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Permalink

<https://escholarship.org/uc/item/38r2w4cd>

Journal

Sleep, 37(5)

ISSN

0161-8105

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Publication Date

2014-05-01

DOI

10.5665/sleep.3654

Peer reviewed

Cortical Gamma-Aminobutyric Acid and Glutamate in Posttraumatic Stress Disorder and Their Relationships to Self-Reported Sleep Quality

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Study Objectives: To test if posttraumatic stress disorder (PTSD) is associated with low brain gamma-aminobutyric acid (GABA) levels and if reduced GABA is mediated by poor sleep quality.

Design: Laboratory study using *in vivo* proton magnetic resonance spectroscopy (1H MRS) and behavioral testing.

Setting: VA Medical Center Research Service, Psychiatry and Radiology.

Patients or Participants: Twenty-seven patients with PTSD (PTSD+) and 18 trauma-exposed controls without PTSD (PTSD-), recruited from United States Army reservists, Army National Guard, and mental health clinics.

Interventions: None.

Measurements and Results: 1H MRS at 4 Tesla yielded spectra from three cortical brain regions. In parieto-occipital and temporal cortices, PTSD+ had lower GABA concentrations than PTSD-. As expected, PTSD+ had higher depressive and anxiety symptom scores and a higher Insomnia Severity Index (ISI) score. Higher ISI correlated with lower GABA and higher glutamate levels in parieto-occipital cortex and tended to correlate with lower GABA in the anterior cingulate. The relationship between parieto-occipital GABA and PTSD diagnosis was fully mediated through insomnia severity. Lower *N*-acetylaspartate and glutamate concentrations in the anterior cingulate cortex correlated with higher arousal scores, whereas depressive and anxiety symptoms did generally not influence metabolite concentrations.

Conclusions: Low brain gamma-aminobutyric acid (GABA) concentration in posttraumatic stress disorder (PTSD) is consistent with most findings in panic and social anxiety disorders. Low GABA associated with poor sleep quality is consistent with the hyperarousal theory of both primary insomnia and PTSD. Our data demonstrate that poor sleep quality mediates low parieto-occipital GABA in PTSD. The findings have implications for PTSD treatment approaches.

Keywords: brain, GABA, glutamate, magnetic resonance spectroscopy, posttraumatic stress disorder, sleep

Citation: 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(5):893-900.

INTRODUCTION

Exposure to combat and civilian trauma is associated with a high risk of mental health problems, including posttraumatic stress disorder (PTSD) and panic disorder. The clinical symptomatology of these stress-related anxiety disorders has been related to long-lasting dysfunction in inhibitory and excitatory neurotransmission and potentially neuronal compromise.^{1,2} Gamma-aminobutyric acid (GABA) and glutamate (Glu) are important neurotransmitters that are intricately linked to neuronal function in general and to memory registration and encoding emotional and fear memory in anxiety disorders in particular.³ Further, Glu neurotoxicity is a central mechanism in neurodegenerative disease. Individuals with low GABA levels in plasma are more vulnerable to acute PTSD.⁴ In recent neuroimaging studies, the concentrations of these amino acids (in addition to density and activity of neurotransmitter receptors and transporters) have been associated with mood disorders and memory dysfunction.⁵ Their concentrations in brain are clinically more meaningful than their plasma and cerebrospinal

fluid (CSF) concentrations, which are inconsistent with each other and do not reflect the regional cerebral heterogeneity of neurotransmitter systems thought to underlie disease symptomatology. Furthermore, quantitation of amino acids from harvested brain tissue — not accessible in healthy controls — is unreliable because of continued GABA synthesis and decreased GABA breakdown during hypoxia. Brain proton magnetic resonance spectroscopy (1H MRS) at high static magnetic fields, however, allows measurement of *in vivo* regional concentrations of GABA and Glu, in addition to another amino acid, *N*-acetylaspartate (NAA), that serves as a neuronal marker. Brain GABA and Glu concentrations have not been measured in PTSD, but they have the potential to illuminate associated biochemistry with behavioral significance.

High-field 1H MRS facilitates the detection of the relatively weak *in vivo* signals from GABA and Glu via increased sensitivity and greater spectral dispersion compared with low-field scanners. 1H MRS-derived GABA concentrations centered on occipital cortex have been shown to be reduced in mood disorders and memory dysfunction⁵ as well as in panic disorder⁶; however, measurements in this and adjacent cortices of patients with PTSD have not been reported. Furthermore, Glu levels were elevated throughout the posterior brain, including parietal lobes of patients with social anxiety disorders,⁷ and potential metabolite level alterations in medial parietal cortex may contribute to altered default mode network activity in PTSD.^{8,9} NAA levels are relatively low in the mesial temporal lobe, including the hippocampus of patients with PTSD,^{10,11} suggesting neuronal

Submitted for publication May, 2013

Submitted in final revised form October, 2013

Accepted for publication November, 2013

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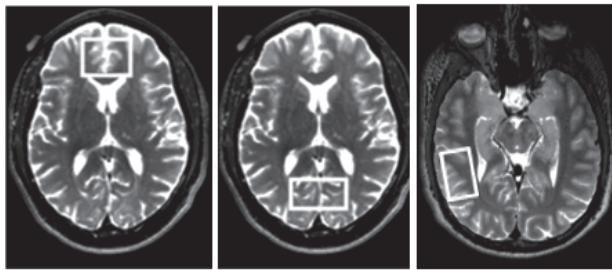


Figure 1—Locations of the volumes of interest on T2-weighted magnetic resonance images (left: ACC; middle: POC; right: TEMP). Examples of stimulated echo spectrum (right, top) and GABA spectrum (right, bottom) analyzed. The GABA spectrum is shown on a different vertical scale than the stimulated echo spectrum. ACC, anterior cingulate cortex; Cho, choline; Cr, creatine; GABA, gamma-aminobutyric acid; Glu, glutamate; Glx, a combination of signals from Glu and glutamine at 3.7 ppm; NAA, *N*-acetylaspartate; POC, parieto-occipital cortex; TEMP, medial temporal cortex.

compromise in this brain region. Some volumetric magnetic resonance imaging (MRI) studies have shown PTSD-related hippocampal atrophy.¹² The medial prefrontal cortex is likely to be affected in acute and chronic stress disorders, given its central role in numerous neural mechanisms related to resilience and stress-vulnerability.¹ In fact, NAA was found to be reduced in the anterior cingulate cortex (ACC) of patients with PTSD.¹¹ However, this measure of neuronal injury does not strongly correlate with behavioral measures in PTSD such as arousal, intrusion, or avoidance. These symptoms also are often accompanied by poor sleep quality, a major complaint by patients with PTSD.¹³ In this context, primary insomnia has been related recently to lower global (including parietal and occipital gray and white matter¹⁴) GABA levels (relative to creatine-containing metabolites) and both lower¹⁵ and higher GABA ratios¹⁶ in occipital and/or anterior cingulate cortices.

The main goals of this study were therefore to measure absolute cortical concentrations of GABA, Glu, and NAA in individuals with PTSD (PTSD+) relative to trauma-exposed individuals without PTSD (PTSD-), and to assess correlations between these metabolite concentrations and measures of insomnia severity and other PTSD symptomatology. Based on the bulk of the previous research, we hypothesized that NAA and GABA levels are lower and Glu levels higher in the ACC, parieto-occipital cortex (POC), and the medial temporal cortex

(TEMP) of PTSD+ than PTSD-, and that these abnormalities represent neurochemical correlates of poor sleep quality and the persistent hyperarousal typical of PTSD. Because PTSD in military veterans is associated with increased levels of depression and alcohol use,^{17,18} and because regional brain amino acid concentrations have been shown to relate to both depressive symptoms and substance use,¹⁹⁻²³ we also tested for the influence of these variables on our main outcome measures.

METHODS

All patients with PTSD and trauma-exposed non-PTSD controls were scanned on a 4 Tesla Bruker Research System (Billerica, MA, USA) with Siemens Trio console (Erlangen, Germany), equipped with an eight-channel transmit-receive radiofrequency head coil. All participants were male and either trauma-exposed American veterans of war or trauma-exposed civilians. They were recruited at the San Francisco VA Medical Center from among northern California United States Army reservists, Army National Guard, and from the Mental Health Service of the San Francisco VA and the Fresno VA, affiliated satellite clinics, regional Veteran Centers, and mental health clinics. All participants voluntarily provided written informed consent that had been approved by the human research committees of the University of California San Francisco, the VA Medical Center in San Francisco, and the Department of Defense. Exclusion criteria were a history of schizophrenia or schizoaffective disorder, alcohol dependence within the past 6 mo, bipolar disorder, or suicidal intention as assessed by a Structured Clinical Interview for Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV) diagnoses.²⁴ Medical exclusion criteria included seizure disorders, head injury associated with any postinjury symptoms or loss of consciousness greater than 10 min, history of stroke or neurodegenerative diseases, or medically unstable injuries related to the indexed critical incident (trauma). Participants prescribed psychiatric medications or hypnotic agents within 2 weeks before MRI, and those individuals with any type of metallic implants, lodged foreign objects, other contraindications for MRI, or likely traumatic reactions to magnetic resonance scanner noise were excluded. Three-dimensional T1-weighted and two-dimensional T2-weighted MRIs were acquired to aid MRS volume-of-interest (VOI) placements, tissue segmentation, and for clinical reads. Single-volume MRS data were acquired from the ACC (medial prefrontal cortex including the anterior cingulate cortex, $35 \times 25 \times 20 \text{ mm}^3 = 17.5 \text{ mL}$), POC (parieto-occipital gray matter, $40 \times 20 \times 20 \text{ mm}^3 = 16 \text{ mL}$) and TEMP (right lateral temporal cortex at the level of the long axis of the hippocampus, which also included significant amounts of mesial temporal lobe white matter adjacent to the hippocampus [$20 \times 40 \times 20 \text{ mm}^3 = 16 \text{ mL}$]). All VOIs were placed to include as much cortical gray matter as possible (see Figure 1). These brain regions were targeted primarily because they were implicated in previous studies of anxiety disorders and insomnia (ACC and POC) and because the hippocampal formation, a region of high interest to PTSD, although not accessible to good-quality GABA MRS, has strong functional and anatomical connections to the lateral temporal cortices.²⁵ One hundred twenty-eight signal averages from NAA, creatine- (Cr) and choline-containing compounds (Cho), myo-Inositol (ml),

and Glu were acquired with a stimulated echo sequence (TR/TE/TM = 1800/15/12 ms),²⁶ followed by obtaining eight averages of a suppressed water signal from the same VOIs for calibration purposes. Immediately afterward, GABA was acquired from the very same VOIs with a modified J-editing sequence.^{27,28} The single-volume GABA J-editing difference acquisition sequence is based on MEGA PRESS²⁷ and has been modified to increase signal-to-noise ratio of the edited spectra and reduce macromolecular signal contribution to the edited GABA signal.²⁸ GABA editing is accomplished by selective inversion of the low-field quintet on alternate scans and observation of its coupled partner at 3.0 ppm, with TR/TE = 2000/71 ms. In the absence of the selective inversion pulse (edit off), the outer lines of the GABA signal at 3 ppm are inverted, while in the presence of the selective inversion pulse (edit on), a signal structure similar to a triplet is observed. Thus, subtraction of signals from alternate scans results in selective observation of the outer lines of the GABA signal at 3 ppm and cancellation of the large overlapping singlet Cr resonance. The Glx system (a combination of signals from Glu and glutamine at 3.7 ppm) is coedited. In all spectra, the GABA resonance can be clearly seen on a flat baseline at 3 ppm, together with a Glx signal at 3.7 ppm. Signal-to-noise ratio varied, depending on experimental conditions and spectral acquisition duration. We usually acquired approximately 13 min per VOI (192 signal averages each edit on and edit off), but if data quality appeared lacking during parameter adjustments and water suppression, data acquisition was increased to 17 min (256 signal averages each). The shorter acquisition yielded spectra with a 14% lower signal-to-noise ratio, but this affected both groups equally as similar proportions of VOIs across the two groups were acquired with the shorter acquisition (approximately 75%). Studies have discussed further details of the data acquisition and spectral processing methods.^{21, 24} In brief, both stimulated echo and J-edited spectra were processed with an in-house program and spectroscopic imaging tools (SITOOLS) using IDL version 6.0. The T1-weighted images were segmented into gray matter, white matter, and CSF,²⁹ and the segmented data were used to estimate tissue and CSF contributions to MRS VOIs. Metabolite concentrations were then calculated from fitted metabolite peak areas (NAA, Cho, Cr, mI, and Glu from STEAM spectra, GABA from J-edited spectra) together with the tissue contributions and individualized system calibration parameters as described.²¹ Signals from scyllo-inositol and Gln were also fit but not included in the statistical analyses because of low signal-to-noise ratio. An additional six low-intensity resonances from macromolecules were included in the model for STEAM spectra but also not analyzed statistically. Gray matter fractions in the VOIs were used as covariates in statistical analyses when they were significantly different between groups.

Both STEAM and J-edited spectra were not always acquired from all three VOIs in any given individual, because not everyone tolerated the time necessary for all acquisitions. Furthermore, of all spectra acquired, not all were of sufficient quality to be included in the final statistical analyses; spectra were excluded when they did not meet our spectral quality assessment criteria. The most common reasons for exclusion were low signal-to-noise ratio, broad line widths that would preclude separate fitting of Cho and Cr resonances, and significant residual water signal that would preclude proper spectral

peak fitting. Between one and three acquired spectra were excluded per VOI and group because of poor spectral quality. This corresponded to < 10% of spectra from any of the VOIs, with no clear pattern of spectral failures across groups or VOIs.

Clinician administered PTSD scores (CAPS) were obtained via interviews. PTSD symptomatology was also assessed with a self-report rating scale (PTSD Checklist, PCL). Insomnia was assessed with the Insomnia Severity Index (ISI).³⁰ The ISI is a valid and reliable self-report measure of perceived insomnia severity. The ISI has seven items that assess sleep onset and sleep maintenance difficulties, satisfaction with sleep, interference with daily functioning, daytime impairment attributable to sleep problem, and degree of distress caused by disturbed sleep. The Alcohol Use Disorder Identification Test (AUDIT) was administered, and lifetime alcohol consumption³¹ and cigarette smoking variables (Fagerstrom Tolerance Test for Nicotine Dependence, FTND³²) were obtained from all participants through structured interviews; the primary outcome measures were considered as possible confounders in our statistical analyses. Drug screens were administered at initial assessment and individuals with positive screens were excluded. To assess the influence of depressive and anxiety symptomatology at the day of the magnetic resonance examination on regional metabolites in the PTSD+ group, we obtained self-report measures of the Beck Depression Inventory (BDI), the Beck Anxiety Inventory (BAI), the State Trait Anxiety Inventory (STAI), and the Symptom Checklist 90R (SCL-GSI), a measure of overall psychological distress, and correlated these to regional metabolite levels.

Statistical Analyses

General linear modeling was used to test our specific primary and directional hypotheses regarding GABA, NAA, and Glu differences between groups, with covariance correction where appropriate (age, monthly lifetime drinking average). Although the primary analyses were directed and based on *a priori* hypotheses, we report the results of two-tailed *t*-tests. However, because the results were not corrected for multiple comparisons (three VOIs), some may consider them preliminary. We also tested a model in which the relationship between PTSD diagnosis and GABA concentration is mediated by insomnia severity as operationalized by the ISI. In all cases and unless otherwise indicated, an alpha level of 0.05 was considered statistically significant. Statistical correlations among metabolite levels and our clinical/behavioral measures used Spearman tests and were not corrected for multiple comparisons. All tests were performed with SPSS version 20 (SPSS Inc., USA) and some exploratory analyses with Stata v12 (StataCorp LP, USA).

RESULTS

Study Participants

We recruited 28 male patients with PTSD symptoms (PTSD+) and 20 male trauma-exposed controls without PTSD symptoms (PTSD-). After data processing and rigorous quality control, we retained quantitative MRS data from two or three VOIs per individual in 27 PTSD+ (35 ± 11 y) and 18 PTSD- participants (37 ± 13 y, *P* = n.s.), with no discernible differences in data quality or signal-to-noise ratio between groups. PTSD+ patients were veterans of foreign wars with trauma related to war zone exposure

Table 1—Behavioral, alcohol, and tobacco use characteristics of both study groups (mean \pm standard deviation)

Variable	PTSD- (n = 18)	PTSD+ (n = 27)	P (two-tailed)
Beck Depression Inventory (BDI)	1.1 \pm 2.0	14.4 \pm 10.1	< 0.0001
Beck Anxiety Inventory (BAI)	1.1 \pm 2.9	12.8 \pm 13.3	0.003
State Trait Anxiety Inventory (STAI)	15.3 \pm 0.8	20.1 \pm 9.3	n.s.*
Insomnia Severity Index (ISI)	2.4 \pm 2.6	14.8 \pm 6.4	< 0.0001
Symptom Checklist 90R – Global Severity Index	0.1 \pm 0.1	1.1 \pm 0.9	< 0.0001
Alcohol Use Disorder Identification Test (AUDIT)	0.9 \pm 1.3	4.6 \pm 6.2	0.04
8-year average of monthly alcohol drinks	10 \pm 14	28 \pm 36	0.04
3-year average of monthly alcohol drinks	15 \pm 31	30 \pm 54	n.s.
1-year average of monthly alcohol drinks	23 \pm 61	19 \pm 35	n.s.
Lifetime average monthly alcohol drinks	15 \pm 23	37 \pm 40	0.04
Lifetime years of regular alcohol drinking ^a	16.0 \pm 11.4	16.7 \pm 11.6	n.s.
Fagerstrom total	2.3 \pm 0.5 (n = 3)	3.0 \pm 1.1 (n = 6)	n.s.
Fagerstrom total # of cigarettes/day	7.5 \pm 5.5 (n = 3)	10.7 \pm 5.0 (n = 6)	n.s.
Fagerstrom life years smoking	20.3 \pm 15.3 (n = 3)	11.7 \pm 6.5 (n = 6)	n.s.

^aRegular drinking defined as > 1 standard alcoholic drink (containing about 13 grams of pure alcohol) per month. *, P = 0.06.

(n = 17, mostly from the wars in Iraq and Afghanistan) and civilians with trauma related to nonmilitary events (n = 10). PTSD- controls were all trauma exposed, but had no meaningful PTSD symptoms (i.e., total CAPS score < 14); 10 of them were veterans and eight were civilians. As expected, PTSD+ had significantly higher CAPS scores than PTSD- (total CAPS: 54.5 \pm 18.6 vs. 3.4 \pm 4.8; arousal: 21.1 \pm 8.0 vs. 1.3 \pm 2.3; avoidance: 19.1 \pm 9.4 vs. 1.4 \pm 3.1; intrusion: 14.3 \pm 6.6 vs. 0.7 \pm 0.8; all P < 0.0001). Of the PTSD+, 14 (52%) were Caucasian (including three Latinos), eight (30%) African American, three (11%) Asian, and one each (4%) Indian and Native American. Of the PTSD-, 11 (62%) were Caucasian (including one Latino), four (22%) Asian, and three (17%) African American. The racial breakdown was not statistically different between the groups.

PTSD+ as a group had a higher AUDIT score than PTSD- (5 vs. 1, P = 0.04), where a score of 8 or higher indicates a strong likelihood of hazardous or harmful alcohol consumption. Based on lifetime drinking history interviews, the PTSD+ group drank moderately at 37 \pm 40 standard alcoholic drinks per mo over lifetime (range 1–181, with only one participant drinking > 100 drinks/mo), whereas the PTSD- group drank significantly less at 15 \pm 23 drinks/mo over lifetime (range 0–100, with no participant drinking > 100 drinks/mo) (P = 0.04). Three (17%) of the PTSD- and six (22%) of the PTSD+ group were cigarette smokers, with FTND total scores between 2 and 5; this indicated a similarly low incidence and degree of nicotine dependence between the two groups. Alcohol drinking and cigarette smoking characteristics as well as some behavioral data of the cohort are summarized in Table 1. PTSD+ had higher measures of depressive and anxiety symptom severities than PTSD-, with group averages in the mild range. PTSD+ also had higher scores on the ISI than PTSD- (14.8 \pm 6.4 vs. 2.4 \pm 2.6, P < 0.0001).

Tissue Contributions and Metabolite Concentrations

The water signal determined from unsuppressed water spectra of the individual VOIs showed no group differences;

this indicates no significant atrophy within the VOIs of PTSD+, consistent with results of the segmentation analyses that showed similar CSF contribution across groups in each VOI (ACC: 18.4 \pm 2.8% in combined groups; POC: 7.8 \pm 2.4%; TEMP: 1.9 \pm 0.1%). Furthermore, white matter (WM) and gray matter (GM) contributions to the VOIs differed by less than 1% between groups (ACC in combined groups: 46.5% \pm 3.7% GM and 34.3 \pm 4.7% WM; POC: 62.6 \pm 3.3% GM and 28.7 \pm 4.3% WM; TEMP: 44.5 \pm 5.3% GM and 52.8 \pm 5.8% WM). Because PTSD+ individuals had a significantly higher AUDIT score and significantly higher average monthly alcohol consumption over lifetime than PTSD-, we used monthly alcoholic drinks over lifetime as a covariate in the general linear model that compared metabolite concentrations between the groups. Age was used as an additional covariate if it explained part of the variance of the model. There were no significant covariate-by-metabolite interactions. Table 2 shows the calculated absolute concentrations of the main metabolites of interest by VOI and group as well as the covariates used in the analyses.

In ACC, NAA was lower in PTSD+ than PTSD- (P = 0.007, uncorrected), but GABA and Glu concentrations were not significantly different between the groups. In the POC, GABA was lower in PTSD+ than PTSD- (P = 0.04, uncorrected), but the groups did not differ significantly on NAA and Glu levels. In the TEMP VOI, GABA was lower (P = 0.04) and Glu higher (P = 0.05, uncorrected) in PTSD+ than PTSD-, with no significant NAA differences between groups. Additional two-tailed *t*-tests showed that neither mI nor Cho or Cr concentrations in any of the VOIs were significantly different between PTSD+ and PTSD- (and are therefore not listed in the table). Furthermore, veterans and civilians did not differ on any of our main metabolite concentration measures.

Metabolite Levels and Self-Reported Sleep Quality

In PTSD+, lower POC GABA related strongly to higher ISI scores (ρ = -0.55, P = 0.008; see Figure 2), whereas TEMP GABA did not (ρ = -0.17, P = 0.20). Given these functional

Table 2—Metabolite concentrations (institutional units, mean \pm standard error) in anterior cingulate cortex, parieto-occipital cortex, and temporal cortex. Statistics used general linear modeling, all P values are two-tailed and uncorrected for multiple comparisons (three volumes of interest). Different models used significant covariates as indicated.

Volume of interest	Metabolite concentration (# of PTSD-, PTSD+)	PTSD-	PTSD+	Effect size/P value	Covariate
ACC	NAA (15, 24)	6.22 \pm 0.24	5.44 \pm 0.17	0.89/0.007	Age, drinks/mo over lifetime
	Glu (15, 24)	4.37 \pm 0.18	4.18 \pm 0.17	0.25/n.s.	None
	GABA (13, 22)	1.11 \pm 0.06	1.13 \pm 0.06	0.08/n.s.	None
POC	NAA (17, 23)	5.58 \pm 0.14	5.67 \pm 0.13	0.15/n.s.	None
	Glu (17, 23)	4.11 \pm 0.11	4.20 \pm 0.12	0.18/n.s.	None
	GABA (17, 22)	1.90 \pm 0.08	1.68 \pm 0.06	0.73/0.04	Age, drinks/mo over lifetime
TEMP	NAA (15, 23)	5.43 \pm 0.14	5.85 \pm 0.26	0.55/n.s.	drinks/mo over lifetime
	Glu (14, 23)	2.94 \pm 0.21	3.50 \pm 0.14	0.77/0.05	Age, drinks/mo over lifetime
	GABA (13, 21)	1.25 \pm 0.05	1.11 \pm 0.05	0.68/0.04	Age, drinks/mo over lifetime

As gamma-aminobutyric acid was obtained by MEGA-PRESS and *N*-acetylaspartate and glutamate by stimulated echo acquisition, their respective concentrations are not on the same scale. Effect size by Cohen *d*. ACC, anterior cingulate cortex; GABA, gamma-aminobutyric acid; Glu, glutamate; NAA, *N*-acetylaspartate; n.s., nonsignificant; POC, parieto-occipital cortex; PTSD, posttraumatic stress disorder; TEMP, temporal cortex. Bold type, statistically significant group differences.

relationships and the aforementioned POC GABA differences, we performed a causal mediation analysis³³ to assess whether the effects of PTSD diagnosis on POC GABA are mediated by insomnia severity. Because depression symptomatology (BDI) across both groups was found to be significantly related to both ISI ($\rho = 0.74$, $P < 0.001$) and POC GABA ($\rho = -0.38$, $P = 0.024$), BDI scores were entered as a covariate in the model. To facilitate interpretation of the mediation effect, the ISI, BDI, and POC GABA values were standardized. The relationship between PTSD diagnosis and POC GABA concentration was found to be fully mediated through insomnia severity (average causal mediation effect = -0.56 , $P < 0.05$; direct effect = -0.06 , $P = 0.90$). Thus, a positive PTSD diagnosis was associated with a decrease in POC GABA of 0.62 z-score units, of which 0.56 z-score units (i.e., 90% of the effect) were mediated through ISI scores.

In the ACC, GABA levels across the combined groups were not correlated with ISI, but they tended to correlate negatively with ISI in PTSD+ only ($\rho = -0.41$, $P = 0.06$). In neither ACC (normal GABA in PTSD+) nor TEMP (low GABA in PTSD+) were the GABA concentrations mediated through ISI scores. Although Glu and Cho levels in the POC were not different as a function of PTSD diagnosis, ISI correlated with POC Glu in PTSD+ only ($\rho = 0.49$, $P = 0.018$) and with POC Cho in both the PTSD+ and combined groups ($\rho > 0.41$, $P < 0.01$). ISI was not correlated with any of the regional NAA levels.

Because POC Glu was also significantly related to ISI, we tested in exploratory analyses the degree to which GABA and/or Glu in any of the VOIs explained variance in ISI (here, a repeated-measures analysis of variance with ISI as the outcome). We found that the addition of Glu to the model did not explain more variance than the model with just GABA and PTSD diagnosis, and that the only significant effects of GABA on ISI were observed in the POC.

Metabolite Levels and Behavioral Measures in PTSD+

In the ACC, lower NAA, Glu, and Cr concentrations correlated with higher arousal scores ($\rho < -0.41$, $P < 0.05$). Across

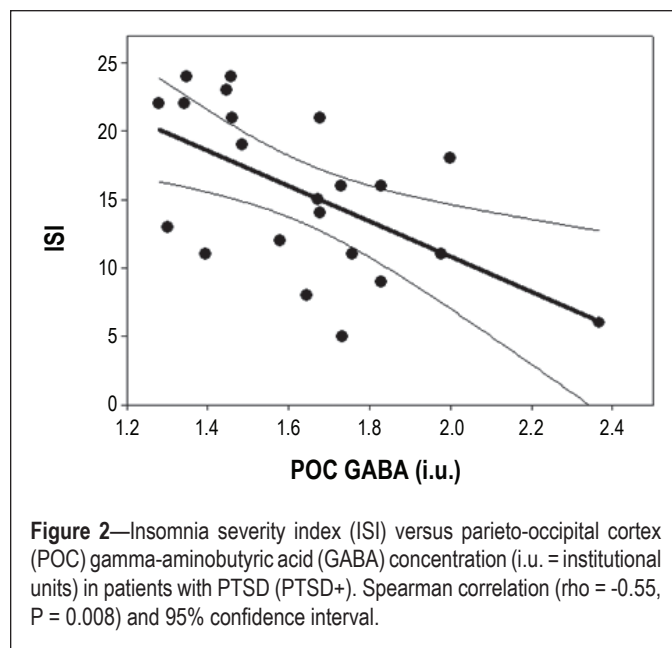


Figure 2—Insomnia severity index (ISI) versus parieto-occipital cortex (POC) gamma-aminobutyric acid (GABA) concentration (i.u. = institutional units) in patients with PTSD (PTSD+). Spearman correlation ($\rho = -0.55$, $P = 0.008$) and 95% confidence interval.

all VOIs of the PTSD+ group, higher mI concentrations correlated with lower intrusion scores (all $\rho < -0.42$, $P < 0.04$). In addition, higher TEMP mI correlated with a lower total CAPS score ($\rho = -0.67$, $P < 0.001$) and most strongly with lower arousal scores ($\rho = -0.54$, $P = 0.006$). CAPS nightmare severity and frequency scores in patients with PTSD were not related to any of the metabolite levels. Also, measures of depressive and anxiety symptom severities and of overall psychological distress (Global Severity Index of the SCL-90) in the PTSD+ group at the day of the magnetic resonance examination did not correlate with the regional metabolite levels.

Neither of the alcohol drinking measures across the combined groups nor the FTND scores of the nine cigarette smokers was related significantly to any of the regional metabolite concentrations. Also, although the PTSD+ group drank more monthly alcoholic drinks over their lifetime than the PTSD- group

(37 ± 40 vs. 15 ± 23 drinks/mo), this modest alcohol consumption did not significantly affect most metabolite group differences except where indicated in Table 2.

Finally, in all VOIs of the PTSD+ group, Glu concentrations correlated strongly and positively with the corresponding NAA (all $\rho > 0.61$, $P < 0.001$) and Cr concentrations (all $\rho > 0.66$, $P < 0.001$). In the POC, lower GABA also correlated with higher Glu ($\rho = -0.41$, $P = 0.05$).

DISCUSSION

This is the first report demonstrating that patients with PTSD compared with trauma-exposed individuals without a PTSD diagnosis have lower GABA concentrations, an *in vivo* measure of inhibitory function, in both POC and TEMP cortices. In TEMP, the GABA reduction was accompanied by a corresponding increase of Glu, a measure of excitatory function. The POC GABA reduction was strongly related to higher ISI scores and could be fully explained by insomnia severity independent of PTSD diagnosis or depressive symptomatology (BDI), reflecting GABA-mediated decrements in sleep quality. Higher ISI scores among patients with PTSD also correlated with higher Glu and Cho concentrations in the POC, but they did not explain insomnia severity. Furthermore, higher mI levels across the different cortices, in particular in the medial temporal lobe, were related to lower total CAPS scores and CAPS sub-scores. Depressive symptoms, alcohol drinking, and cigarette smoking, conditions that are common in PTSD+ patients and have been shown to influence metabolite measures in different neuropathologies when more severe,^{19–23} did not significantly affect the metabolite concentrations in this PTSD sample. Together with the behavioral correlates of our concentration measures, our findings demonstrate significant disturbances of GABA-ergic and Glu-ergic tone in PTSD and point to potential treatment approaches for reducing PTSD symptoms and associated sleep problems.

Our additional findings of lower concentrations of the neuronal marker NAA in the ACC of PTSD+ replicate a previous report from this laboratory¹¹ in a different cohort. In this study, NAA, Glu, and Cr concentrations in the ACC were associated with higher CAPS arousal scores; thus, given that these metabolites reflect neuronal integrity and oxidative phosphorylation,^{34–37} these ACC data suggest significant prefrontal neuronal injury related to hyperarousal in PTSD. Given the role of the ACC in self-monitoring as well as in regulation of emotional and affective tone and behavior,^{38,39} these prefrontal metabolic abnormalities are likely of behavioral significance in trauma-exposed patients with PTSD. NAA levels in the lateral temporal cortices of this sample were not reduced, in contrast to previous reports of NAA reductions in mesial temporal lobe including the hippocampus.^{10,11}

Brain GABA and Glu Levels in PTSD

Long-lasting dysfunction in Glu-ergic and GABA-ergic neurotransmission and related neuronal injury contribute to the clinical symptomatology of stress-related anxiety disorders.^{1,2,7} MRS-derived GABA concentration was found to be reduced in the occipital cortex of patients with panic disorder,⁶ but cerebral GABA reductions and/or Glu elevations, as hypothesized *a priori* for this analysis, have not yet been demonstrated in PTSD. Both GABA and Glu are important for memory registration and

for encoding emotional and fear memory.³ The medial prefrontal cortex is a critical site involved in these processes via its central role in numerous neural mechanisms related to resilience and stress vulnerability.¹ In addition, the observed prefrontal neuronal injury in (suggested by low NAA concentration in the ACC) may affect regulatory processes of memory and fear extinction subserved by temporal structures, which showed elevated Glu and low GABA suggestive of neurotransmitter imbalances.

Low cortical GABA levels in PTSD are consistent with low cortical and subcortical benzodiazepine receptor binding in patients with PTSD and panic disorder, which has been described as a genetic risk factor for the development of PTSD.¹ Whether low cortical GABA reflects a predisposing vulnerability associated with increased PTSD risk or is a consequence of posttraumatic stress and/or sleep problems cannot be answered by these cross-sectional studies. Low cortical GABA, however, does not appear to relate directly to PTSD symptomatology.

Brain GABA and Sleep Quality

Our analyses demonstrate that low GABA in POC (but not in TEMP) of patients with PTSD is mediated through poor sleep quality. This relationship is consistent with the hyperarousal theory in both PTSD⁴⁰ and primary insomnia.⁴¹

Congruent with our findings are two studies from the same laboratory^{14,15} that showed patients with primary insomnia, compared with healthy sleepers, have significantly lower levels of GABA/Cr throughout the brain, lower GABA/Cr in the occipital cortex (a region close to our POC VOI), and lower but still significant reductions in the ACC; before correction for multiple comparisons, lower occipital GABA/Cr correlated significantly with insomnia severity. These findings suggest that the low POC GABA concentration observed in our patient cohort may be related more to insomnia than PTSD diagnosis or traditional PTSD symptomatology; in fact, our mediation analysis is consistent with this finding. A recent study¹³ measured higher GABA/Cr in the occipital cortex of patients with primary insomnia compared with controls, but confirmed decreasing GABA/Cr levels with higher wake after sleep onset on polysomnography and a trend for lower GABA/Cr with poorer sleep quality as assessed by the Pittsburgh Sleep Quality Index. In a commentary,¹⁵ it was suggested that these divergent group differences in primary insomnia may be explained chiefly by variable occipital GABA levels across the day (low in the morning and high in the evening). In our study, almost all GABA measurements were obtained in the late afternoon/early evening.

Study Limitations

Although most brain GABA is intracellular, it is not clear whether the decreased GABA concentrations reflect reduced GABA production or inhibited uptake by an intact population of neurons or a reduction in GABA-ergic cell number. The negative correlations of GABA with NAA and Glu in the POC ($r < -0.41$, $P < 0.05$), the absence of coherent metabolite differences in the POC (where GABA is decreased, but NAA unchanged) and in the ACC (where NAA is decreased, but GABA unchanged) do not suggest GABA-ergic neuron death in PTSD. Furthermore, without specifically comparing patients with PTSD, generalized anxiety disorder, or primary

insomnia on these exact metabolite and sleep measures, it is very difficult to determine unequivocally whether or not our high-field 1H MRS metabolic signatures are specific to PTSD or if they cut across anxiety and sleep disorders in general. Finally, depressive symptom levels may have been too mild and alcohol and tobacco exposure may have been too modest in this PTSD sample to have had significant effects on cortical metabolite levels. Despite these limitations, our results, obtained in a mixed veteran-civilian sample that is relatively low in trauma and PTSD severities, should generalize quite well to large sections of the populations with the disorder.

CONCLUSIONS

Our MRS findings— independent of depressive symptomatology, moderate alcohol drinking, and low smoking prevalence— suggest neuronal injury in the ACC of PTSD+ patients compared to trauma-exposed controls without PTSD symptoms and associated with hyperarousal. Our results confirm and extend previous reports of cortical NAA deficits in PTSD¹¹ and survive correction for multiple comparisons (three VOIs). NAA, Glu, and Cr levels in the ACC, metabolites that are closely tied to cellular oxidative phosphorylation,^{34–37} all are moderately strong related to arousal, suggesting that a general compromise of bioenergetics/metabolism of prefrontal tissue is associated with the hyperarousal commonly seen in PTSD. This interpretation is also consistent with recent findings of lower phosphocreatine in cortical gray matter throughout the brain, including frontal and parieto-occipital cortices.⁴² Currently unreported high Glu in the temporal cortex of PTSD+ patients support our *a priori* hypothesis of abnormalities within excitatory mechanisms, albeit without measurable effect on the neuronal marker NAA or the high-energy metabolite Cr in the same region. Importantly, cortical GABA reductions in the temporal and parieto-occipital cortices (uncorrected) suggest disturbances of GABA-ergic tone in PTSD, perhaps with neurotransmitter involvement, which may have implications for altered fear conditioning, extinction, and memory encoding in PTSD. In the parieto-occipital cortex, low GABA concentration is related to both worse sleep quality and higher depressive symptomatology and is almost entirely mediated through ISI scores. Thus, low GABA in this region does not appear to be a specific biomarker of PTSD but a phenomenon possibly related to sleep disturbance and/or anxiety in general. These new findings may imply that enhancing endogenous GABA levels may be a good strategy to alleviate PTSD sleep problems.

ACKNOWLEDGMENTS

The authors thank Clare Henn-Haase, Charles Marmar, Maryann Lenoci, and all volunteers for their participation in this research.

DISCLOSURE STATEMENT

This was not an industry-supported study. This project was supported by grants from the Department of Defense DAMD17-03-1-0532 (to Dr. Meyerhoff), National Institutes of Health AA10788 (to Dr. Meyerhoff), MH073978 (to Dr. Neylan), MH077667 (to Dr. Neylan), and the Mental Illness Research and Education Clinical Center (MIRECC) of the US Veterans Health Administration. This material is the result of

work supported with resources and the use of facilities at the Veterans Administration Medical Center, San Francisco, California. Dr. Neylan has received study medication from Actelion for a project funded by the Department of Defense and from Glaxo-Smith Kline for a study funded by the Department of Veterans Affairs. The other authors have indicated no financial conflicts of interest.

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