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Abnormal *N*-acetylaspartate in hippocampus and anterior cingulate in posttraumatic stress disorder

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Abstract

Magnetic resonance spectroscopic imaging (MRSI) studies suggest hippocampal abnormalities in posttraumatic stress disorder (PTSD), whereas findings of volume deficits in the hippocampus, as revealed with magnetic resonance imaging (MRI), have been inconsistent. Co-morbidities of PTSD, notably alcohol abuse, may have contributed to the inconsistency. The objective was to determine whether volumetric and metabolic abnormalities in the hippocampus and other brain regions are present in PTSD, independent of alcohol abuse. Four groups of subjects, PTSD patients with ($n=28$) and without ($n=27$) alcohol abuse and subjects negative for PTSD with ($n=23$) and without ($n=26$) alcohol abuse, were enrolled in this observational MRI and MRSI study of structural and metabolic brain abnormalities in PTSD. PTSD was associated with reduced *N*-acetylaspartate (NAA) in both the left and right hippocampus, though only when normalized to creatine levels in the absence of significant hippocampal volume reduction. Furthermore, PTSD was associated with reduced NAA in the right anterior cingulate cortex regardless of creatine. NAA appears to be a more sensitive marker for neuronal abnormality in PTSD than brain volume. The alteration in the anterior cingulate cortex in PTSD has implications for fear conditioning and extinction.

Keywords

Magnetic resonance imaging; Magnetic resonance spectroscopy; Brain metabolites; Brain atrophy; Alcoholism

1. Introduction

Many neuroimaging studies of posttraumatic stress disorder (PTSD) have focused on potential abnormalities in the hippocampus, which is known to play a crucial role in the biological response to stress (Sapolsky, 2000). Using magnetic resonance imaging (MRI) investigators have found smaller hippocampal volumes in Vietnam veterans with combat-related PTSD

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(Bremner et al., 1995; Gurvits et al., 1996; Gilbertson et al., 2002; Hedges et al., 2003) and adults with a history of physical and/or sexual childhood abuse (Bremner et al., 1997; Stein et al., 1997; Villarreal et al., 2002a). However, some studies have not found hippocampal volume deficits in PTSD (De Bellis et al., 1999; Bonne et al., 2001; Schuff et al., 2001b; Fennema-Notestine et al., 2002; Golier et al., 2005; Yehuda et al., 2007), particularly in traumatized children (De Bellis et al., 1999), in recent onset PTSD (Bonne et al., 2001), and in subjects with mild or subsyndromal symptoms (Yamasue et al., 2003). In our previous research, we used both structural MRI and proton magnetic resonance spectroscopic imaging (^1H MRSI) to investigate hippocampal characteristics in Vietnam veterans with PTSD (Schuff et al., 2001b). We documented reduced hippocampal *N*-acetylaspartate (NAA), a putative marker of neuronal integrity, without hippocampal volume deficits in PTSD. In the absence of tissue deficits, reduced NAA concentration may indicate disproportionately less neuronal than glial tissue or neuronal dysfunction without frank loss of neurons. In this regard, NAA changes would be expected to be more sensitive to the detection of neuronal alterations in PTSD than volumetric measures. Other studies have also reported reduced hippocampal NAA in PTSD (Freeman et al., 1998; Villarreal et al., 2002b; Mohanakrishnan Menon et al., 2003). Furthermore, hippocampal NAA measures correlated strongly with PTSD symptoms in prisoners of war (Brown et al., 2003), but these results could not be replicated in a second study on a similar population (Freeman et al., 2006). However, previous studies have not investigated potential effects of alcohol abuse on NAA reduction in PTSD. Some studies of PTSD have attempted to address the issue of comorbid alcohol abuse by matching or statistically controlling for the effects of alcoholism (Bremner et al., 1995) and have continued to find hippocampal abnormalities in PTSD. Other studies have excluded subjects with recent alcohol abuse altogether and have usually failed to detect hippocampal abnormalities in PTSD (De Bellis et al., 1999, 2000b; Schuff et al., 2001b; Fennema-Notestine et al., 2002). It is also possible to experimentally determine the independent contribution of alcoholism to PTSD and the interaction between the two conditions by comparing four groups, subjects positive or negative for PTSD and with or without alcoholism. However, such studies have been rare. A recent study, comparing veterans positive or negative for PTSD and with or without alcohol abuse found a trend for a PTSD effect on hippocampal volume regardless of alcoholism, though this effect disappeared when adjusted for head or brain size (Woodward et al., 2006a). To our knowledge, there has been no NAA study directly comparing subjects positive or negative for PTSD and with or without alcoholism. The primary goal of this study was to test for the independent effects of PTSD, alcohol, and their interaction on hippocampal NAA as well as on hippocampal volume by comparing MRSI and MRI data from four groups, subjects positive or negative for PTSD and with or without a diagnosis of alcohol abuse or dependence.

While most MRI studies of PTSD focused initially on the hippocampus, other brain regions may also be involved. PTSD patients have been found to have difficulty with selective attention, and have also been shown to have heightened fear conditioning and weaker fear extinction (Orr et al., 2000). The relevant neuroanatomical basis for these behaviors may involve the anterior cingulate cortex (ACC) for attention, the amygdale for fear conditioning and the frontal cortex for fear extinction (Hamner et al., 1999). The role of the ACC in selective attention may be especially relevant to PTSD, because of potential treatment implications. Functional neuroimaging studies detected reduced activity of the ACC in response to trauma-related stimuli in individuals with PTSD compared with trauma-exposed controls (Shin et al., 2001), and a structural MRI study reported reduced ACC volumes in veterans with PTSD (Woodward et al., 2006b). However, whether metabolic deficits accompany the functional and structural abnormalities of the ACC in PTSD or whether alcohol is a confounding factor is not clear. Several MRS studies reported abnormal metabolite levels of ACC in adult PTSD, though the findings were not always consistent. Whereas one study found reduced ratios of NAA relative to creatine in the ACC in PTSD patients (Mahmutyazicioglu et al., 2005), another study found increased choline to creatine ratios, suggesting glial rather than neuronal alterations

(Seedat et al., 2005). Although one MRS study reported reduced NAA of the ACC in abused children with PTSD (De Bellis et al., 2000a) without complications from alcohol abuse, the implications and interpretation of the finding were limited by lack of comparisons to a group of abused children without PTSD. A second goal of the present study was therefore to determine the metabolic characteristics of the ACC and other cortical regions in adult PTSD regardless of alcoholism by comparisons between subjects positive or negative for PTSD and with or without a diagnosis of alcohol abuse or dependence.

2. Methods

2.1. Subject recruitment and clinical assessment

The study design required recruitment of the following four groups of subjects: two groups positive for PTSD with (P+A+) and without (P+A-) alcohol abuse, and two groups negative for PTSD with (P-A+) and without (P-A-) alcohol abuse. Both men and women were eligible for the study. Veterans participating in the study were interviewed by a clinical psychologist with extensive experience using the Clinician Administered PTSD Scale (CAPS) (Blake et al., 1995) for diagnosis of PTSD and the Structured Clinical Interview for DSM-IV Diagnosis (SCID) (Spitzer et al., 1992) for assessment of past or current alcohol or drug abuse or dependence. An interview version of the Life Stressor Checklist—Revised (LSC-R) (Wolfe et al., 1996) was also used to determine exposure to childhood trauma before the age of 14 years. The LSC-R assesses 21 stressful life events (e.g., experiencing or witnessing serious accidents, illnesses, sudden death, physical and sexual assault). A second rater listened to 20% of the taped CAPS interviews, and interrater reliability for CAPS was obtained. The intraclass correlation coefficient for the two raters was 0.98. Veterans with a diagnosis of alcohol abuse in the past 5 years were included in the A+ groups, while the A- groups consisted of veterans with no history of alcohol abuse. The 5-year cutoff was chosen because the interviewers did not probe back beyond 5 years based on the assumption that earlier reporting would be unreliable, especially in heavy drinkers. To describe quantitative drinking behavior, alcohol consumption was quantified by total cumulative drinks per year over the past 5 years. In addition, current alcohol consumption was assessed by cumulative drinks during the last month before the study. Information on alcohol consumption was obtained from the subject and by reviewing medical records. Veterans with past but not current PTSD or current subsyndromal PTSD, defined as exhibiting at least one symptom each from the three symptom clusters of PTSD, were excluded. Subjects were also excluded if they had a lifetime history of psychotic disorder or bipolar disorder, and drug abuse or dependence within the previous 6 months. Other exclusion criteria included a medical record of neurological illness, head trauma with loss of consciousness, medical disorders affecting brain function, and standard MRI exclusion criteria. A clinical neuroradiologist further reviewed the MRI scans for additional exclusionary neuropathological conditions, such as brain tumors, white matter lesions, and small vessel disease. Fifty-five participants with PTSD and 49 without PTSD were included in the study. The sample characteristics are summarized in Table 1. Those with PTSD suffered from traumas related to combat in Vietnam ($n=34$), combat in the 1992 Gulf War ($n=4$), other military events, e.g. accidents, imprisonment ($n=11$), and civilian events ($n=6$). Thirty-three of the 55 subjects with PTSD also reported exposure to childhood trauma. The majority of participants without PTSD also had a history of adulthood trauma exposure, including Vietnam combat ($n=4$), other military events ($n=10$) and civilian events ($n=22$). Only 13 subjects without PTSD had no traumatic event during adulthood. However, of those 13 subjects without adult trauma, two reported exposure to childhood trauma. Overall, 19 of the 49 subjects without PTSD reported childhood trauma. Combat exposure of veterans with or without PTSD was verified by reviewing military discharge records. The mean ages of PTSD-positive subjects with ($n=28$) and without ($n=27$) alcohol abuse in the past 5 years were 49.2 ± 7.1 years and 48.9 ± 8.6 years, respectively. The mean ages of PTSD-negative subjects with ($n=23$) and without ($n=26$)

alcohol abuse in the past 5 years were 43.2 ± 12.6 years and 47.8 ± 2.6 years, respectively. About 78% of the PTSD-positive subjects had a history of lifetime depression, compared with 40% in those negative for PTSD. Nearly 30% of PTSD-positive subjects also had episodes of current depression compared with 12% of those negative for PTSD. Psychiatric medications were used currently by 52% of those positive for PTSD and by 49% of those positive for alcohol abuse. In detail: Antidepressants were used by 64% of subjects in the P+/A+ group, 41% in the P+/A- group, 30% in the P-/A+ group, and 12% in the P-/A- group. Anticonvulsants were used by 14% in the P+/A+ group, 7% in the P+/A- group, and 4% in the P-/A+ group, but by no one in the P-/A- group. Hypnotics were used by 3% in the P+/A+ group, 11% in the P+/A- group, 9% in the P-/A+ group, and 11% in the P-/A- group. Finally, anti-psychotics were used by one subject in the P+/A+ group but by no one else. A total of 15 subjects across the groups were treated with more than one type of psychiatric medication. Subjects were recruited from the outpatient mental health clinic of the San Francisco Veterans Affairs Medical Center and from the community by advertisement. After a complete description of the study, all subjects voluntarily provided written informed consent that had been approved by the human research committees of the University of California and the VA Medical Center, San Francisco. Participants received \$140 for time and efforts spent completing the study.

2.2. MRI and ^1H MRSI acquisition

The subjects were scanned on a 1.5 T VISION™ MR system (Siemens Inc., Iselin, NJ). Structural MRI included a volumetric magnetization prepared rapid gradient echo (MPRAGE) sequence (TR/TE/TI=10/4/300 ms timing, flip angle=15°, inplane resolution=1.0×1.0 mm², 1.4-mm coronal slices), yielding T1-weighted images and an axially oblique dual spin echo sequence (TR/TE₁/TE₂=2500/20/80 ms, 1.0×1.4 mm² inplane resolution; 3-mm-thick slices without gap), yielding pseudo density and T2-weighted images. A Point Resolved Spectroscopy (PRESS) ^1H MRSI (Bottomley, 1987) sequence was used to acquire water-suppressed ^1H MR spectra simultaneously from the left and right hippocampal region, whereas a multislice ^1H MRSI sequence was used to acquire ^1H MR spectra from contiguous regions of the frontal and parietal lobes including surface cortex, which is difficult to accomplish with the box-like volume selection of PRESS. The timing of both PRESS and multislice MRSI was TR/TE=1800/135 ms. Spatial sampling with PRESS MRSI was accomplished using 24×24 circularly bounded encoding steps across a 210×210 mm² field of view, yielding a nominal inplane resolution of 8.5×8.5 mm². A 15-mm-thick slice was aligned approximately along the long axis of the hippocampus. Similarly, spatial sampling with multislice MRSI was accomplished using 36×36 circularly bounded encoding steps across a 280×280 mm² field of view, yielding a nominal spatial resolution of 7.8×7.8 mm² inplane. Two 15-mm-thick axially oblique slices were oriented at a 10 degree angle away from the anterior-posterior commissure line (Fig. 1).

2.3. Volume measurements of the hippocampus

Hippocampal determination was based on MPRAGE images and carried out semi-automatically using a high dimensional brain-mapping tool (Medtronic, Louisville, CO), which combines a coarse and then a fine transformation to match brain MR images with a reference anatomy template, in which the left and the right hippocampi are segmented. Hippocampal determination is guided by 22 manually placed landmarks around the hippocampal structure: one at the hippocampal head, one at the tail, and four per image (i.e. at the superior, inferior, medial and lateral boundaries) on five equally spaced images perpendicular to the long axis of the ipsilateral hippocampus. The steps are repeated for the contralateral hippocampus. A coarse transformation is first applied for initial landmark matching and then refined using a high-dimensional fluid image matching transformation for hippocampal morphometry. We (Hsu et al., 2002) and other researchers (Haller et al., 1997) independently validated this method by comparing hippocampal volumes of healthy subjects, patients with Alzheimer's disease, and

patients with schizophrenia with volumes obtained by tracing the hippocampus manually. Correlations between manual and semi-automated volume measurements were consistently better than 90%. Two readers performed the hippocampal measurements for this study, achieving 96% inter-rater reliability. In addition, an experienced reader (without knowledge of clinical information) visually reviewed the markings scan by scan and manually corrected misregistrations, further reducing potential discrepancies between manual and automated measurements. No hippocampal volume measurements had to be discarded.

2.4. Volume measurements of the major brain lobes

Volume measurements of gray matter and white matter of the major brain lobes were determined as follows: First, a probabilistic classification to segment MPRAGE images into gray matter, white matter, and CSF was used, as described and validated by Cardenas et al. (Cardenas et al., 2005). Second, a deformation algorithm (Studholme et al., 2003) was used to register individual MRI scans to a reference anatomy template, in which the brain hemispheres and the major lobes (frontal, temporal, parietal) were identified. The transformation was then inverted and applied to the template labels to demarcate subject-specific regions of interest on each MRI scan, yielding gray matter, white matter, and CSF volumes within each lobe region. Lastly, the volumes of the brain lobes and all CSF spaces were summed to obtain for each subject an index of the intracranial volume (ICV). All automated marked MRI scans were visually checked to insure that the automated markings were accurate. Data from seven subjects had to be excluded because of incorrect anatomical markings or incomplete brain coverage by MRI.

2.5. Spectral processing and MRI-MRSI co-analysis

Processing of ^1H MRSI data has been described before in detail (Schuff et al., 2001a). In brief, peak areas of NAA, creatine (Cr) and choline (Cho) containing compounds were estimated using fully automated spectral fitting software. For measurements of metabolite concentrations in the hippocampus and ACC, first the amounts of gray matter, white matter, and CSF in the MRSI voxels as well as the tissue contribution from the hippocampus and the ACC was estimated, based on information from tissue-segmented MRI data that were aligned to MRSI data. Then, the intensity of the metabolite signal was converted to metabolite concentration (in arbitrary units) by normalizing the metabolite signal to CSF intensity on density-weighted MRI data (first spin-echo image of the dual spin-echo data). To account for variable amounts of gray matter and white matter in MRSI voxels encompassing the hippocampus or the ACC, the gray/white matter volume fractions in each voxel were used as covariates in the analysis of metabolite concentrations. For metabolite measurements in the major brain lobes, linear regression was used to express variations of the metabolite signal from many MRSI voxels as a function of the gray/white matter volume fractions, as previously described (Schuff et al., 2001a). This approach yields estimates of metabolite concentrations for 100% gray matter and 100% white matter in each lobe. Overall, MRSI data from 19 subjects had to be discarded because of poor quality of MR spectra or difficulties in aligning MRSI to MRI.

2.6. Statistical analysis

Volumes (from MRI) and metabolites (from ^1H MRSI) were analyzed within a linear model, accounting for the effects of PTSD diagnosis, alcohol, and a PTSD by alcohol interaction. Age, gender, education, childhood trauma, lifetime depression, current depression and voxel-tissue composition of MRSI were added into the model as covariates when appropriate to account for these effects on the variability of the dependent measure. *F*-tests were used to determine if factors added explanatory power and were therefore appropriate for inclusion in the model. Three nested models were fitted by maximum likelihood: the first (base) model included all covariates plus presence/absence of alcohol abuse in the last 5 years and the subsequent models

added a PTSD effect and then a further PTSD by alcohol interaction, respectively. The resulting fits were compared sequentially via F -tests to determine whether PTSD, alcohol, or a PTSD by alcohol status interaction added significant ($P<0.05$) explanatory power to the base model. No corrections for multiple comparisons were applied for tests involving the hippocampus and the ACC, since the primary hypothesis of a PTSD effect related to both structures. For exploratory tests involving other brain structures than hippocampus and ACC, Bonferroni correction for multiple comparisons was applied.

3. Results

Demographic characteristics, PTSD severity, alcohol use in the past 5 years and current as well as presence of lifetime or current depression are summarized by group in Table 1. The groups, did not differ by age ($F_{3,100}=2.17$, $P>0.09$) or sex ($\chi^2>0.09$), but the P+A+ group had fewer years of education than the other groups ($F_{3,100}=4.8$, $P<0.004$). The results were similar for those with useable MRS data. As expected, the PTSD-positive group had higher CAPS scores than the negative groups ($F_{3,100}=123.9$, $P<0.001$), but differences of the CAPS between those with and without alcohol abuse were not significant. The groups differed substantially by alcohol consumption in the past 5 years ($F_{3,100}=14.1$, $P<0.001$) but not by current alcohol consumption ($F_{3,100}=1.5$, $P=0.2$). Furthermore, more subjects with PTSD than without PTSD had a history of lifetime depression ($F_{3,100}=7.0$, $P<0.001$) or suffered from current depression ($F_{3,100}=4.1$, $P<0.01$).

3.1. Brain volumes

Results from modeling volumes of the hippocampus and gray and white matter of the major lobes as functions of diagnostic and demographic measures are summarized in Table 2. Means and standard deviations of volume measures by group are listed in Table 4. PTSD alone was not significantly associated with smaller volumes of the left ($F_{6,97}=0.27$, $P>0.6$) or right ($F_{6,97}=0.46$, $P>0.5$) hippocampus. There was a strong association between increasing age and smaller hippocampal volume (left: $F_{6,97}=9.8$, $P=0.002$; right: $F_{6,97}=11.2$, $P<0.001$). There was a trend for smaller hippocampi in subjects exposed to childhood trauma (left: $F_{6,97}=3.2$, $P=0.08$; right: $F_{6,97}=3.3$, $P=0.07$). To further test if PTSD is associated with smaller hippocampal volume, we limited the analysis to the 24 subjects with severe PTSD symptoms (CAPS larger than 65), but still did not detect a significant effect. Dropping the women from the analysis also did not substantially alter the results. To investigate the cost of A+/A- dichotomization for detecting a PTSD effect, we also performed an analysis of the entire sample by using long-term alcohol-drinking behavior, expressed as total cumulative drinks per year, as a continuous variable, but still did not detect a significant effect of PTSD on hippocampal volume (both left and right side: $F_{4,104}=2.9$, $P>0.09$). Outside the hippocampus, we found a trend for smaller volumes of frontal lobe white matter in PTSD (right: $F_{7,89}=4.1$, $P=0.03$; left: $F_{7,89}=3.2$, $P=0.06$) after accounting for intracranial volume, age, sex, and child abuse and Bonferroni correction. No other associations between brain regions and PTSD were found, not even in those 24 subjects with severe PTSD (CAPS>65). Alcohol diagnosis as well as childhood trauma, or lifetime or current depression had no significant effect on brain volumes outside the hippocampus.

3.2. Brain metabolites

Results from modeling variations of regional NAA concentration as a function of diagnostic and demographic measures are presented in Table 3. Means and standard deviations of NAA measures by group are listed in Table 4. PTSD was associated with reduced NAA concentration in hippocampal regions (right: $F_{5,79}=5.8$, $P=0.02$; left: $F_{5,79}=5.6$, $P=0.02$) when accounting for Cr variations and age. The inclusion of Cr into the model for NAA added explanatory power ($P<0.001$). However, absolute NAA concentration, without accounting for Cr, was not

significantly reduced in PTSD (left: $F_{5,79}=0.3$; $P=0.6$; right: $F_{5,79}=1.1$, $P=0.3$). The findings for NAA, either absolute or relative to Cr, remained the same when the analysis was limited to the 17 subjects with severe PTSD symptoms (CAPS above than 65). Outside the hippocampal region, PTSD was associated with reduced NAA concentration in the ACC region (right: $F_{5,79}=4.4$, $P=0.04$; left: $F_{5,79}=4.9$, $P=0.03$) when accounting for Cr variations and age. The inclusion of Cr into the model added explanatory power ($P<0.001$). Furthermore, absolute NAA concentration, without accounting for Cr, remained significantly reduced in the right ACC in PTSD ($F_{5,79}=4.9$, $P=0.03$), while it became a trend in the left ACC ($F_{5,79}=2.1$, $P=0.09$). Treating long-term alcohol consumption as a continuous variable did not alter the results for the hippocampus and the ACC. Furthermore, childhood trauma or lifetime or current depression had no significant effect on metabolite concentrations, nor did we find a significant effect on metabolites from use of psychiatric medications. No other significant metabolite abnormalities were found in PTSD.

4. Discussion

We have two major findings: First, PTSD is associated with reduced NAA/Cr in the hippocampus in the absence of significant reduction of hippocampal volume. This is in agreement with previous MRSI findings in PTSD, but contrasts with some MRI studies that reported reduced hippocampal volumes in PTSD. Second, outside the hippocampus, PTSD is associated with reduced NAA concentration in the ACC. Collectively, these findings have implications for understanding abnormalities in cognition, emotion regulation, and both fear conditioning and fear extinction in PTSD.

The finding of reduced NAA/Cr in the hippocampal region in the absence of a significant reduction of hippocampal volume is in agreement with our previous MRSI study of Vietnam veterans with PTSD (Schuff et al., 2001b). However, we did not replicate our earlier observations of reduced absolute NAA concentration in the hippocampus in PTSD. Measurements of absolute metabolite concentration are notoriously challenging with MRSI. In addition, MRSI voxels unavoidably extend into adjacent non-hippocampal regions, because of the irregular shape of the hippocampus and poor spatial resolution of MRSI. Although we accounted for hippocampal tissue in MRSI voxels, this may not have eliminated regional contamination for different subjects. The result that hippocampal NAA is not reduced unless normalized to Cr implies that Cr is increased in PTSD. The biological underpinning of increased hippocampal Cr is unclear. Nonetheless, the finding of reduced NAA/Cr in the hippocampal region is consistent with many other spectroscopy reports in PTSD (Freeman et al., 1998; Villarreal et al., 2002b; Brown et al., 2003; Mohanakrishnan Menon et al., 2003).

We replicated our finding that hippocampal volume deficits seem less sensitive than metabolic alterations in PTSD (Schuff et al., 2001b). The association between PTSD and a smaller hippocampus remains controversial with several MRI studies reporting no evidence for smaller hippocampal volumes in PTSD (De Bellis et al., 1999; Bonne et al., 2001; Schuff et al., 2001b; Fennema-Notestine et al., 2002; Golier et al., 2005; Yehuda et al., 2007), while others found smaller hippocampi in PTSD (Bremner et al., 1995; Gurvits et al., 1996; Bremner et al., 1997; Gilbertson et al., 2002; Hedges et al., 2003; Woodward et al., 2006a) even when alcohol and drug abuse are non-existent (Emdad et al., 2006). The semi-automated method used for tracing the hippocampus is unlikely the explanation for the lack of volumetric findings, because results were visually inspected by an expert rater, who could manually adjust the markings if they were misregistered to the anatomical boundaries of the hippocampus. The manifestation of PTSD is also unlikely a reason for lack of volumetric findings, because we still did not detect smaller hippocampal volumes when limiting the analysis to those subjects with severe (CAPS>65) PTSD. The inclusion of a trauma control group could potentially be a reason for the lack of volumetric findings, because there is evidence of reduced hippocampal volumes in

trauma-exposed but PTSD-negative subjects (Karl et al., 2006). We conclude that hippocampal volumetry is less sensitive than metabolic measures in PTSD. However, we found a trend toward smaller hippocampi in subjects exposed to childhood trauma after accounting for PTSD, alcohol, age, gender, and depression, suggesting that developmental factors may play a prominent role in hippocampal size. Whether a small hippocampus is more characteristic for childhood trauma than for adult PTSD requires further investigation.

We also designed this study to address the potential impact of alcohol abuse, on the interpretation of structural and metabolic hippocampal differences in PTSD. Specifically, because of evidence from MRS that NAA can increase within 1 month of abstinence from alcohol after long-term alcohol dependence (Durazzo et al., 2006), we included only subjects without recent heavy drinking in the month before the study to contrast effects from PTSD on the brain with those from long-term alcohol abuse. The findings suggest that alcohol abuse over at least the past 5 years cannot explain the metabolic abnormalities in PTSD.

The finding of reduced NAA in the ACC region could have important theoretical and treatment implications. The ACC has extensive afferent connections to the hippocampus, the amygdala, and the sensory cortex. The ACC, in particular, is known for its role in selective attention (prioritizing stimuli) and for extinguishing conditioned fear responses. It has been argued that a failure of extinction (i.e. mediated by the ACC) could be a mechanism for persistent hyper-reactivity to trauma cues in PTSD (Hamner et al., 1999). Functional imaging has suggested altered ACC function in PTSD, and one study in particular suggested a diminished response in the ACC in the presence of emotionally relevant stimuli (viewing combat related words) in combat-related PTSD (Shin et al., 2001). There have been several previous studies examining metabolites in the ACC in PTSD subjects (Mahmutyazicioglu et al., 2005; Seedat et al., 2005). Our data are consistent with findings of reduced NAA/Cr of the ACC in abused children with PTSD (De Bellis et al., 2000a). Because we compared subjects with and without PTSD, our data further suggest that low NAA in the ACC is related to PTSD and not generally a consequence of exposure to traumatic stress. Moreover, we found normal NAA values globally in frontal lobe gray matter and white matter in PTSD, suggesting a selective abnormality of the ACC. Recent reports indicate that the ACC may also be structurally affected in PTSD (Yamasue et al., 2003; Woodward et al., 2006b). However, we have currently no validated protocol for parcellation of this region and could therefore not determine if our PTSD subjects had reduced ACC volumes. This will be the objective of future data analysis. Nevertheless, the NAA loss could imply neuronal damage to the ACC in PTSD that is beyond neuronal dysfunction as suggested by functional imaging data. This could have implications for treatment strategies in PTSD. For example, one model for the therapeutic benefit of exposure therapy, in which the patient confronts real life and imaginal reminders of a trauma, is that the ACC becomes more activated (responsive) and in turn inhibits amygdala-based fear responses to reminders of the trauma. With repetition, this process leads to the extinction of conditioned fear memories of the trauma and hence less terror, horror and helplessness. Such attempts to restore ACC function could be limited if the damage to the ACC in PTSD involves loss of neurons. One way to test this hypothesis would be to determine if NAA levels of the ACC predict response to exposure therapy.

The present study has several limitations: About 25% of the subjects without PTSD were never traumatized, but these individuals might go on to develop PTSD if traumatized. Hence, the sensitivity of the contrast to the PTSD group per se could be reduced. Alcohol consumption before the 5-year window surveyed may have produced permanent effects on the hippocampus or other brain structures. In addition, individual alcohol tolerance may have biased the findings. It cannot completely be ruled out that comorbid alcoholism facilitates the effects of PTSD on metabolites in the hippocampus and the ACC. We could not match the groups regarding lifetime and current depression. Depression may therefore explain in part some metabolite and

volume differences between the groups, despite efforts to account statistically for depression in our models for PTSD. Moreover, we did not collect detailed data on nicotine use, which could potentially confound metabolite differences between the groups.

In conclusion, this study replicates previous findings suggesting that metabolite measures are a more sensitive marker for neuronal abnormality in PTSD than volumetric measures of the brain. Moreover, the reduction of NAA in the ACC indicates that neuronal abnormalities in PTSD can extend outside the hippocampus. More studies, including prospective observations, are needed to clarify whether abnormalities are a function of exposure to traumatic stressors or premorbid factors that increase susceptibility to developing PTSD.

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Fig. 1. Slice arrangement of PRESS (A) and multislice (B) MRSI. Localizations of MRSI voxels in regions of the hippocampus (A and C) and anterior cingulate (B and D) are depicted in bold on the MRSI grids.

Table 1

Demographics

	P+A+	P+A-	P-A+	P-A-
Number of subjects	28	27	23	26
% Male	96	85	96	77
Age (years)	49.2±7.1	48.9±8.6	43.2±12.6	47.8±2.6
Education (years)	13.5±1.8 ^a	14.8±2.2	14.5±2.4	15.6±1.8
CAPS	66.4±20.8 ^b	63.3±16.2 ^b	5.6±6.3	1.3±2.6
Last 5 years drinks (total/year)	221±196 ^c	104±127	120±91 ^c	42±80
Current drinks ^d (total/month)	34±76	13±22	34±58	19±38
% Lifetime depression	78 ^b	78 ^b	52	27
% Current depression	25 ^e	35 ^e	16	8
Types of trauma ^f				
○ Vietnam War Combat	18	16	2	2
○ 1992 Gulf War Combat	1	3	0	0
○ Other Military events	6	5	5	5
○ Civilian events	4	2	12	10
○ No adulthood trauma	0	0	4	9
○ Childhood abuse	19	14	10	9

^a $P < 0.004$ P+A+ compared with other groups.

^b $P < 0.001$ P+ compared with P-.

^c $P < 0.001$ A+ compared with A-.

^d Last month prior to the study.

^e $P < 0.01$, P+ compared with P-.

^f Listed are the number of subjects in each trauma category; note some subjects are listed multiple times if they were exposed to several traumatic events.

Table 2
Predicted brain volumes in association with PTSD, alcohol abuse (A), PTSD*A interaction and other confounding factors

Dependent variable	Independent variables	Regression coefficients		Standard error		P-value	
		Left	Right	Left	Right	Left	Right
Hippocampus	Age	-8.3	-11.9	2.9	3.1	<0.006	<0.001
	Child abuse	-33.6	-36.8	18.9	20.3	0.08	0.07
	A	-21.4	-52.1	26.1	28.7	0.4	0.07
	PTSD	-11.2	-17.2	27.1	29.0	0.7	0.6
Frontal lobe white matter	PTSD*A	-40.7	-33.9	25.9	27.7	0.1	0.2
	Age	-7.9	-6.6	1.4	1.3	<0.001	<0.001
	A	0.8	2.4	13.1	12.1	0.9	0.8
	PTSD	-25.4	-25.7	12.9	11.9	0.06	0.03
	PTSD*A	15.9	16.2	12.3	11.3	0.2	0.2

Note: Variables are listed in the order they were entered into the models. Hippocampus and ICV results are from all 104 subjects; White matter results are from 97 subjects, who had complete lobar volume data. Results include adjustments for total intracranial volume (ICV) and sex.

Table 3
Predicted regional NAA concentration in association with PTSD, alcohol abuse (A), a PTSD*A interaction and other confounding factors

Dependent variable	Independent variables	Regression coefficients		Standard error		P-value	
		Left	Right	Left	Right	Left	Right
NAA, hippocampus	Creatine	14.1	13.4	1.0	0.9	<0.001	<0.001
	Age	-0.1	-0.3	0.1	0.1	0.1	<0.001
A	A	0.8	0.2	0.7	0.6	0.3	0.7
	PTSD	-2.1	-1.8	0.9	0.7	0.02	0.02
PTSD*A	PTSD*A	-0.5	0.3	0.8	1.1	0.5	0.8
	Creatine	10.4	12.7	2.3	1.7	<0.001	<0.001
NAA, anterior cingulated	Age	0.0	0.0	0.1	0.1	0.7	0.6
	A	0.2	0.1	0.3	0.1	0.4	0.8
PTSD	PTSD	-0.5	-0.7	0.2	0.3	0.03	0.04
	PTSD*A	-0.2	-0.2	0.2	0.3	0.4	0.4

Note: Hippocampal NAA includes adjustment for creatine variations while NAA in the anterior cingulate is independent of creatine variations. Variables are listed in the order they were entered into the models. MRSI results included 85 subjects, who had MRSI data of acceptable quality.

Table 4
Means and standard deviations of volumes and metabolite in subjects positive (P+) or negative (P-) for PTSD and with (A+) or without (A-) alcohol abuse

Group	Hippocampus volume ^a	Frontal WM volume ^a	Hippocampus NAA/Cr ^b	Anterior cingulate NAA ^c
P+A+	0.413±0.031	19.0±1.9	1.64±0.21	1.67±0.34
P+A-	0.413±0.051	18.7±1.8	1.70±0.18	1.71±0.32
P-A+	0.412±0.045	18.5±1.3	1.84±0.26	1.91±0.37
P-A-	0.428±0.046	18.7±2.4	1.80±0.17	1.93±0.33

^aTotal (left and right) volume in percent of intracranial volume.

^bLeft and right averaged.

^cConcentration in arbitrary units.