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A preliminary examination of cortical neurotransmitter levels associated with heavy drinking in posttraumatic stress disorder

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ABSTRACT

Posttraumatic stress disorder (PTSD) patients have low cortical concentrations of γ-aminobutyric acid (GABA) and elevated glutamate (Glu) as measured by proton magnetic resonance spectroscopy (^{1}H MRS). Alcohol use disorder (AUD) is highly comorbid with PTSD, but the neurobiological underpinnings are largely unknown. We wanted to determine if PTSD patients with AUD have normalized cortical GABA and Glu levels in addition to metabolite alterations common to AUD. We compared brain metabolite concentrations in 10 PTSD patients with comorbid AUD (PAUD) with concentrtations in 28 PTSD patients without AUD and in 20 trauma-exposed controls (CON) without PTSD symptoms. We measured concentrations of GABA, Glu, *N*-acetylaspartate (NAA), creatine- (Cr) and choline-containing metabolites (Cho), and myo-Inositol (mI) in three cortical brain regions using ^{1}H MRS and correlated them with measures of neurocognition, insomnia, PTSD symptoms, and drinking severity. In contrast to PTSD, PAUD exhibited normal GABA and Glu concentrations in the parieto-occipital and temporal cortices, respectively, but lower Glu and trends toward higher GABA levels in the anterior cingulate cortex (ACC). Temporal NAA and Cho as well as mI in the ACC were lower in PAUD than in both PTSD and CON. Within PAUD, more cortical GABA and Glu correlated with better neurocognition. Heavy drinking in PTSD is associated with partially neutralized neurotransmitter imbalance, but also with neuronal injury commonly observed in AUD.

1. Introduction

Among individuals with posttraumatic stress disorder (PTSD), up to 85% suffer from alcohol use disorders (AUD) (Kessler et al., 1995; Baker et al., 2009; Javidi and Yadollahie, 2012). The co-occurrence of these disorders is associated with worse psychosocial and medical outcomes, higher rates of hospitalization and typical substance use-related problems (McCarthy and Petrakis, 2010). Although the recent biological literature on PTSD and AUD has each grown substantially (Volkow and Li, 2005; Spanagel, 2009; Pitman et al., 2012), little is known about the neurobiological underpinnings associated with *comorbid* PTSD and AUD (PAUD). The purpose of this study is to contrast neuroimaging-based brain metabolite concentrations in PTSD patients with and without AUD.

In vivo proton magnetic resonance spectroscopy (^{1}H MRS) is an invaluable tool for non-invasive quantitation of regional brain metabolite levels related to the neuropathology of a disease. ^{1}H MRS has been used to investigate the deregulation of the glutamate and γ-aminobutyric acid (GABA) pathways posited to be involved in the pathophysiology of PTSD (Hageman et al., 2001). In a recent ^{1}H MRS study comparing PTSD patients with trauma-exposed individuals without PTSD symptoms, we found lower GABA levels in the lateral temporal (TEMP) and parieto-occipital cortices (POC), higher glutamate in TEMP cortex, and lower *N*-acetylaspartate levels (NAA, a marker of neuronal viability) in prefrontal cortex (Meyerhoff et al., 2014).

Other brain metabolites such as myo-inositol (mI), creatine- (Cr), and choline-containing compounds (Cho) serve as intracellular

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markers of membrane abnormalities and high-energy metabolism in psychiatric disorders (Vion-Dury et al., 1994). PTSD brain studies have mainly targeted regions with functional (Shin et al., 2001; Shin et al., 2004) and structural abnormalities (Pitman et al., 2012), namely the hippocampus and anterior cingulate cortex (ACC). A meta-analysis of 16 ^{1}H MRS studies that compared PTSD patients with healthy controls (Karl and Werner, 2010) revealed lower left and right hippocampal NAA measures (both NAA relative to Cr and absolute NAA concentration), reduced NAA concentration in the ACC, and higher left hippocampal Cho/Cr. These abnormalities indicate neuronal injury and membrane alterations in regions of the brain associated with memory encoding, fear extinction, and emotional control (Hamner et al., 1999).

Brain metabolite concentrations are also altered in individuals with AUD, primarily in the frontal lobes (Sullivan, 2000; Meyerhoff et al., 2004; Durazzo and Meyerhoff, 2007; Buhler and Mann, 2011; Mon et al., 2012). Using ^{1}H MRS methods identical to those employed in this study, we showed (Mon et al., 2012) lower concentrations of Glu, NAA, and Cr in the ACC of recently detoxified alcohol-dependent individuals compared with non-drinking or light-drinking controls, and normal ACC GABA and mI concentrations; however, metabolite levels in the dorsolateral prefrontal cortex and POC were not abnormal in these alcohol-dependent individuals (Mon et al., 2012).

One ^{1}H MRS study of PTSD investigated the effects of alcohol consumption on brain metabolite concentrations (Schuff et al., 2008). Both PTSD patients with little or no alcohol consumption and PTSD patients with a history of alcohol abuse within the 5 preceding years had low NAA/Cr in the ACC and mesial temporal lobe including the hippocampus. Given that we detected NAA deficits only in heavy drinkers who consumed at least 90 standard alcoholic drinks per month for extended periods (Meyerhoff et al., 2004), this was not necessarily surprising: The alcohol-drinking PTSD patients of the study of Schuff et al. consumed <20 standard alcoholic drinks/month averaged over 5 years and only 34 drinks the month before the study. Such an amount of alcohol consumption is far below what is considered “at risk” or “heavy” drinking according to NIH/NIAAA guidelines (Willenbring et al., 2009).

Therefore, to our knowledge, no research has investigated the effects of heavy drinking on brain metabolite concentrations in PTSD patients with a current AUD diagnosis. This high comorbidity exists, at least in part, because alcohol use may be an attempt to “self-medicate” and/or respond to symptoms such as insomnia, anxiety, and hyperarousal (Leeies et al., 2010; Ouimette et al., 2010). Therefore, we hypothesized that the cortical neurotransmitter imbalances we described in PTSD patients without AUD (Meyerhoff et al., 2014) are attenuated in PTSD patients with AUD. Specifically, we hypothesized that GABA and Glu concentrations would be less abnormal in our comorbid sample than in patients with PTSD only. Additionally, we expected that cortical NAA, typically reduced in individuals with AUD, would also be reduced in patients with comorbid PTSD and AUD (PAUD) compared to both PTSD patients and trauma-exposed controls without AUD (CON). We also explored the degree to which the regional cortical metabolite levels reflected neurocognitive function, PTSD symptoms, and sleep quality.

2. Methods

2.1. Participants

All participants voluntarily provided written informed consent before the study, which 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. All PTSD, PAUD, and non-PTSD (CON) individuals were either trauma-exposed American veterans of war or trauma-exposed civilians recruited at the San Francisco VA Medical Center, from among Northern California United States Army reservists, Army National Guard, or the Mental Health Service of the San Francisco and Fresno VA, regional Veteran Centers and mental health clinics. Exclusion criteria were a history of schizophrenia or schizoaffective disorder, past and current AUD (CON only), AUD and substance use disorder within the past 6 months (PTSD only), suicidal intention, or bipolar disorder as assessed by the Structured Clinical Interview for DSM-IV (First et al., 1998). Medical exclusion criteria included pregnancy, seizure disorders, head injury associated with post-injury memory loss for >24 h or loss of consciousness >10 min, history of stroke or neurodegenerative diseases, HIV infection, or medical instability. Participants were excluded if they were prescribed psychiatric medications or hypnotics within 2 weeks before magnetic resonance imaging (MRI), had any kind of metallic implants, lodged foreign objects, other contraindications for MRI, or likely traumatic reactions to MR scanner noise.

2.2. Clinical assessment

All participants completed a structured clinical interview to yield basic demographic information. PTSD diagnosis and symptom severity were measured with the Clinician-Administered PTSD Checklist (CAPS; Blake et al., 1995), a 30-item structured interview based on the DSM-IV. The CAPS instrument is divided into sections based on typical symptom clusters: Exposure to a traumatic event; Re-experiencing; Numbing and avoidance; Hyper-arousal; Chronology; and Functional impairment. A criterion was considered present if a participant endorsed a symptom with a score ≥ 1 in frequency and ≥ 2 in severity rating. Insomnia was assessed with the Insomnia Severity Index (ISI; Bastien et al., 2001), a valid and reliable self-report measure of perceived insomnia severity. Harmful and hazardous drinking was assessed using the Alcohol Use Disorder Identification Test (Saunders et al., 1993). Alcohol consumption was assessed using the Time Line Follow Back (Sobell and Sobell, 1992) interview, which yielded average drinks consumed over 90 days before the MRI study. To assess the influence of self-reported depressive and anxiety symptoms on regional metabolite levels, we administered the Beck Depression Inventory-II (Beck, 1978) and Beck Anxiety Inventory (Beck et al., 1988) on the day of the MRI examination.

2.3. Neurocognitive assessment

Within 3 days before the MRI study, PAUD participants completed a neurocognitive battery consisting of the following: Trail Making Test A and B (Reitan and Wolfson, 1985), a measure of processing speed and divided attention, Hopkins Verbal Learning Test-Revised (Brandt, 1991), including total recall and delayed recall which measure auditory-verbal learning and memory, and the Balloon Analogue Risk Task (Lejuez et al., 2002), a task-based measure of risk taking. Neither CON nor PTSD participants underwent neuropsychological testing.

2.4. MRI acquisition and processing

MR data were acquired on a 4-Tesla Bruker MedSpec system with a Siemens Trio console (Siemens, Erlangen, Germany) using an eight-channel transmit-receive head coil. Three-dimensional sagittal T1-weighted and 2D axial T2-weighted images were acquired using Magnetization Prepared Rapid Gradient imaging ($1\times1\times1$ mm^3 resolution) and turbo spin-echo ($0.9\times0.9\times3$ mm^3 resolution) sequences, respectively. ^{1}H MRS evaluated 3 volumes of interest (VOIs) known to be associated with PTSD and AUD, the ACC, TEMP and POC. These VOIs were evaluated because the ACC is metabolically abnormal in PTSD (Karl and Werner, 2010) and critically involved in the development and maintenance of all forms of addictive disorders (e.g., Goldstein et al., 2009; Volkow et al., 2012). The TEMP is functionally connected to the hippocampus, and together they contribute to the mesial temporal lobe memory system in humans (Kahn et al., 2008) associated with PTSD (Hamner et al., 1999). The POC has been targeted traditionally in ^{1}H MRS studies to measure levels of the inhibitory neurotransmitter GABA in various populations, and this general brain region has been recently implicated in altered neural activity in PTSD (Sripada et al., 2012; Chen and Etkin, 2013). MRS VOIs were placed over the ACC ($35\times25\times20$ mm^3), POC ($20\times40\times20$ mm^3) and right TEMP ($20\times40\times20$ mm^3), maximizing gray matter content as displayed on the structural MR images. Fig. 1 (top) shows typical VOI locations on T2-weighted MR images, midline for ACC and POC, and always patient right for TEMP. NAA, Cr, Cho, mI and Glu signals were acquired at 12-ms echo time with a Stimulated Echo Acquisition Mode sequence (Frahm et al., 1987). Immediately afterwards, a reference water signal was collected from the same VOI with the same Stimulated Echo Acquisition Mode sequence but without water suppression and used for normalizing all metabolite peak areas across participants. Signals from GABA were acquired from the same VOIs with a J-editing sequence modified for optimal GABA signal-to-noise and improved suppression of water and macromolecular signal (Kaiser et al., 2008). MR images were segmented into gray matter, white matter, and cerebrospinal fluid (Van Leemput et al., 1999) to estimate tissue fraction and cerebrospinal fluid contributions to each VOI. Metabolite and J-edited spectra were processed by operators blind to participant diagnosis to yield metabolite levels in institutional units as peak area ratios relative to the unsuppressed voxel tissue water

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(i.e., not corrected for relaxation times). A full description of the spectral processing and metabolite quantitation methods can be found elsewhere (Mon et al., 2012). The metabolite spectra yielded concentrations for NAA, Cr, Cho, mI and Glu, whereas GABA concentrations were derived from the J-edited spectra as described. Example spectra are given in Fig. 1 (bottom). Mostly due to time constraints, not all participants had spectral data acquired from all three VOIs, so that after data processing and rigorous quality control (Meyerhoff et al., 2014), the number of participants contributing to quantitative MRS data varied by group and VOI as indicated in Table 2.

2.5. Statistical analyses

Separate univariate analyses of covariance were performed for three VOIs and six metabolites (NAA, Cr, Cho, mI, Glu, GABA). Follow-up planned pairwise comparisons tested for group differences in metabolite concentrations among PAUD, PTSD, and CON. Each three-group-comparison was covaried for age and gray matter-tissue contribution to the VOI, as differences in these variables can affect metabolite levels (e.g., Schuff et al., 2001; Jansen et al., 2006). We left age and/or tissue contribution in the final model only when they predicted significant group differences. Analyses of covariance were also used to test for differences in participant characteristics. In pairwise group comparisons of metabolite levels, we accounted for the multiplicity of metabolite measures in each VOI by correcting alpha levels via a modified Bonferroni procedure (Sankoh et al., 1997). This approach yields adjusted alpha levels for each VOI separately using the number of metabolites under investigation (six) and their average inter-correlation coefficients (ACC: $r=0.35$, POC: $r=0.32$, TEMP: $r=0.26$); the corresponding adjusted alpha levels for pairwise group comparisons were 0.014 for ACC, 0.013 for POC and 0.012 for TEMP. Effect sizes were calculated via Cohen's d (Cohen, 1988). In PAUD, we correlated VOI-specific metabolite concentrations with the raw scores of our neurocognitive measures using Spearman's rho, and in both PTSD groups we also related metabolite concentrations to ISI and CAPS scores (p-values uncorrected). All analyses were completed with SPSS v20.

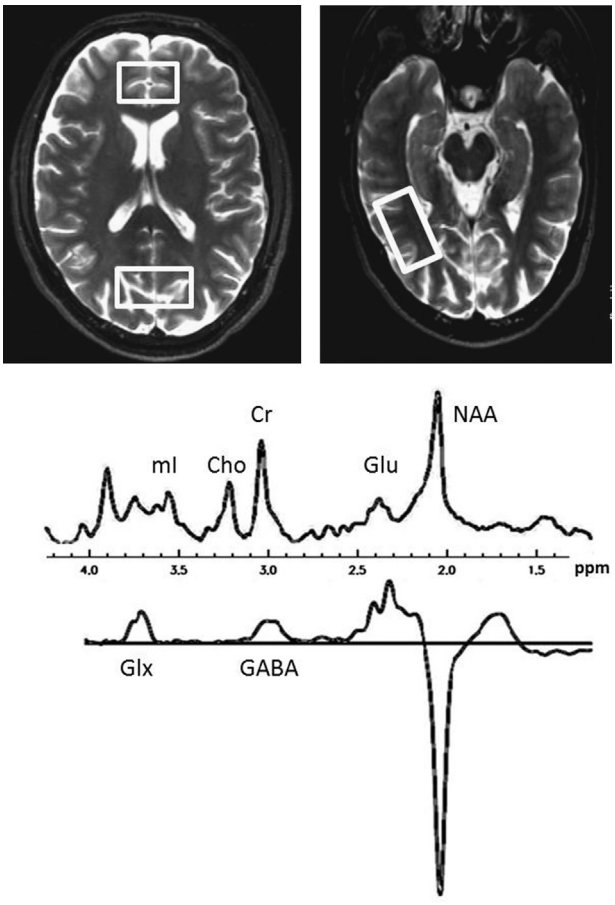

Fig. 1. Locations of volumes of interest on T2-weighted magnetic resonance Images (left: ACC and POC; right: TEMP). Examples of analyzed stimulated echo acquisition mode spectrum (top) and J-edited spectrum (bottom) from the POC. Spectra shown on different vertical scales for clarity. Major metabolite signals are labeled. The N-acetylaspartate (NAA) signal is fit seprately from the neighboring N-acetylaspartylglutamate signal.

3. Results

3.1. Participant characteristics

Characteristics of the PAUD, PTSD, and CON groups are shown in Table 1. PAUD participants were older than both CON and PTSD participants, who were of similar age. Nine of the 10 PAUD participants were Caucasian, including one Latino, and one African American. The group of 28 PTSD patients comprised 14 Caucasians (50%), including three Latinos, eight African Americans (29%), three Asians (11%), two Native Americans (7%), and one Indian (3%). Of the 19 CON participants, 10 were Caucasians (53%), including one Latino, six Asian Americans (32%), two African Americans (11%), and one Pacific Islander (5%). All PAUD and PTSD were veterans of foreign wars in Vietnam, the Gulf Wars, and wars in Iraq and Afghanistan with war-zone and/or civilian related trauma exposure. CON participants (including 10 veterans) were all exposed to non-military trauma, but had no meaningful PTSD symptoms (i.e., total CAPS score <14). PAUD participants had higher CAPS scores reflecting greater non-specific PTSD symptom severity than the PTSD group, but similar arousal scores. Both PTSD groups had significantly higher depressive symptoms on the Beck Depression Inventory and anxiety symptoms on the Beck Anxiety Inventory than CON, with PAUD having higher Beck Depression Inventory and Beck Anxiety Inventory scores than PTSD. CON did not differ from PTSD on any drinking variables, but – by design – the PAUD group consumed more standard alcoholic drinks over the last 90, 30 and 7 days before study than either the CON or PTSD group.

3.2. Three-group comparison of regional metabolite concentrations

Univariate tests were significant for group differences in the ACC: NAA ($p=0.048$), Cho ($p=0.008$), mI ($p<0.001$), Glu ($p=0.001$), and GABA ($p=0.046$); in the TEMP: NAA ($p<0.001$), Cho ($p=0.040$), and Glu ($p=0.006$); and in the POC: GABA ($p=0.050$). Table 2 shows mean metabolite concentrations by VOI and group, pairwise group statistics, and effect sizes.

In planned pairwise comparisons, PAUD showed normal GABA and Glu levels in both POC and TEMP. This was in contrast to PTSD, who had higher Glu in TEMP ($p=0.009$) and a trend toward lower GABA ($p=0.026$) in the POC compared with CON. Thus, TEMP Glu was also significantly lower in PAUD than PTSD ($p=0.009$). In the ACC, PAUD had lower Glu ($p\leq 0.001$) and tended to have higher GABA levels than both PTSD and CON ($p\leq 0.027$), whereas PTSD had normal Glu and GABA levels in the ACC.

In PAUD, TEMP NAA concentration was lower than in PTSD and CON ($p\leq 0.001$) and ACC NAA levels tended to be lower compared with CON levels ($p=0.024$). In addition, concentrations of mI and Cho in the ACC were much lower in PAUD than in both CON and PTSD (all $p\leq 0.005$), whereas PTSD tended to have only lower than normal NAA in the ACC ($p=0.059$). Similarly, Cho and mI tended to be lower in the TEMP of PAUD compared with both PTSD and CON ($p<0.092$). Effect sizes for all significant group differences were strong (effect sizes $=0.91$–2.13), in particular in the ACC. The total CAPS, Beck Depression Inventory, and Beck Anxiety Inventory scores, which were significantly higher in PAUD than PTSD, did not contribute significantly to the described regional metabolite group differences.

3.3. Correlations among main outcome measures within PAUD

3.3.1. Metabolite concentrations and neurocognition (See Table 3)

Within the 10 PAUD participants, ACC Glu was strongly related to divided attention (Trail Making Test-B: $r=0.73$, $p=0.025$) and GABA to auditory-verbal learning/memory (Hopkins Verbal

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Table 1
Patient characteristics (mean ± standard deviation).

Variable	PAUD	PTSD	CON	PAUD vs. PTSD *p*-value	PAUD vs. CON *p*-value	PTSD vs. CON *p*-value
***n* (all male)**	**10**	28	20	–	–	–
Age [years]	51.9 ± 13.9	35.4 ± 10.5	36.3 ± 12.4	< 0.001	0.001	NS
Education [years]	14.6 ± 2.1	15.1 ± 2.4	15.8 ± 2.2	NS	NS	NS
Smoker *n* (%)	5 (50)	8 (29)	5 (25)	–	–	–
Insomnia Severity Index	16.7 ± 7.3	14.8 ± 6.4	2.5 ± 2.6	NS	< 0.001	< 0.001
Beck depression Inventory	23.7 ± 10.5	14.4 ± 10.1	1.1 ± 2.0	0.009	< 0.001	< 0.001
Beck Anxiety Inventory	21.3 ± 12.9	12.8 ± 13.3	1.1 ± 2.9	0.057	< 0.001	0.003
AUDIT Score	25.1 ± 9.3	4.6 ± 6.2	0.9 ± 1.3	< 0.001	< 0.001	NS
Total# of alcoholic drinks[a]						
last week	39 ± 34	4 ± 8	2 ± 4	< 0.001	< 0.001	NS
last 30 days	169 ± 81	17 ± 34	10 ± 16	< 0.001	< 0.001	NS
last 90 days	588 ± 291	50 ± 101	29 ± 47	< 0.001	< 0.001	NS
CAPS Total	78.6 ± 17.9	55.2 ± 18.3	2.7 ± 4.5	0.000	< 0.001	< 0.001
Intrusion	19.5 ± 7.4	14.8 ± 6.6	0.5 ± 1.6	0.036	< 0.001	< 0.001
Avoidance	34.0 ± 6.2	19.3 ± 9.2	1.1 ± 2.8	< 0.001	< 0.001	< 0.001
Arousal	25.1 ± 6.6	21.0 ± 7.8	1.1 ± 2.1	NS	< 0.001	< 0.001
ACC GM-tissue (% of VOI)[b]	49 ± 3	45 ± 4	48 ± 3	0.009	NS	0.013
POC GM-tissue (% of VOI)[b]	64 ± 3	62 ± 3	63 ± 4	NS	NS	NS
TEM GM-tissue (% of VOI)[b]	52 ± 7	44 ± 6	45 ± 5	0.001	0.003	NS

[a] Standard alcoholic drink defined as containing 13.6 g of pure alcohol.
[b] Volume of interest.

Table 2
Mean and standard deviation of metabolite concentrations (institutional units) by group and volume of interest.

Region	Metabolite	PAUD (*n*)	PTSD (*n*)	CON (*n*)	PAUD vs. PTSD *p*-value (ES)	PAUD vs. CON *p*-value (ES)	PTSD vs. CON *p*-value (ES)
ACC	NAA	5.08 ± 0.89 (10)	5.39 ± 0.83 (23)	5.92 ± 0.81 (14)	NS (0.36)	0.024[a] (0.99)	0.059[a] (0.64)
	Cr	3.72 ± 0.80 (8)	4.08 ± 0.80 (24)	4.44 ± 0.80 (14)	NS (0.45)	0.049[a] (0.90)	NS (0.45)
	Cho	1.03 ± 0.25 (9)	1.32 ± 0.25 (24)	1.34 ± 0.25 (14)	0.004 (1.16)	0.005 (1.25)	NS (0.08)
	mI	2.68 ± 0.66 (9)	4.08 ± 0.66 (24)	3.93 ± 0.66 (14)	< 0.001 (2.13)	< 0.001 (1.90)	NS (0.23)
	Glu	3.06 ± 0.78 (9)	4.13 ± 0.78 (24)	4.38 ± 0.78 (14)	0.001 (1.38)	< 0.001 (1.70)	NS (0.32)
	GABA	1.43 ± 0.32 (9)	1.14 ± 0.31 (22)	1.10 ± 0.32 (12)	0.027[a] (0.92)	0.023[a] (1.05)	NS (0.13)
POC	NAA	5.50 ± 0.59 (10)	5.67 ± 0.59 (24)	5.64 ± 0.59 (16)	NS (0.29)	NS (0.24)	NS (0.05)
	Cr	4.46 ± 0.60 (10)	4.38 ± 0.56 (24)	4.28 ± 0.54 (16)	NS (0.14)	NS (0.32)	NS (0.18)
	Cho	0.82 ± 0.11 (10)	0.78 ± 0.11 (24)	0.75 ± 0.11 (16)	NS (0.38)	NS (0.65)	NS (0.27)
	mI	3.00 ± 0.58 (10)	3.34 ± 0.57 (24)	3.18 ± 0.58 (16)	NS (0.59)	NS (0.31)	NS (0.28)
	Glu	3.92 ± 0.50 (10)	4.17 ± 0.50 (24)	4.17 ± 0.50 (16)	NS (0.50)	NS (0.50)	NS (0.00)
	GABA	1.88 ± 0.31 (10)	1.67 ± 0.31 (23)	1.90 ± 0.31 (16)	NS (0.68)	NS (0.06)	0.026[a] (0.75)
TEMP	NAA	4.58 ± 0.66 (10)	5.65 ± 0.66 (23)	5.52 ± 0.66 (14)	< 0.001 (1.62)	0.001 (1.42)	NS (0.20)
	Cr	3.53 ± 0.71 (10)	3.86 ± 0.70 (23)	3.71 ± 0.71 (14)	NS (0.47)	NS (0.25)	NS (0.21)
	Cho	0.81 ± 0.15 (10)	0.96 ± 0.15 (23)	0.94 ± 0.15 (14)	0.014[a] (0.97)	0.058[a] (0.84)	NS (0.13)
	mI	2.69 ± 0.59 (10)	3.09 ± 0.59 (23)	3.12 ± 0.59 (14)	0.082[a] (0.68)	0.092[a] (0.73)	NS (0.05)
	Glu	2.56 ± 0.83 (10)	3.43 ± 0.77 (23)	2.73 ± 0.76 (14)	0.009 (1.08)	NS (0.21)	0.009 (0.91)
	GABA	1.23 ± 0.23 (6)	1.10 ± 0.22 (22)	1.22 ± 0.22 (12)	NS (0.58)	NS (0.04)	NS (0.55)

[a] Trend ($p < 0.10$) after adjusting alpha levels to 0.014 for ACC, 0.013 for POC, and 0.012 for TEMP.

Learning Test-Revised-Total Recall: $r=0.69$, $p=0.040$; Hopkins Verbal Learning Test-Revised-Delay Recall: $r=0.89$, $p=0.002$). ACC Cho was negatively associated with auditory-verbal memory (Hopkins Verbal Learning Test-Revised-Delay Recall: $r=-0.89$, $p=0.002$). In the TEMP of PAUD, GABA was positively associated with processing speed (Trail Making Test-A: $r=0.87$, $p=0.019$). Brain metabolite concentrations did not significantly correlate with measures of risk-taking (Balloon Analog Risk Task) in this small group.

Table 3
Significant ($p < 0.04$) correlations (r) between metabolite concentrations and neurocognition in PAUD.

Region	Metabolite	Neurocognitive domain			
		Auditory-verbal learning	Auditory-verbal memory	Divided-attention	Processing speed
ACC	Cho	−0.89			
	Glu			0.73	
	GABA	0.69	0.89		
TEMP	GABA				0.87

3.3.2. *Metabolite concentrations and PTSD symptomatology, sleep and drinking measures*

Within PAUD participants, there were no significant associations of ACC, POC, and TEMP metabolite levels with CAPS measures or the ISI score. However, in the larger PTSD group, lower TEMP Cho levels had a moderately strong relationship to high CAPS total scores ($r=-0.64$, $p=0.001$) and to high arousal scores ($r=-0.49$, $p=0.017$). Similarly, low Glu in the ACC related to high CAPS total ($r=-0.41$, $p=0.048$) and arousal scores ($r=-0.59$, $p=0.002$). High arousal scores also correlated moderately strong with lower NAA ($r=-0.43$, $p=0.040$) and Cr ($r=-0.48$, $p=0.018$) in the ACC. High

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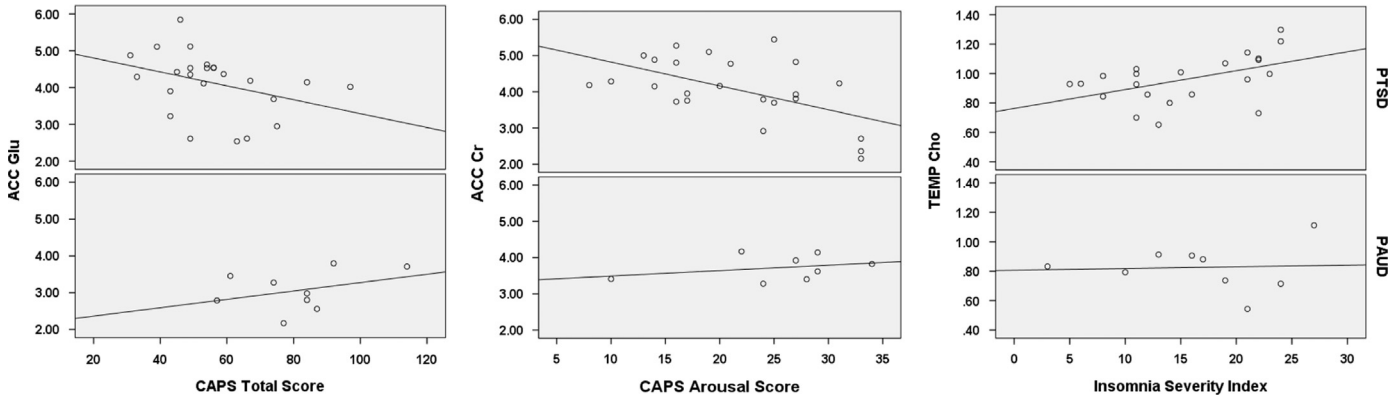

Fig. 2. Examples of different relationships between metabolite concentrations, PTSD symptomology and sleep problems by PTSD group.

intrusion scores related to low mI in the ACC ($r=-0.60$, $p=0.002$) and POC ($r=-0.49$, $p=0.015$) as well as low POC Cr ($r=-0.43$, $p=0.034$) in PTSD. Whereas in the smaller PAUD group none of the regional metabolite concentrations correlated significantly with ISI, PTSD exhibited moderately strong positive correlations between ISI and POC Glu ($r=0.49$, $p=0.018$) and Cho ($r=0.54$, $p=0.007$), as well as a negative association of ISI with POC GABA ($r=-0.55$, $p=0.008$). TEMP Cho in the PTSD group was also positively associated with ISI ($r=0.50$, $p=0.018$). Examples of these relationships are illustrated in Fig. 2. Self-reported alcohol consumption in the PAUD group over 90 days before the study did not correlate significantly with any of the regional metabolite concentrations, PTSD symptom measures, or ISI.

4. Discussion

We used high-field ^{1}H MRS to compare brain metabolite concentrations in frontal, parietal, and temporal cortices of PTSD patients with and without alcohol use disorder. PTSD patients showed considerable metabolic variability in the ACC and TEMP as a function of AUD diagnosis. As hypothesized, we found normal GABA and Glu concentrations in the TEMP and POC of PTSD patients with AUD (PAUD), metabolite levels that were previously shown to be lower (GABA) and higher (Glu in TEMP) in PTSD patients without AUD (Meyerhoff et al., 2014). Furthermore, PAUD had lower Glu and tended to have higher GABA levels in the ACC than both PTSD and CON, whereas PTSD did not differ from CON on these prefrontal measures. In PAUD, higher TEMP GABA and higher ACC GABA and Glu levels were related to better neurocognitive performance. In total, these findings demonstrate significant effects of comorbid AUD on cortical GABA and Glu levels in PTSD. Importantly, these findings suggest that this PAUD population may be consuming alcohol in an attempt to regulate PTSD-associated glutamatergic and GABAergic deficits throughout the lateral cortices (POC and TEMP), thereby inadvertently damaging these systems in medial prefrontal cortex (ACC) and promoting neuronal injury.

In addition to these neurotransmitter alterations, the PAUD group demonstrated dramatically lower NAA concentrations in the TEMP as well as lower Cho and mI concentrations in the ACC and TEMP compared with both the PTSD and CON groups. The PTSD group, on the other hand, was indistinguishable from CON on these same measures. These results indicate that PAUD have metabolite alterations that are associated with their AUD diagnosis (i.e., they are above and beyond those abnormalities related to PTSD alone), but that are not related to quantitive estimates of alcohol consumption. Since we did not include a matched AUD group without PTSD in this analysis, we do not know if these differences of moderate to strong effect size are greater in comorbid PAUD than in AUD alone. However, an AUD population without PTSD, which we earlier studied with ^{1}H MRS (Meyerhoff et al., 2004) and which had consumed similar amounts of alcohol to our PAUD group, did not exhibit measurable frontal or temporal gray matter NAA reductions. Taken together, this suggests greater metabolic injury in PTSD participants with comorbid AUD than in individuals with AUD alone.

Within PAUD, higher GABA in the ACC correlated with better performance in auditory-verbal learning and memory, while high GABA in the TEMP was equally beneficial to processing speed. Although higher Glu in the ACC was related to better performance on a task of divided attention in PAUD, this group exhibited lower ACC Glu levels than both CON and PTSD. This pattern, along with our findings above, suggests that chronic drinking in PTSD is associated with better cortical GABAergic function but worse glutamatergic abnormalities related to cognitive performance typically associated with PTSD (Golier and Yehuda, 2002). As we did not test neurocognition in PTSD or CON, a direct comparison between participant groups could not be made.

Interestingly, PTSD symptoms and sleep quality in PAUD were not strongly related to metabolite concentrations, whereas both were significantly associated with metabolite concentrations in the PTSD group (see Fig. 2). Inasmuch as different group sizes (10 PAUD and 28 PTSD participants) were not the main reason for these different associations, the observation suggests that a comorbid AUD diagnosis modulates these relationships, consistent with our *a priori* hypothesis. Specifically, this different correlation pattern across both PTSD groups suggests that drinking in PTSD may positively influence sleep quality via normalizing GABA and Glu levels in the POC. On the other hand, as lower concentrations of NAA, Glu, and Cr in the ACC of PTSD were robustly associated with higher PTSD symptom scores, the corresponding metabolite reductions seen in PAUD likely did not serve to alleviate PTSD symptoms overall. To the contrary, PAUD had generally greater PTSD, depression, and anxiety severities than PTSD in addition to similar ISI scores. Although AUD may partially modulate PTSD symptoms, the associated level of drinking is not related to any overall symptom relief. This suggests a complex relationship between an AUD diagnosis and PTSD symptoms that is modulated by other factors not examined in this study.

As chronic drinking in PTSD appears to be associated with neutralized parieto-occipital and temporal cortical neurotransmitter levels but also with more severe PTSD symptoms, our findings only partly support the theory that individuals use psychoactive substances to successfully cope with psychiatric distress (Hall and Queener, 2007). Glutamatergic and GABAergic pathways are involved in the mechanism for encoding memory, and they are likely affected by extreme stress related to trauma (Hageman et al.,

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2001). Although still unclear, the downregulation of the inhibitory GABA system is likely mediated by the experience of trauma, which also implies excessive activation of the excitatory glutamate system, a pattern reflected in metabolite levels measured in the TEMP of PTSD patients (Meyerhoff et al., 2014). Here, we showed that inasmuch as the measured static metabolite concentrations reflect corresponding metabolic processes, glutamatergic and GABAergic processes in PAUD were attenuated in two of the three cortical brain regions examined. Although this study links the presence of AUD to altered inhibitory and excitatory processes in PTSD, we cannot assume this link to be causal. The PAUD participants investigated here could simply share a greater common liability to developing both disorders (Berenz and Coffey, 2012) or AUD may have been present before the defining traumatic event.

4.1. Study limitations

The presented comparisons of PTSD and PAUD groups were retrospective and the data were obtained for two different projects without an original intent to compare the groups. Therefore, we did not have data on the onset of AUD in PAUD. However, our analyses were directed by *a priori* hypotheses based on previous reports, and our group comparisons were valid, as data acquisition and processing methodologies were identical and most of the data for the two projects were acquired contemporaneously. Since the PAUD group was small, probing for significant associations between outcome measures was probably underpowered. However, we did observe rather large effect sizes in group comparisons; this should be considered even when the comparisons did not meet statistical significance after controlling for multiple comparisons. Additionally, we did not obtain cognitive data in our CON or PTSD groups to illuminate further the functional relevance of metabolite concentrations. Nevertheless, our analyses underscore clear metabolic and symptomatic differences between PTSD patients with and without AUD.

Given the high prevalence of PTSD and AUD in recently returning veterans (Hoge et al., 2004; Seal et al., 2011), there is an urgent need to improve the treatment approaches to these co-occurring disorders. However, there is a lack of consensus on the optimal use of medications for treating these comorbid conditions (McCarthy and Petrakis, 2010). Given our novel findings of cortical GABA and Glu differences between PTSD patients with and without AUD and differential associations with cognition and various diseases symptoms, our findings need to be confirmed in larger samples. Although any conclusions must be speculative at this time, further supporting evidence for group differences of neurotransmitter levels would obviate the need for advancing targeted treatment approaches for PAUD that are different from those traditionally used to treat PTSD or AUD. A better understanding of the GABAergic and glutamatergic processes in PAUD could inform future pharmacotherapy and behavioral intervention studies, thus enhancing specialized treatment of PAUD.

4.2. Conclusions

Heavy drinking in PTSD is associated with normal GABA and Glu levels in the POC and TEMP, levels which are abnormal in non-drinking PTSD patients. Several regional metabolite levels associated with drinking in PTSD were altered in such a way as to favor better sleep; however, other metabolite levels in PAUD, in particular in the ACC, served to worsen PTSD symptoms or sleep quality. Thus, our data overall can only be interpreted to partly support the self-medication hypothesis in anxiety disorders. Equally as important, PTSD patients with AUD have metabolic abnormalities that are consistent with neuronal, specifically glutamatergic, injury in prefrontal and temporal cortical gray matter not seen in PTSD patients without AUD. The significant abnormalities in the ACC may have implications for self-monitoring as well as regulation of emotional and affective tone and behavior, which is highly relevant to both PTSD and alcohol misuse (Bush et al., 2000; Bush et al., 2002). These prefrontal alterations may affect fear conditioning, extinction, and memory encoding in PTSD, which are subserved by temporal brain structures that also show metabolite abnormalities. Altogether, these differences may relate to the more severe PTSD, depression, and anxiety symptoms of the PTSD patients with AUD in this study. If futher substantiated, the observed metabolic group differences suggest, that along with their relationships to neurocognition, PTSD and insomnia symptoms, different treatment strategies – both pharmacological and behavioral – should be considered for PTSD patients with and without a comorbid AUD diagnosis.

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