew W Goddard

Andrew W Goddard, Department of Psychiatry, UCSF Fresno Medical Education and Research Program, Fresno, CA 93701, United States

Author contributions: Dr. Goddard AW reviewed the literature and wrote the manuscript.

Conflict-of-interest statement: Royalties for manuscript production for UpToDate. No financial support for the current review paper.

Open-Access: This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: http://creativecommons.org/licenses/by-nc/4.0/

Correspondence to: Andrew W Goddard, MD, Professor of Psychiatry, Department of Psychiatry, UCSF Fresno Medical Education Program, 155 N. Fresno Street, Fresno, CA 93701, United States. agoddard@fresno.ucsf.edu

Received: October 27, 2015
Peer-review started: November 3, 2015
First decision: December 4, 2015
Revised: December 18, 2015
Accepted: January 27, 2016
Article in press: January 29, 2016
Published online: March 22, 2016

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

© The Author(s) 2016. Published by Baishideng Publishing Group Inc. All rights reserved.

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.

Goddard AW. Cortical and subcortical gamma amino acid butyric acid deficits in anxiety and stress disorders: Clinical implications. World J Psychiatr 2016; 6(1): 43-53 Available from: URL: http://www.wjgnet.com/2220-3206/full/v6/i1/43.htm DOI: http://dx.doi.org/10.5498/wjp.v6.i1.43
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%\textsuperscript{[1]}. Moreover, in their lifetime, over 25% of the United States population is expected to suffer from at least one anxiety disorder\textsuperscript{[2]}. Anxiety disorders are responsible for long-term morbidity, and are now thought to be even more chronic than either substance use or mood disorders\textsuperscript{[3]}. Similar observations have been reported from surveys conducted around the globe\textsuperscript{[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\textsuperscript{[6]}.

Over the last three decades, diagnostic assessment and treatment options for morbidity 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 inhibitory neurotransmitter in the central nervous system (CNS)] and its excitatory counterpart, glutamate, in anxiogenesis and anxiety. 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\textsuperscript{[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\textsuperscript{[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)\textsuperscript{[9]}

Improvements in proton magnetic resonance spectroscopy (\textsuperscript{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\textsuperscript{[10]}. Evaluating extraversion/introversion and neuroticism in healthy subjects, another group reported a negative correlation between frontal GABA/creatinine ratios (data acquired at 3 Tesla), and extraversion\textsuperscript{[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\textsuperscript{[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\textsuperscript{[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\textsuperscript{[14]}. However, studying female subjects, others did not observe a relationship between low insula GABA and inclination toward fearfulness\textsuperscript{[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 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. 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. 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 receptors as a risk factor for anxiety-proneness. For example, genetically induced deficits (moderate reductions) in expression of GABA receptor 2 subunits (by heterozygous knockdown or knockout), were associated with neophobic behaviors, behavioral inhibition, or exaggerated defensive responses to mild threat. More recently, the same group demonstrated that 2-containing GABA receptor subpopulations are also implicated in the defensive response to mild threat, in that mice lacking 2 subunits exhibited anxious phenotype. 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 1H-MRS, GABA benzodiazepine receptor single photon emission computed tomography (SPECT), and positron emission tomography (PET) studies. 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. (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 and obsessive compulsive disorder (OCD) (thalamo and mPFC deficits respectively). 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 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. Thus, low cortical GABA could be an important ongoing vulnerability factor conferring panic-proneness. 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. 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%, 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; 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 receptor PET imaging findings in PD, work evaluating the potential role of GABA receptor genes (GABRA2, GAD1, and GABRA4) in anxiety spectrum disorders has thus far been negative. 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. 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. Finally, an association study in a cohort of n = 238 anxiety patients (84% with PD), and n = 267 healthy subjects recently linked several SNPs (rs23930152, rs2697153, and rs956053) of the GAT1 transporter gene, SLC6A1, with panic attacks. The odds ratio of the association increased in more severely
GABA deficits in anxiety disorders

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

| Method          | Finding                                      | Design                                      | Clinical significance                                      | Ref.   |
|-----------------|----------------------------------------------|---------------------------------------------|------------------------------------------------------------|--------|
| 123I-o-methyleneprenicnine  | Decreased L hippocampus and precuneus GABA+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+ receptor density or function | Bremner et al[28] |
| 1H-MRS          | Decreased OC GABA in PD                      | 14 PD vs 14 matched HCs (historical)        | Could be related to impaired production of GABA or of GABA-glutamate cycling in PD | Goddard et al[29] |
| GABA, 1.5T      | No change in OC GABA pre and post-acute and chronic BZD Rs | 10 PD vs 9 HCs | Suggests low GABA is a trait-like biomarker for PD | Goddard et al[29] |
| 1H-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[29] |
| 1H-GABA, 4T     | Reduced mPFC GABA in OCD                      | 11 PD vs 21 HCs | Interoceptive/somaticsensitivity in PD likely could be insula-mediated | Cameron et al[29] |
| 1H-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 | Pellack et al[29] |
| 1H-GABA, 4T     | Reduced frontal, temporal, parietal Cx GABA+ binding pot. in PD | 15 BZD-naive PD vs 18 HCs | Generalized cortical impairment in GABA+ 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[29] |
| 1H-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[29] |
| 1H-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] |
| 1H-MRS GABA, 3T | Reduced ACC/mPFCx GABA in PD                 | Parallel gp 11 PD vs 8 matched HCs          | Effect size greater in FH+ PD ACC GABA negatively correlated with enhanced ACC-precuneus, connectivity, 2 DMN nodes | Long et al[29] |
| 1H-MRS GABA, 4T | Low R A’ 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[29] |
| 1H-MRS GABA, 3T | 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[29] |
| 1H-MRS GABA, 3T | Elevated DLPFC and ACC GABA and glutamine 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[29] |

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[30]. However, none of these studies assessed cortical GABA as part of 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 isofrom 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[30]. 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 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,
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 ventromPFC[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 amygdalar 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: 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.

Table 2  Gamma amino acid system butyric acid effects of psychotropics with anxiolytic activity

| 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. Preclinical study of BZD effects on conditioned and unconditioned fear in mice | fMRI study in 16 healthy male subjects α2 subunit-containing GABA_A receptors sufficient for BZD anxiolysis of conditioned fear | Leicht et al[46]                  |
| SSRIs         | Increased cortical GABA levels                   | Clinical MRS-GABA in MDD patients CSF ALLO increases in SSRI treated MDD patients (n = 15) | Chronic medication admin fMRI activation study in 16 healthy humans | Sanacora et al[76] Uznova et al[74] |
| TCAs          | Increased release of GABA levels in the CNS      | Preclinical; desipramine effects studied |                                                                 | Korf et al[77] Paslawski et al[76] |
| MAOIs         | Increase total brain GABA                       | Preclinical study of phenelzine effects |                                                                 | Petrilli et al[77] Auppere et al[78] |
| Gabapentin    | Increased cortical GABA                          | MRS-GABA in seizure patients PGB decreases activation of left insula and amygdala in response to emotional images | Chronic medication admin fMRI activation study in 16 healthy humans |                                                                 |
| PGB           | Increased release of GABA                        | PGB decreases activation of left insula and amygdala in response to emotional images | Chronic medication admin fMRI activation study in 16 healthy humans |                                                                 |
| 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[89] |
symptomatology. 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. 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. 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.

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). 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 receptor 1 subunit expression changes that were gender-dependent. 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 pathology in the adolescence and beyond. 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. 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. Finally, in a CSF assessment study of unmedicated MDD patients, those with anxious features tended to have abnormally low CSF GABA levels in contrast to patients without anxious features. Thus, abnormal GABA function could be an important mediator of anxiety across multiple neuropsychiatric syndromes, and these preliminary data suggests the potential efficacy of Gabaaergic 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 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.), 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. 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[10]. 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 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 intraneuronal GABA in a large ROI, while current SPECT and PET methodologies offer the ability to study post-synaptic GABA 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 receptor complex is well known[60]. Preclinical work has further defined the role of discrete GABA 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, amnesic, 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 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)[55]. 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[65]. Neuroactive steroids synthesized from this pathway, such as allopregnenolone, act as positive allosteric modulators at a specific neurosteroid site within the GABA 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-
ACKNOWLEDGMENTS

Many thanks are due to our departmental research manager, Barbara Price, RN, for her contributions to the compilation of this paper.

REFERENCES

1. Kessler RC, Berglund P, Demler O, Jin R, Merikangas KR, Walters EE. Lifetime prevalence and age-of-onset distributions of DSM-IV disorders in the National Comorbidity Survey Replication. *Arch Gen Psychiatry* 2005; 62: 593-602 [PMID: 15939837 DOI: 10.1001/archpsyc.62.6.593]

2. Kessler RC, Avenevoli S, Costello EJ, Georgiades K, Green JG, Gruber MJ, He JP, Koretz D, McLaughlin KA, Petukhova M, Sampson NA, Zaslavsky AM, Merikangas KR. Prevalence, persistence, and sociodemographic correlates of DSM-IV disorders in the National Comorbidity Survey Replication Adolescent Supplement. *Arch Gen Psychiatry* 2012; 69: 372-380 [PMID: 22147808 DOI: 10.1001/archgenpsychiatry.2011.160]

3. Angst J. Modern epidemiology of anxiety: Results of the zurich cohort study. *Hum Psychopharmacol Clin Exp* 1999; 14: S29-S37 [DOI: 10.1002/schc.1040140102](https://doi.org/10.1002/schc.1040140102)

4. McEvoy PM, Grove R, Slade T. Epidemiology of anxiety disorders in the Australian general population: findings of the 2007 Australian National Survey of Mental Health and Wellbeing. *Aust N Z J Psychiatry* 2011; 45: 957-967 [PMID: 22044173 DOI: 10.3109/00048674.2011.624083]

5. Steel Z, Marnane C, Ipanpour C, Chey T, Jackson JW, Patel V, Silove D. The global prevalence of common mental disorders: a systematic review and meta-analysis 1980-2013. *Int J Epidemiol* 2014; 43: 476-493 [PMID: 24648481 DOI: 10.1093/ije/dyu038]

6. Greenberg PE, Sisitsky T, Kessler RC, Finkelstein SN, Berndt ER, Davidson JR, Ballenger JC, Fyer AJ. The economic burden of anxiety disorders in the 1990s. *J Clin Psychiatry* 1999; 60: 427-435 [PMID: 10453795]

7. Heldt SA, Mou L, Ressler KJ. In vivo knockdown of GAD67 in the amygdala disrupts fear extinction and the anxiolytic-like effect of diazepam in mice. *Transl Psychiatry* 2012; 2: e181 [PMID: 23149445 DOI: 10.1038/tp.2012.101]

8. Lange MD, Jängling K, Paulukat L, Vieler M, Gaburro S, Sosulina L, Blassen P, Sreepathi HK, Ferraguti F, Pape HC. Glutamic acid decarboxylase 65: a link between GABAergic synaptic plasticity in the lateral amygdala and conditioned fear generalization. *Neuropsychopharmacology* 2014; 39: 2211-2220 [PMID: 24663011 DOI: 10.1038/npp.2014.72]

9. Ikeda T, Makino Y, Yamada MK. 17α-estradiol is generated locally in the male rat brain and can regulate GAD65 expression and anxiety. *Neuropsychopharmacology* 2015; 40: 9-14 [PMID: 25446575 DOI: 10.1016/j.neuroph.2014.10.019]

10. Kim JH, Kim JE, Cho G, Song IC, Bae S, Hong SJ, Yoon SJ, Lyoo IK, Kim TS. Associations between anterior cingulate cortex glutamate and gamma-aminobutyric acid concentrations and the harm avoidance temperament. *Neurosci Lett* 2009; 464: 103-107 [PMID: 19660524 DOI: 10.1016/j.neulet.2009.07.087]

11. Goto N, Yoshimura R, Moriya J, Kakeda S, Hayashi K, Ueda N, Ikenouchi-Sugita A, Umene-Nakano W, Oonari N, Korogi Y, Nakamura J. Critical examination of a correlation between brain gamma-aminobutyric acid (GABA) concentrations and a personality trait of extroversion in healthy volunteers as measured by a 3 Tesla proton magnetic resonance spectroscopy study. *Psychiatry Res* 2010; 182: 53-57 [PMID: 20227251 DOI: 10.1016/j.psychres.2009.11.002]

12. Hasler G, van der Veen JW, Grillon C, Drevets WC, Shen J. Effect of acute psychological stress on prefrontal GABA concentration determined by proton magnetic resonance spectroscopy. *Am J Psychiatry* 2010; 167: 1226-1231 [PMID: 20634372 DOI: 10.1176/appi.ajp.2010.09070994]

13. Streeter CC, Whitfield TH, Owen L, Rein T, Karri SK, Yakhkind A, Perlmutter R, Prescot A, Renshaw PF, Ciraulo DA, Jensen JE. The relationship between fearfulness, GABA+, and fear-related BOLD responses in the insula. *PLoS One* 2015; 10: e0120101 [PMID: 25811453 DOI: 10.1371/journal.pone.0120101]

14. Shekhar A, Keim SR, Simon JR, McBride WI. Dorsomedial hypothalamic GABA dysfunction produces physiological arousal following sodium lactate infusions. *Pharmacol Biochem Behav* 1996; 55: 249-256 [PMID: 8951961 DOI: 10.1016/s0091-3057(96)00077-9]

15. Johnson PL, Truitt WA, Fitz SD, Lowry CA, Shekhar A. Neural pathways underlying lactate-induced panic. *Neuropsychopharmacology* 2008; 33: 2003-2107 [PMID: 18059441 DOI: 10.1038/sj.njnp.1001261]

16. Johnson PL, Truitt W, Fitz SD, Minick PE, Districh A, Sanghani S, Träskman-Bendz L, Goddard AW, Brundin L, Shekhar A. A key role for orexin in panic anxiety. *Nat Med* 2010; 16: 111-115 [PMID: 20037593 DOI: 10.1038/nm.2075]

17. Crestani F, Lopez M, Baer K, Essrich C, Benke D, Laurent JP, Belzung C, Fritschy JM, Lüscher B, Mohler H. Decreased hypothalamic GABA dysfunction produces physiological arousal following sodium lactate infusions. *Pharmacol Biochem Behav* 1996; 55: 249-256 [PMID: 8951961 DOI: 10.1016/s0091-3057(96)00077-9]

18. Miech T, Estepi B, Davies JF, Marjaška M, Doyon J, Bajouq M, Northoff G. GABA in the insula - a predictor of the neural response to interoceptive awareness. *Neuroimage* 2014; 86: 10-18 [PMID: 23618604 DOI: 10.1016/j.neuroimage.2013.04.042]

19. Lipp I, Evans CJ, Lewis C, Murphy K, Wise RG, Casera X. The relationship between fearfulness, GABA+, and fear-related BOLD responses in the insula. *PLoS One* 2015; 10: e0120101 [PMID: 25811453 DOI: 10.1371/journal.pone.0120101]

20. Crestani F, Lopez M, Baer K, Essrich C, Benke D, Laurent JP, Belzung C, Fritschy JM, Lüscher B, Mohler H. Decreased GABA- receptor clustering results in enhanced anxiety and a bias for threat cues. *Nat Neurosci* 1999; 2: 833-839 [PMID: 10461223 DOI: 10.1038/12070]

21. Koester C, Rudolph U, Haenggi T, Papilloud A, Fritschi JM, Crestani F. Dorsal raphe. The role of dopamine-sensitive γ-aminobutyric acid type A receptors in defensive behavioral reactivity to mild threat. *Pharmacol Biochem Behav* 2013; 103: 541-549 [PMID: 23067879 DOI: 10.1016/j.pbb.2012.10.004]

22. Goddard AW, Mason GF, Almair A, Rothman DL, Behar KL, Petroff OA, Charney DS, Krystal JH. Reductions in occipital cortex GABA levels in panic disorder detected with 1H-magnetic resonance spectroscopy. *Arch Gen Psychiatry* 2001; 58: 556-561
spectroscopy. *Depress Anxiety* 2014; 31: 115-123 [PMID: 23861191 DOI: 10.1002/da.22155]

Meyerhoff DJ, Mon A, Metzler T, Neylan TC. Cortical gamma-aminobutyric acid and glutamate in posttraumatic stress disorder and their relationships to self-reported sleep quality. *Sleep* 2014; 37: 893-900 [PMID: 24790267 DOI: 10.5665/sleep.3654]

Michels L, Schulte-Vels T, Schick M, O’Gorman RL, Zeffiro T, Hasler G, Mueller-Pfeiffer C. Preferential GABA and glutathione imbalance in posttraumatic stress disorder: preliminary findings. *Psychiatry Res* 2014; 224: 288-295 [PMID: 25448399 DOI: 10.1016/j.psychres.2014.09.007]

Feng M, Sheng G, Li Z, Wang J, Ren K, Jin X, Jiang K. Postnatal maternal separation enhances tonic GABA current of cortical layer 5 pyramidal neurons in juvenile rats and promotes genesis of GABAergic neurons in neocortical molecular layer and subventricular zone in adult rats. *Behav Brain Res* 2014; 260: 74-82 [PMID: 24304720 DOI: 10.1016/j.bbr.2013.11.040]

León Rodríguez DA, Duenas Z. Maternal Separation during Breastfeeding Induces Gender-Dependent Changes in Anxiety and the GABA-A Receptor Alpha-Subunit in Adult Wistar Rats. *PLoS One* 2013; 8: e68010 [PMID: 23826356 DOI: 10.1371/journal.pone.0068010]

King EC, Pattwell SS, Glatt CE, Lee FS. Sensitive periods in fear learning and memory. *Stress* 2014; 17: 13-21 [PMID: 23611461 DOI: 10.3109/10253890.2013.796355]

Almeida-Suáett CP, Prager EM, Pidoplichko V, Figueredo TH, Mariní AM, Li Z, Eiden LE, Braga MF. Reduced GABAergic inhibition in the basolateral amygdala and the development of anxiety-like behaviors after mild traumatic brain injury. *PLoS One* 2014; 9: e102627 [PMID: 25047645 DOI: 10.1371/journal.pone.0102627]

Rocha L, Alonso-Vanegas M, Martínez-Juárez E, Orozco-Suárez S, Escalante-Santiago D, Feria-Romero IA, Zavala-Tecuapetla C, Cisneros-Franco JM, Buentello-Garcia RM, Cienfuegos J. GABAergic alterations in neocortex of patients with pharmacoresistant temporal lobe epilepsy can explain the comorbidity of anxiety and depression: the potential influence of clinical factors. *Front Cell Neurosci* 2014; 8: 442 [PMID: 25601827 DOI: 10.3389/fncel.2014.00442]

Mann JJ, Quemodo MA, Watson BJ, Erritzoe D, Wilson SJ, Cunningham VJ, Riano Barros D, Hammers A, Turkheimer FE, Stokes PR, Myers JF, Kalk NJ, Lee FS. Sensitive periods in fear learning and memory. *Stress* 2014; 17: 1091-1095 [PMID: 23688914 DOI: 10.1016/j.stres.2013.04.026]

Leicht G, Muler C, Eser D, Sämann PG, Erti M, Laenger A, Karch S, Pogrell C, Meindl T, Crisch M, Ruppert M. Benzodiazepines counteract rostral anterior cingulate cortex activation induced by cholecystokinin-tetrapeptide in humans. *Biol Psychiatry* 2013; 73: 337-344 [PMID: 23059050 DOI: 10.1016/j.biopsych.2012.09.004]

Smith KS, Engin E, Meloni EG, Rudolph U. Benzodiazepine-induced anxiolysis and reduction of conditioned fear are mediated by distinct GABAA receptor subtypes in mice. *Neuropharmacology* 2012; 63: 250-258 [PMID: 22465203 DOI: 10.1016/j.neuropharm.2012.01.032]

Sancarova G, Mason GF, Rothman DL, Krystal JH. Increased occipital cortex GABA concentrations in depressed patients after therapy with selective serotonin reuptake inhibitors. *Am J Psychiatry* 2002; 159: 663-665 [PMID: 11925309 DOI: 10.1176/appi.ajp.159.4.663]

Uzunova V, Sheline Y, Davis JM, Rasmussen A, Uzunov DP, Costa E, Guidotti A. Increase in the cerebrospinal fluid content of neurosteroids in patients with unipolar major depression who are receiving fluoxetine or fluvoxamine. *Neuropharmacology* 2012; 63: 3239-3244 [PMID: 22032516 DOI: 10.1016/j.neuropharm.2011.12.007]

Korf J, Venema K. Desmethylimipramine enhances the release of endogenous GABA and other neurotransmitter amino acids from the rat thalamus. *J Neurochem* 1983; 40: 946-950 [PMID: 6131937 DOI: 10.1111/j.1471-4159.1983.tb08078.x]

Polaszek T, Treit D, Baker GB, George M, Couuts RT. The antidepressant drug phenelzine produces anti-anxiety effects in the plus-maze and increases in rat brain GABA. *Psychopharmacology (Berl)* 1996; 127: 19-24 [PMID: 8880939]

Petrow OA, Rothman DL, Behar KL, Lamoureux D, Mattson RH. The effect of gabapentin on brain gamma-aminobutyric acid in patients with epilepsy. *Ann Neurol* 1996; 39: 95-99 [PMID: 8572673 DOI: 10.1002/ana.410390114]

Aupperle RL, Ravindran L, Tankersley D, Flagan T, Stein NR, Simmons AN, Stein MB, Paulus MP. Pregabalin influences insula

Borsch B1,2,3,4,5,6. Sex differences in the effects of allopregnanolone on the rat and human amygdala. *Neuroscience* 1994; 59: 489-495 [PMID: 8059725 DOI: 10.1016/0306-4522(94)90054-1]
and amygdala activation during anticipation of emotional images. Neuropsychopharmacology 2011; 36: 1466-1477 [PMID: 21430645 DOI: 10.1038/npp.2011.32]

Evans KC, Simon NM, Dougherty DD, Hoge EA, Worthington JJ, Chow C, Kaufman RE, Gold AL, Fischman AJ, Pollack MH, Rauch SL. A PET study of tiagabine treatment implicates ventral medial prefrontal cortex in generalized social anxiety disorder. Neuropsychopharmacology 2009; 34: 390-398 [PMID: 18536708 DOI: 10.1038/npp.2008.69]

Zwanzger P, Baghai TC, Schuele C, Ströhle A, Padberg F, Kathmann N, Schwarz M, Möller HJ, Rupprecht R. Vigabatrin decreases cholecystokinin-tetrapeptide (CCK-4) induced panic in healthy volunteers. Neuropsychopharmacology 2001; 25: 699-703 [PMID: 11682253 DOI: 10.1016/S0893-133X(01)00266-4]

P- Reviewer: Grof P, Hosak L
S- Editor: Qiu S L- Editor: A E- Editor: Jiao XK
