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Prefrontal GABA and glutathione imbalance in posttraumatic stress disorder: Preliminary findings

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ABSTRACT

Although posttraumatic stress disorder (PTSD) is associated with a variety of structural and functional brain changes, the molecular pathophysiological mechanisms underlying these macroscopic alterations are unknown. Recent studies support the existence of an altered excitation–inhibition balance in PTSD. Further, there is preliminary evidence from blood-sample studies suggesting heightened oxidative stress in PTSD, potentially leading to neural damage through excessive brain levels of free radicals. In this study we investigated PTSD ($n=12$) and non-PTSD participants ($n=17$) using single-voxel proton magnetic resonance spectroscopy (MRS) in dorsolateral prefrontal cortex (DLPFC) and anterior cingulate cortex (ACC). We found significantly higher levels of γ-amino butyric acid (GABA) (a primary inhibitory neurotransmitter) and glutathione (a marker for neuronal oxidative stress) in PTSD participants. Atypically high prefrontal inhibition as well as oxidative stress may be involved in the pathogenesis of PTSD.

1. Introduction

Posttraumatic stress disorder (PTSD), a serious psychiatric condition that can develop in the aftermath of a traumatic event, has substantial impact on both quality of life and physical health status (Zatzick et al., 1997). Hallmark symptoms of PTSD are persistent re-experiencing of traumatic memories (e.g., nightmares or flashbacks), avoidance of stimuli reminiscent to the traumatic event (e.g., avoiding activities that arouse recollections of the trauma), negative cognitions and mood (e.g., excessive self-blaming and shame) and heightened arousal (e.g., sleep disturbances, irritability, concentration problems, hyper-vigilance, and exaggerated startle response) (American Psychiatric Association, 2013). There is compelling evidence from neuroimaging studies showing structural and functional brain alterations in PTSD in dorsolateral and ventrolateral prefrontal cortex, anterior cingulate cortex (ACC), hippocampus, amygdala, and insula (Karl et al., 2006; Bremner et al., 2008; Robinson and Shergill, 2011; Pitman et al., 2012). Even so, the pathogenesis of these structural and functional abnormalities at a molecular level, and their relationship to traumatic stress, are far from clear (Pitman et al., 2012).

A growing body of evidence suggests that γ-amino butyric acid (GABA) is involved in the conditioning and extinction of fear (Bermudo-Soriano et al., 2012; Harvey and Shahid, 2012; Steckler and Risbrough, 2012). Given the prominent role of fear conditioning and extinction in theoretical models for PTSD (Keane et al., 1985), GABA is of potential interest in PTSD research. Preliminary measurements of GABA in PTSD are inconsistent with studies showing similar (Fujita et al., 2004; Rosso et al., 2013) or decreased (Bremner et al., 2000; Geuze et al., 2008; Rosso et al., 2013) cortical GABA concentration or GABA(A) benzodiazepine receptor levels. Reduced GABA (A) benzodiazepine receptor levels might also be explained by increased GABA concentration leading to secondary down-regulation of the GABA receptor complex (Geuze et al., 2008).

Another potential mechanism underlying the observed brain abnormalities in PTSD involves neural cellular damage caused by excessive brain levels of free radicals (Tezcan et al., 2003; Ceprnja et al., 2011). These highly reactive agents might interact with

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molecules from important cellular components such as cell membranes, thereby resulting in cellular damage (Henningsen et al., 2012). The concept of oxidative stress refers to an imbalance of oxidants and antioxidants beyond typical physiological limits. Reactive oxygen species, e.g., nitric oxide and hydrogen peroxide, are synthesized during interaction with oxygen in the context of cellular mitochondrial energy generation. Antioxidants, such as glutathione, neutralize reactive oxygen species, thereby maintaining oxidative balance and preventing potential cellular damage (Berk et al., 2008). Glutathione is a central marker of oxidative stress and the most abundant, predominantly intracellular, antioxidant in the central nervous system (Livingstone and Davis, 2007). Recently, glutathione is detectable by magnetic resonance spectroscopy (MRS) (Wijtenburg et al., 2014; Godlewska et al., 2014). In brief, glutathione reduces disulfide bonds to cysteine by serving as an electron donor, thereby detoxifying reactive oxygen species. During this process, reduced glutathione is converted to its oxidized form. Once oxidized, glutathione can be reduced back by glutathione reductase, using nicotinamide adenine dinucleotide phosphate as an electron donor (Couto et al., 2013). Maintaining glutathione homeostasis seems not only relevant with respect to antioxidant defense mechanisms, but also to memory function, synaptic plasticity, and learning (Cruz et al., 2003). A potential role for oxidative stress in the pathophysiology of PTSD is suggested by some preliminary evidence from blood-sample studies observing lower concentrations of protein carbonyls, markers for protein oxidation, in PTSD patients (Ceprnja et al., 2011) and an association between antioxidative enzyme concentrations and PTSD symptom severity (Tezcan et al., 2003).

In the current study we used single-voxel proton magnetic resonance spectroscopy (^{1}H MRS) to measure GABA and glutathione concentrations in the dorsolateral prefrontal cortex (DLPFC) and the ACC in both PTSD and non-PTSD participants. Given the growing evidence from imaging studies for an involvement of the DLPFC and the ACC in the pathophysiology of PTSD (Shin et al., 2011; Aupperle et al., 2012), we expected to find altered levels of GABA and glutathione in PTSD participants.

2. Methods

2.1. Participants

While the initial sample consisted of 39 subjects, reliable MRS spectra for both regions of interest (ACC and DLPFC) could only be achieved in 29 subjects, as described below. Participants in this sample were right-handed (Oldfield, 1971), trauma-exposed individuals with ($n=12$) and without ($n=17$) a current Diagnostic and Statistical Manual of Mental Disorders, 4th edition (DSM-IV) PTSD diagnosis, as assessed with the Clinician-Administered PTSD Scale (CAPS) (Blake et al., 1995). The groups were similar regarding gender ($p=0.474$) and age ($p=0.739$, $t_{(27)}=0.267$). A CAPS total score of greater than 50 was required for PTSD participants and of less than 34 for non-PTSD participants. Trauma history was assessed using the trauma checklist from the Posttraumatic Diagnostic Scale (PDS) (Foa et al., 1997) and the Childhood Trauma Questionnaire (CTQ) (Bernstein et al., 2003). Across all examined subjects, trauma experience was as follows: sexual violence ($n=7$), physical violence ($n=5$), accidents ($n=9$), natural disaster ($n=2$), and war/terror ($n=6$).

PTSD onset was defined as the time when the subject met the full set of diagnostic criteria for the first time following the traumatic event (lifetime diagnosis), as retrospectively evaluated using the CAPS. Current Axis I disorders were assessed by the Structured Clinical Interview for DSM-IV Axis I Disorders (SCID-I) and the Structured Clinical Interview for DSM-IV Dissociative Disorders-Revised (SCID-D-R) (Steinberg, 1994). All participants were free of neurological or other major medical conditions. Four PTSD and one non-PTSD participant were currently medicated with antidepressants, including selective serotonin or noradrenaline reuptake inhibitors. Six PTSD participants were taking other medication, including a non-opioid analgesic ($n=1$), an antiretroviral ($n=1$), thyroid substitutes ($n=2$), a calcium channel blocker ($n=1$), and an anti-asthmatic ($n=1$). One non-PTSD participant took both an angiotensin II receptor antagonist and a thyroid substitute. None of the subjects were taking nicotine (current smokers: five PTSD participants, three non-PTSD participants; $p=0.092$, Chi-Square test) or alcohol for at least 12 h before the ^{1}H MRS recording.

Participants completed the CTQ (Bernstein et al., 2003), the Multidimensional Inventory of Dissociation (MID) (Dell, 2006), the trait portion of the State-Trait Anxiety Inventory (STAI) (Spielberger et al., 1970), and the Beck Depression Inventory (BDI) (Beck et al., 1961). Standard cognitive tests were administered using Hogrefe Test System 4 software (Hogrefe, 2006) and included the Viennese Matrices Test (Formann and Piswanger, 1979), an adapted version of the Raven Progressive Matrices (Raven, 1947), the Test of Word Power (Schmidt and Metzler, 1992), and the d2 Test of Attention (Brickenkamp and Zillmer, 1998). All measures were validated German-adapted versions. Table 1 summarizes the participants' demographic and clinical characteristics (including information on comorbidities and substance use). The study protocol was approved by the institutional review board of the County of Zurich, Switzerland. All participants provided written informed consent after full explanation of the procedures.

2.2. Acquisition of MR data

Magnetic resonance imaging (MRI) and spectroscopy were performed with a 3T General Electric HDxt MRI scanner (General Electric Medical Systems, Milwaukee, WI) equipped with TwinSpeed gradients and an eight-channel receive-only head coil. The scanning protocol included a high-resolution anatomical scan (three-dimensional FSPGR BRAVO; repetition time (TR)=10.9 ms, echo time (TE)=4.6 ms; field of view=24 cm; flip angle=13°; 156 axial slices; thickness=1.2 mm; 352×224 matrix) used for localization of the spectroscopy voxels. Single voxel edited ^{1}H MRS spectra were acquired from a $25\times40\times30$ mm^3 voxel of interest positioned in the left DLPFC, with the ventral boundary not extending into the insular cortex or ventrolateral prefrontal cortex, and the lateral boundary not extending into the premotor cortex, and a $28\times30{,}225$ mm^2 voxel of interest positioned in the ACC, with the ventral boundary not extending into the subgenual ACC and the lateral boundary not extending into the genu of the corpus callosum (Fig. 1). Methods included a frequency-selective editing technique combined with a point-resolved spectroscopy sequence (PRESS) method (called MEGA-PRESS) (Mescher et al., 1998; Edden and Barker, 2007). MEGA-editing was achieved using 16-ms Gaussian editing pulses applied at 1.9 ppm and 7.5 ppm in alternate spectral lines. Edited spectra were acquired with a TR of 1800 ms, a TE of 68 ms, an eight-step phase cycle, and either 320 (DLPFC) or 640 (ACC) spectral averages. DLPFC and ACC voxels were prescribed according to previously described localization methods (Stone et al., 2009; O'Gorman et al., 2011; Michels et al., 2012). The spectra were coil combined with weighting factors derived from the first point of the unsuppressed water free induction decay signal from each coil. Metabolite ratios relative to creatine (Cr) were derived with LCModel version 6.3-1B (Provencher, 1993), using the sptype='mega-press-2' option for optimized estimation of GABA. The edited spectra were analyzed with a simulated basis set including basis spectra for GABA, glutathione, *N*-acetyl aspartate (NAA), glutamine, and *N*-acetyl aspartyl glutamate, and the edit-off sub-spectra were analyzed with a basis set including spectra for GABA, glutathione, NAA, glutamate, glutamine, Cr, choline, lactate, and myo-inositol.

While the editing pulses for MEGAPRESS were centered on the GABA multiplet at 1.9 ppm, the bandwidth was sufficiently wide that the editing pulses also co-edited the glutathione multiplet at 2.1 ppm, the glutamate and glutamine multiplets at 2.1 ppm and the macromolecule at 1.7 ppm. All four of those moieties (glutamate, glutamine, glutathione, and the macromolecule) are co-edited with GABA, but glutamate, glutamine, and glutathione can also be assessed from the edit OFF (TE=68 ms) subspectra. For the present study, GABA/Cr values were derived from the edited spectra, while glutathione/Cr, NAA/Cr, and glutamate/Cr values were derived from the edit OFF spectra (PRESS, TE=68 ms).

MRS recordings were only performed if the line width was below 12 Hz, as estimated from the auto-prescan. Each spectrum was visually inspected for the presence of artifacts or fitting errors, and spectra with poorly fitted metabolite peaks (Cramer–Rao minimum variance bounds of more than 10% for Cr or NAA or more than 20% for any of the other metabolites) in any of the examined regions and spectra with visible artifacts were excluded from further analysis. Examples of representative MRS spectra are shown in Figs. 2 and 3 (ppm range is shown from 1 to 4 ppm, i.e., not for the full (ppm) range analyzed).

2.3. Statistical analysis

For the analysis of clinical measures, we used chi-squared tests to compare proportions of nominal variables, and *t*-tests to compare continuous variables between groups. Because of the small number of participants with well-fitted glutamine peaks (24 of 39 [61.5%] for the DLPFC and 19 of 39 [48.7%] for the ACC), glutamine was excluded from the statistical analysis. The final set of metabolites included in the statistical analysis consisted of GABA ($n=12/17$), glutathione ($n=11/15$), NAA ($n=12/17$), and the combination of glutamate and glutamine ($n=12/17$). A repeated measures analysis of variance (ANOVA) was performed separately for each metabolite with region (left DLPFC, ACC) included as a repeated factor and group (PTSD, non-PTSD) as a between-group factor. Cohen's *d* effect sizes were calculated for between-group differences of metabolite concentrations. Associations between metabolite concentrations in the left DLPFC and ACC, cognitive performance scores and clinical measures (CAPS, CTQ, MID, STAI, BDI scores, and duration since PTSD onset) were assessed by non-parametric correlations (Spearman's rho) in PTSD participants. The critical threshold was set at $p=0.05$ (two-tailed). Statistical analyses were performed in IBM SPSS Statistics V. 21.0 (IBM Corporation, Armonk, NY).

Table 1
Demographic characteristics, cognitive performance and clinical measures of PTSD and non-PTSD participants.

	Group				Analysis
	PTSD ($n=12$)		Non-PTSD ($n=17$)		
Measure	*N*	%	*N*	%	*p*
Female	11	91.7	16	94.1	0.474
Current Axis I comorbidity					
Depressive disorder	5	41.7	0	0	
Dysthymia	3	25.0	0	0	
Panic disorder	1	8.3	0	0	
Social phobia	1	8.3	0	0	
Specific phobia	1	8.3	0	0	
Generalized anxiety disorder	1	8.3	0	0	
Depersonalization disorder	3	25.0	0	0	
Medication					
Antidepressant	4	30.0	1	5.9	
For physical medical conditions	6	50.0	1	5.9	

	Group				Analysis	
	PTSD ($n=12$)		Non-PTSD ($n=17$)			
Measure	Mean	S.D.	Mean	S.D.	*p*	Cohen's *d*
Age (years)	38.8	13.1	40.4	12.3	0.739	−0.125
Education (years)	14	3.2	14.4	2.7	0.711	−0.135
EHI: Right handedness	13.8	0.45	13.8	0.97	0.962	0
EHI: Left handedness	0.9	1.1	1.2	1.8	0.596	−0.201
Cognitive performance						
WMT: Total number of correct responses (non-verbal intelligence)	10.5	4.8	12.5	5	0.335	−0.408
WST: Number of recognized words (verbal intelligence)	30.7	3.9	33.1	2.2	0.042	−0.757
d2: Total number of items processed (processing speed)	394.1	91.7	501.7	113.6	0.012	−1.042
d2: Total number of errors (accuracy)	6.6	5.8	12.4	12.8	0.154	−0.583

	Group				Analysis	
	PTSD ($n=12$)		Non-PTSD ($n=17$)			
Measure	Mean	S.D.	Mean	S.D.	*p*	Cohen's *d*
CAPS: Total	67.3	15	7.7	10.8	<0.001	4.560
CAPS: Re-experiencing	20.8	7.1	2.7	4.3	<0.001	3.083
CAPS: Avoidance	24.6	9	1.7	3	<0.001	3.413
CAPS: Hyperarousal	22	5.5	3.4	4.9	<0.001	3.570
PDS: Number of self-reported single trauma	1.6	1.4	1.5	0.8	0.921	0.087
PDS: Number of self-reported prolonged and repeated trauma	1.5	1.1	0.3	0.5	<0.001	1.404
Illness duration (months)	73.3	67.2	–	–		–
CTQ: Total	62.5	26.8	41.5	14.2	0.011	0.979
MID: Total	15.6	10.7	1.5	1.9	<0.001	1.834
STAI: Trait anxiety	47.9	11.3	33.1	10.3	0.001	1.368
BDI: Total	21.6	12.4	7	4.9	<0.001	1.584

EHI: Edinburgh Handedness Inventory; WMT: Viennese Matrices Test; WST: Test of Word Power; d2: d2 Test of Attention; CAPS: Clinician-Administered PTSD Scale; PDS: Posttraumatic Diagnostic Scale; CTQ: Childhood Trauma Questionnaire; MID: Multidimensional Inventory of Dissociation; STAI: State-Trait Anxiety Inventory; BDI: Beck Depression Inventory.

3. Results

As shown in Figs. 2 and 3, using MEGAPRESS and LCModel resulted in reliable fits of GABA, GSH, Glu, and other metabolites. With no correction for partial volume effects, there were no between-group differences in Cr/H_2O across both regions ($p=0.900$, $t=0.126$; unpaired *t*-tests). GABA concentrations were 12.65% higher in both regions in PTSD participants, evidenced by a significant main effect of group ($F_{(1,27)}=5.035$, $p=0.033$). There was a main effect of region ($F_{(1,27)}=4.991$, $p=0.034$) with 10.08% higher GABA concentration in the left DLPFC than the ACC. There was no GABA concentration group × region interaction ($F_{(1,27)}=0.542$, $p=0.468$). Glutathione concentration was 22.73% higher (across both regions) in PTSD participants as evidenced by a significant main effect of group ($F_{(1,24)}=5.757$, $p=0.025$). There was neither a significant main effect of region ($F_{(1,24)}=0.309$, $p=0.583$) nor a significant group × region interaction ($F_{(1,24)}=0.565$, $p=0.459$) for glutathione concentration. No significant main effect of group or group × region interaction effects were found for NAA ($F_{(1,27)}=1.277$, $p=0.268$, $F_{(1,27)}=0.834$, $p=0.369$) or glutamate/glutamine ($F_{(1,27)}=1.324$, $p=0.260$; $F_{(1,27)}=3.919$, $p=0.058$). The results remained similar after exclusion of participants taking antidepressants (GABA: main effect of group: $F_{(1,22)}=4.754$, $p=0.004$; glutathione: main effect of group: $F_{(1,19)}=4.036$, $p=0.049$). Covarying for cigarette smokers ($n=8$), a repeated measures ANOVA for GSH and GABA including both regions still revealed a main effect of group ($F_{(1,27)}=10.27$, $p=0.004$) but no region × nicotine interaction ($p=0.153$). Metabolite concentrations are presented in Table 2 and Fig. 4.

No correlation was found between CAPS or any other clinical (MID, STAI, BDI, and CTQ) scores and GABA ($r \leq 0.320$, $p \geq 0.119$) or

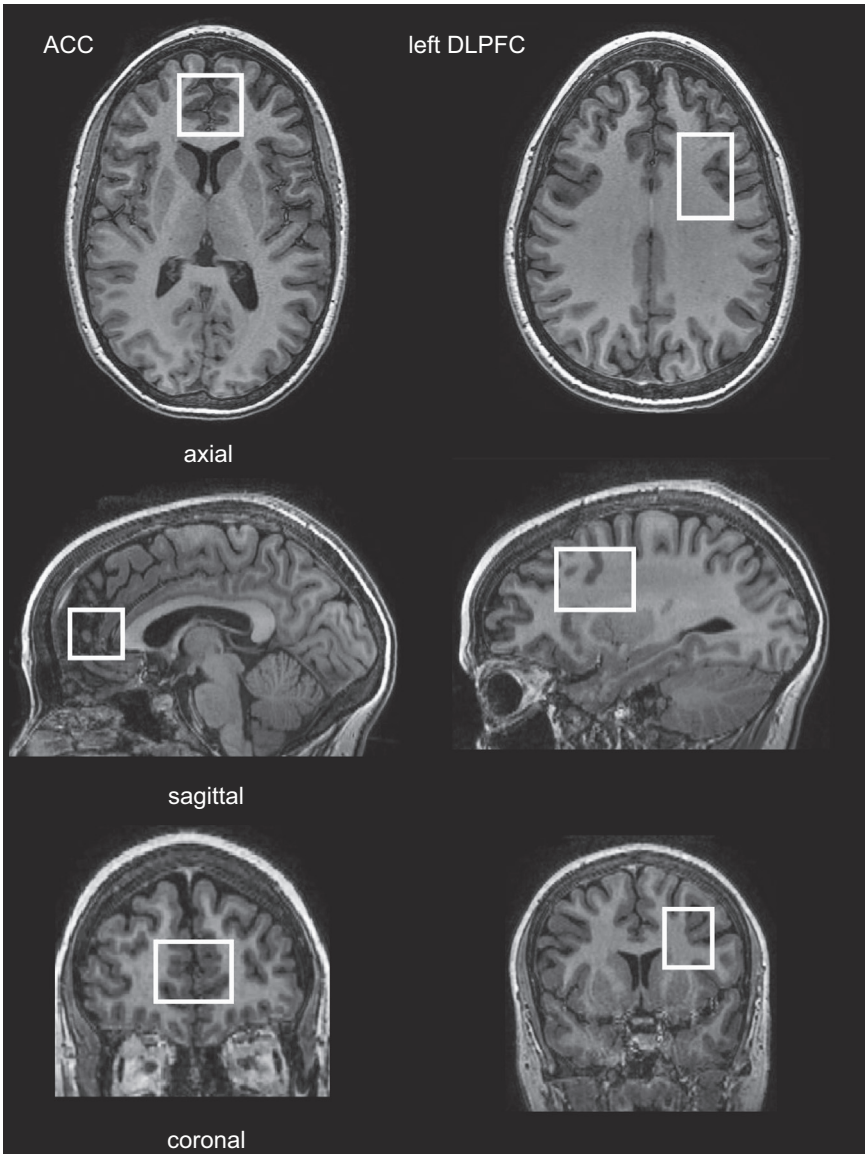

Fig. 1. Schematic illustration of the voxel's position on axial (top) and sagittal (middle), and coronal (bottom) views. Voxel positions are shown for both the anterior cingulate cortex (ACC) (left panel) and the left dorsolateral prefrontal cortex (DLPFC, right panel). For the ACC, the ventral boundary was not extending into the subgenual ACC and the lateral boundary was not extending into the genu of the corpus callosum. For the left DLPFC, the ventral boundary was not extending into the insular cortex or ventrolateral prefrontal cortex and the lateral boundary was not extending into the premotor cortex.

glutathione ($p \geq 0.085$, $r \geq 0.517$) concentration, respectively, in PTSD participants. However, we observed a significant moderate correlation between illness duration and glutathione concentration in the left DLPFC ($r = 0.638$, $p = 0.025$).

4. Discussion

We used single-voxel proton MRS to investigate whether cortical concentrations of primary inhibitory and excitatory neurotransmitters and metabolites related to oxidative stress were altered in PTSD. We found elevated GABA levels in the DLPFC and ACC in PTSD participants, indicating enhanced inhibitory neurotransmission. Furthermore, we found higher levels of the antioxidant glutathione in DLPFC and ACC in PTSD participants, suggesting an imbalance of glutathione homeostasis and oxidative status in PTSD. Cortical levels of NAA, a marker for neuronal integrity, and glutamate/glutamine, primary excitatory neurotransmitters, did not differ between PTSD and non-PTSD participants.

4.1. Interpretation: GABA

Potential involvement of the GABA system in PTSD has been examined in pre-clinical, pharmacological, and neuroimaging studies. In an animal model using a stress and re-stress paradigm, stress evoked a sustained decrease in hippocampal GABA levels (Harvey et al., 2004). Rodents also responded with a decrease of cortical and hippocampal GABA-A receptor function after inescapable food shocks (Lippa et al., 1978; Medina et al., 1983; Drugan et al., 1986). In humans, low plasma GABA levels after a traumatic event might be predictive of subsequent development of PTSD (Vaiva et al., 2004). Neuroimaging studies of the benzodiazepine-$GABA_A$ receptor using single photon emission computed tomography (SPECT) in veterans with PTSD revealed lower levels of [^{123}I]iomazenil in the prefrontal cortex (Bremner et al., 2000). However, this finding was not confirmed in another SPECT study in veterans (Fujita et al., 2004). More recently, Geuze et al. (2008) found lower cortical (including midline and lateral prefrontal cortex), thalamic, and hippocampal GABA-A benzodiazepine receptor binding in patients with PTSD relative to trauma-exposed

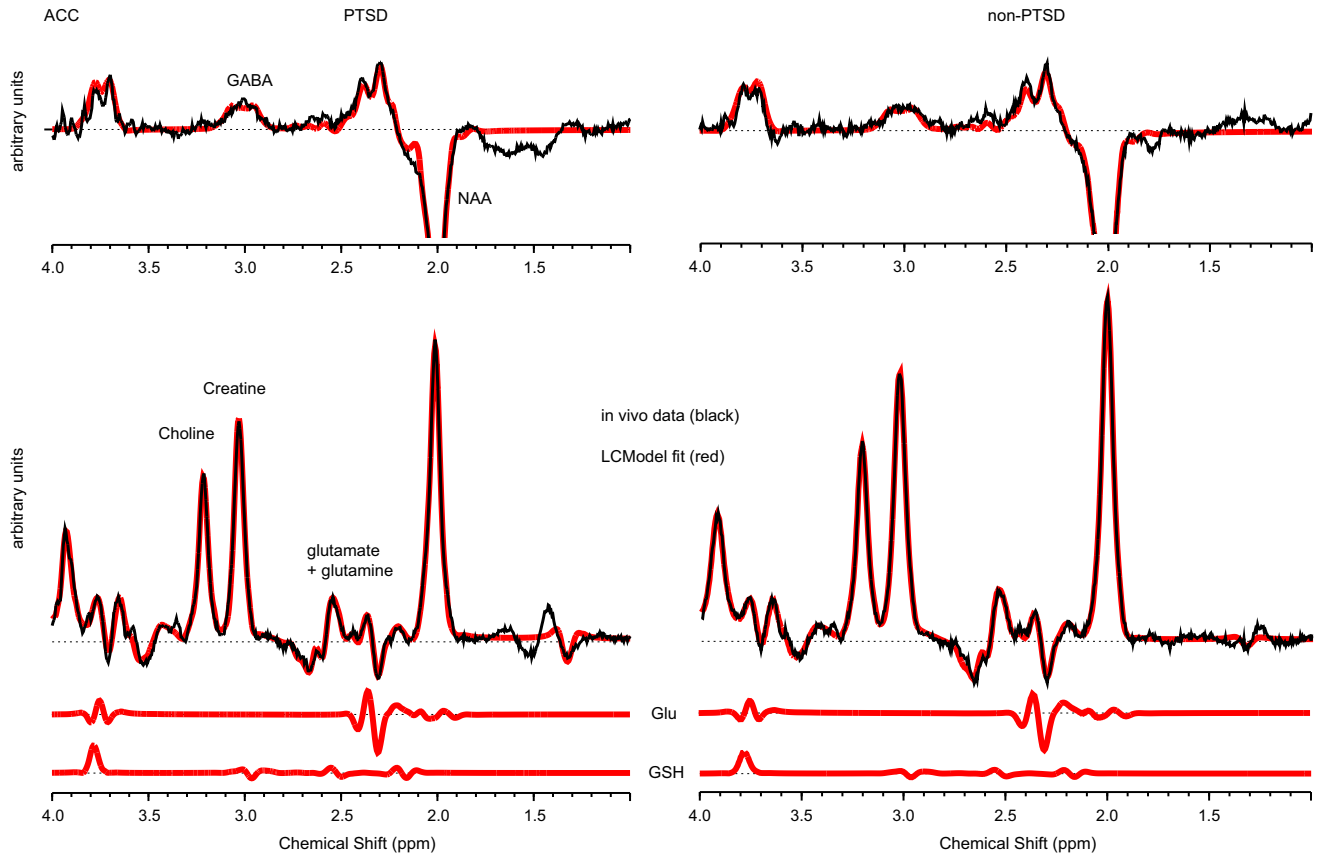

Fig. 2. Single-voxel proton magnetic resonance spectroscopy (^{1}H MRS) spectrum of one PTSD participant (left panel) and one non-PTSD participant (right panel) for the anterior cingulate cortex (ACC). Top row: MEGAPRESS edited spectra. The raw data (black curve) is well fitted by LCModel (red curve) for all major metabolites, including glutathione *N*-acetyl-aspartate (NAA) and GABA. Middle row: MEGAPRESS unedited sub-spectra. Bottom rows: individual fits of Glu and GSH. In this region, individual Cramer lower bounds for the PTSD participant are 6% (GABA) and 13% (GSH), and for the non-PTSD participant 6% (GABA) and 12% (GSH). The ppm range is only shown from 1 to 4 ppm, i.e., not for the full (ppm) range analyzed. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

controls using positron emission tomography (PET), a method which provides higher spatial selectivity in detection of the benzodiazepine-$GABA_A$ receptor than SPECT (Geuze et al., 2008). Our study results are consistent with their proposition of down-regulation of the GABA-A receptor complex by increased levels of GABA. Our results differ from recent ^{1}H MRS GABA findings of lower levels in insula and similar levels in ACC in PTSD participants (Rosso et al., 2013). However, while the ACC voxel in this study was positioned anteriorly to the genu of the corpus callosum (Fig. 1), Rosso and colleagues positioned the voxel in a more posterior and superior location. Thus, regional differences in GABA levels might explain the apparent discrepancy between the present results and those reported by Rosso et al. (2013).

Since GABA is synthesized from the alpha-decarboxylation of glutamate by glutamic acid decarboxylase, our results suggest that elevated GABA levels in PTSD could result from two potential physiological processes that are not mutually exclusive: (1) overexpression of glutamate or glutamic acid decarboxylase, or (2) decreased clearance of GABA from the synaptic cleft, possibly as a result of altered neurotransmitter recycling.

4.2. Interpretation: glutathione

Our finding of higher prefrontal glutathione levels in PTSD participants provides further evidence for a link between glutathione dysregulation and hyperarousal states such as anxiety, suggested by previous animal research. Overexpression of the genes glyoxalase 1 and glutathione reductase 1 (GSHr) in the cingulate cortex of mice is associated with increased levels of anxiety-like behavioral phenotypes (Hovatta et al., 2005). Glyoxalase 1 uses reduced glutathione as a co-factor in detoxification of reactive species, thereby lowering reduced glutathione concentration, while glutathione reductase 1 resynthesizes glutathione, thereby replenishing reduced glutathione levels. Thus, one potential explanation for the observed elevated glutathione levels in PTSD might be overexpression of genes involved in the glutathione metabolism that might increase vulnerability for developing PTSD after a traumatic event.

Alternatively, higher glutathione levels might be an acquired feature in PTSD, reflecting a compensation mechanism for maintaining oxidative homeostasis in neurons in the presence of increased production of reactive oxygen species due to excessive mitochondrial energy generation. Increased turnover of free radical detoxification by glutathione, which is enzymatically catalyzed by glutathione peroxidase, is suggested by a reported positive correlation between plasma glutathione peroxidase activity and PTSD symptom severity (Tezcan et al., 2003). Maintained oxidative homeostasis in PTSD would also be in agreement with the absence of oxidative cellular damage as suggested by our finding of similar NAA concentrations in PTSD and non-PTSD participants.

It has been argued that two main processing systems affected in PTSD are attention (MacLeod et al., 2002) and memory (Amir et al., 1996), findings that are consistent with the lower performance we observed with respect to attention and estimated verbal intelligence in PTSD participants. However, we did not find a correlation between cognitive performance scores and glutathione levels in PTSD

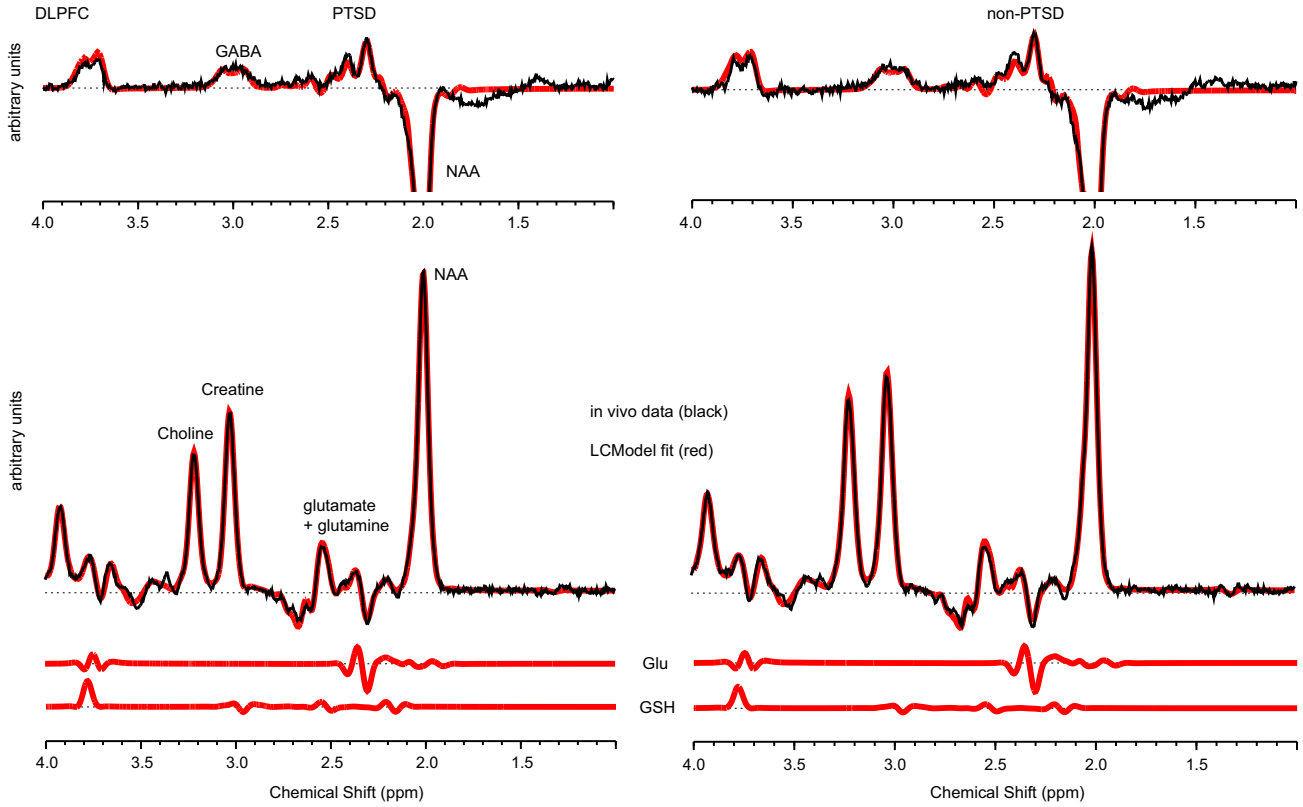

Fig. 3. Single-voxel proton magnetic resonance spectroscopy (^{1}H MRS) spectrum of one PTSD participant (left panel) and one non-PTSD participant (right panel) for the dorsolateral prefrontal cortex (DLPFC). Top row: MEGAPRESS spectra. The raw data (black curve) are well fitted by LCModel (red curve) for all major metabolites, including glutathione *N*-acetyl-aspartate (NAA) and GABA. Middle row: MEGAPRESS unedited sub-spectra. Bottom rows: individual fits of Glu and GSH. In this region, individual Cramer lower bounds for the PTSD participant are 4% (GABA) and 7% (GSH), and for the non-PTSD participant 5% (GABA) and 7% (GSH). The ppm range is only shown from 1 to 4 ppm, i.e., not for the full (ppm) range analyzed. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 2
Brain metabolite/creatine ratios in PTSD and non-PTSD participants.

	Group				Analysis
	PTSD ($n=12$)		Non-PTSD ($n=17$)		
Metabolite	Mean	S.D.	Mean	S.D.	Cohen's *d*
ACC					
GABA	0.29	0.06	0.24	0.04	0.98
Glutathione	0.15	0.03	0.11	0.03	1.33
Glutamate/glutamine	1.01	0.14	0.89	0.21	0.67
NAA	0.97	0.21	1.10	0.17	0.68
Left DLPFC					
GABA	0.31	0.05	0.27	0.04	0.88
Glutathione	0.14	0.03	0.11	0.03	1.00
Glutamate/glutamine	0.78	0.09	0.77	0.14	0.08
NAA	1.54	0.20	1.56	0.19	0.10

GABA and glutathione concentrations were higher in PTSD than non-PTSD participants across regions as evidenced by a significant main effect of group (GABA: $F_{(1,27)}=5.035$, $p=0.033$; Glutathione: $F_{(1,24)}=5.757$, $p=0.025$). There was no main effect of group on concentrations of the NAA ($t_{(27)}=1.45$, $p=0.158$) and glutamate/glutamine ($t_{(27)}=0.55$, $p=0.584$). There was no significant group by region interaction for any neurometabolite ($p \geq 0.369$). PTSD: post-traumatic stress disorder; ACC: anterior cingulate cortex; DLPFC: dorsolateral prefrontal cortex; NAA: *N*-acetyl-aspartate; GABA: α-amino butyric acid.

participants, suggesting that glutathione homeostasis and oxidative status may not be directly involved in the pathophysiology of attention and memory deficits in PTSD. Nevertheless, we found a positive correlation between illness duration and glutathione levels. This might reflect the time-integrated effect of neurodegenerative processes or increased metabolic compensatory activity acting to reduce oxidative stress in patients with long illness duration. Wood et al. (2009) described the biochemical interactions in the glutathione cycle in the context of schizophrenia (Wood et al., 2009). One of the factors potentially leading to increased oxidative stress, evidenced as higher accumulation of the free radical O_2, is altered neurotransmission. In our study, GABA levels were elevated, possibly reflecting a disturbed excitation–inhibition balance in PTSD. Based on data from the present study, it is not possible to confirm a link between elevated GABA and glutathione, but the biochemical association between neurotransmitter cycling, free radical production, oxidative stress, and glutathione cycling suggests a potential link between these metabolic measures.

4.3. Limitations

One limitation of the current study is that the optimal MRS protocol for detecting GABA (the MEGAPRESS protocol used here) is not optimal for detecting glutathione. However, while the accuracy and reliability of glutathione detection is expected to be greater with an optimized protocol, the signal-to-noise benefits of the large voxel size used in the present study, coupled with a spectral fitting approach using prior knowledge, appear to provide adequate sensitivity to glutathione. This sensitivity was confirmed by a study of phantom spectra acquired from phantoms with varying concentrations of glutathione. The spectral fitting approach has also been applied successfully to measure glutathione from short echo STEAM spectra (Terpstra et al., 2005). However, a further limitation of the current study is that we could not discriminate reduced and oxidized forms of glutathione using our MRS protocol. We assume that the measured GSH signal represents reduced glutathione, since the MRS signal from oxidized

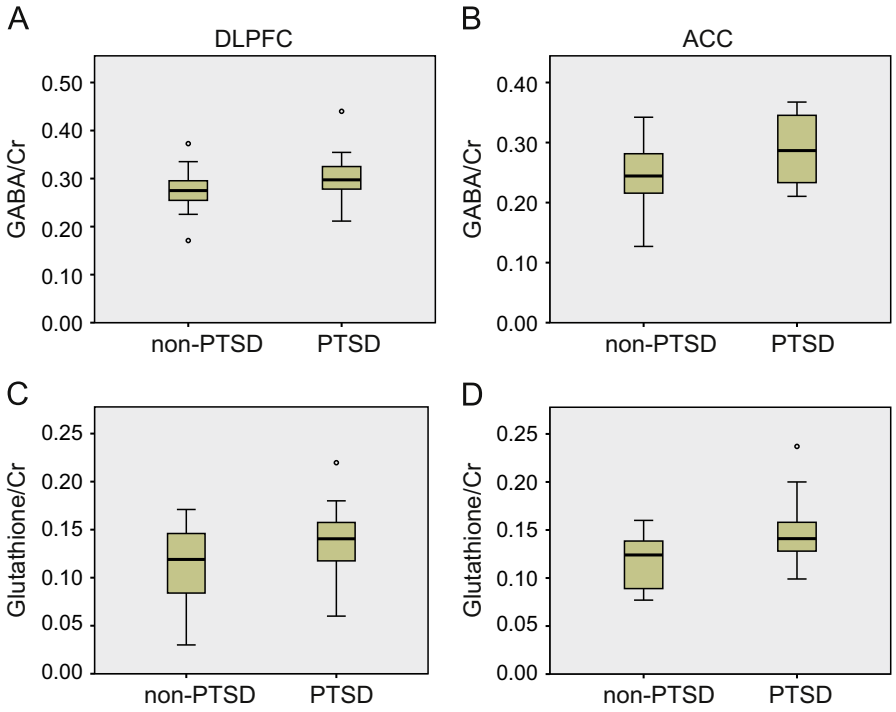

Fig. 4. GABA (A and B) and glutathione (C and D) levels in patients with PTSD and non-PTSD in left DLPFC (A and C) and ACC (B and D). GABA: gamma-amino butyric acid, Cr: Creatine, DLPFC: dorsolateral prefrontal cortex, and ACC: anterior cingulate cortex. °: outlier (not included in analysis).

glutathione in vivo is thought to be negligible (Terpstra et al., 2006). Antidepressants, as taken by four participants in this study, might influence neurometabolite concentrations. However, repeating the analyses after exclusion of participants taking antidepressants did not change our findings. The sample size was small, leaving open the possibility that moderate between-group effects might have been missed. Also, we only examined trauma-exposed but not trauma-unexposed healthy controls. Finally, the reported correlations between illness duration and GSH concentrations were not corrected for multiple comparisons, which enhances the risk that the finding may reflect a false-positive correlation.

In conclusion, our observation of increased prefrontal GABA and glutathione levels in PTSD provides evidence for a role of atypically high inhibitory effects and oxidative stress in the pathogenesis of functional abnormalities in PTSD. Future research is needed to investigate whether the observed neurotransmitter imbalance reflects either a pre-existing, possibly innate, vulnerability factor for the development of PTSD after trauma, or rather a compensation mechanism for maintaining neurotransmitter and oxidative homeostasis in PTSD. Improved understanding of the molecular pathways involved in the pathophysiology of PTSD may provide the basis for the development of novel treatment targets (Berk et al., 2008).

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