Do Morphological Differences in Brain Structures in PTSD Arise from a Familial Predisposition or from the Disorder Itself?

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ABSTRACT

The cingulate cortex, the hippocampus, and the amygdala have each been implicated in posttraumatic stress disorder (PTSD). This study examines morphological differences in these regions of interest in monozygotic twins discordant for trauma exposure and PTSD. We hypothesized that reduced volumes in the cingulate would represent acquired characteristics of PTSD and that reduced volumes in the hippocampus would represent a familial predisposition to the disorder. Subjects included identical twin pairs in which (1) the combat-exposed co-twin developed PTSD and the combat-unexposed co-twin did not; and (2) the combat-exposed co-twin never developed PTSD and the combat-unexposed cotwin also did not have PTSD. Differences between PTSD pairs and control pairs will indicate a familial predisposition to abnormalities in the cingulate, hippocampus, and amygdala. Differences between trauma-exposed and unexposed subjects will reflect characteristics acquired after trauma. If individuals with PTSD show significantly different volumes from their brothers as well as from subjects in control pairs, it will indicate that these brain abnormalities are an acquired characteristic of the disorder. In the right rostral and right dorsal anterior cingulate, we found main effects of PTSD Diagnosis in which PTSD twin pairs had larger volumes than non-PTSD pairs. In the right rACC, there was a PTSD Diagnosis x Exposure interaction in the rACC indicating that larger right rACC volumes may be an acquired characteristic of PTSD. Subjects with PTSD but not their brothers had significantly larger right rACC volumes than combat-exposed and unexposed controls. There was an inconsistent result in the left dACC that showed greater volumes in non-PTSD pairs. We also found larger posterior cingulate thickness non-PTSD pairs than PTSD pairs. There

was also a main effect of Exposure in the right posterior cingulate with unexposed subjects showing larger volumes than exposed subjects. There was a main effect of Exposure in the right hippocampus in which exposed subjects exhibited larger volumes than unexposed subjects. There were no significant effects in the amygdala. These findings overall did not support our hypotheses and conflict with previous literature on these regions of interest. These results require replication, however may be explained by low symptom severity, effects of treatment, or may demonstrate abnormalities found in unremitting forms of PTSD. The mixed results found in this investigation point to the need for further study of structural differences in these regions of interest.

INTRODUCTION

Posttraumatic stress disorder (PTSD) occurs in some individuals who experience an event that threatened death or serious injury and caused them to feel intense fear, horror, or helplessness. Symptoms include the subjective experience of reliving the trauma or disturbing dreams about the trauma, avoidance of situations that remind the patient of the trauma, the inability to remember important details about the trauma, hypervigilance and increased feelings of arousal (American Psychiatric Association, 2000). Several brain regions have been implicated in PTSD, including the anterior cingulate cortex (ACC), the amygdala, and the hippocampus (Shin and Handwerger 2009). Whether the abnormalities in these regions are acquired signs of PTSD or familial risk factors that predispose individuals to developing the disorder is at issue.

Amygdala:

The amygdala is involved in regulating behavior, assessing threat, emotional memory formation, and fear, pointing to its potential role in PTSD (Woon and Hedges, 2009). The fear responses present in PTSD may be due to abnormal structure or function of the amygdala.

Amygdala Structure in PTSD:

Investigations of volumetric differences in the amygdala have yielded mixed results. Some studies have found no differences in amygdala volume (Hara et al., 2008; Fennema-Notestine et al., 2002; Bonne et al., 2001; Bremner et al., 1997), and one meta-analysis examining nine research studies comparing trauma-exposed subjects with PTSD, trauma exposed subjects without PTSD, and trauma-unexposed subjects found no group differences in amygdala volume (Woon and Hedges, 2009). Conversely, in a quantitative meta-analysis of fifty studies (of the hippocampus and other regions including the amygdala,) Karl et al. (2006) found that subjects with PTSD did show smaller amygdala volumes compared to trauma exposed and unexposed controls. That Karl et al. (2006) used pediatric data whereas Woon and Hedges (2009) did not may account for the differences in findings. Whether differences in amygdala volume, when demonstrated, represent a familial risk factor or an acquired sign of PTSD is unknown.

Amygdala Function in PTSD:

According to one review by Koenigs and Grafman (2009), previous research suggests that heightened amygdala activation paired with failure of the medial prefrontal cortex to inhibit this response may contribute to some of the symptoms of PTSD. For

example, Shin et al. (2005) found that PTSD subjects exhibited heightened amygdala responsivity and lower medial prefrontal cortex responsivity when viewing images of overt facial expressions. Conversely, Gilboa et al. (2007) did not find evidence consistent with the idea that the ACC fails to inhibit an exaggerated amygdala response in subjects with PTSD. It is possible that failure of the ACC to inhibit a hyperresponsive amygdala may account for volumetric abnormalities in the amygdala, however the relationship between structure and function in the ACC and amygdala is not fully understood.

Anterior Cingulate Cortex (ACC):

The ACC is a medial prefrontal structure that is highly interconnected with the amygdala and is involved in extinction of fear responses (Shin et al., 2006). The ACC is split into several subdivisions, including the rostral anterior cingulate (rACC) and dorsal anterior cingulate (dACC) (Shin et al., 2009).

ACC Structure in PTSD:

Several neuroimaging studies have found smaller ACC volumes in PTSD than in control subjects, implying that the ACC may be failing to modulate fear responses (Woodward et al., 2005; Felmingham et al., 2009; Rauch et al., 2003; Kasai et al., 2008). In a study comparing combat veterans with PTSD to combat exposed veterans without PTSD, Woodward et al. (2005) found smaller rACC and dACC volumes in subjects with PTSD. Felmingham et al. (2009) also found significant reductions in the rACC compared to trauma exposed controls. In each study, the control group was trauma-exposed; there was no unexposed control group without PTSD. Consequently, these control groups may

represent a subgroup of resilient individuals who were resistant to PTSD. In an MRI study on female subjects who served as combat nurses in the Vietnam war, Rauch et al. (2003) found decreased pregenual ACC volumes in women with PTSD but no significant differences in the dACC. In a study on victims of sexual abuse, Kitayama et al. (2006) found smaller dACC volumes in PTSD subjects versus unexposed controls. In a voxel-based morphometry study, Kasai et al. (2008) found pregenual ACC grey matter loss in monozygotic twins with PTSD compared to their unexposed cotwins, leading them to conclude that these volumetric differences were acquired traits of the disorder.

ACC Function in PTSD:

The rostral ACC (rACC) is active during tasks with emotional stimuli and during emotional states (Shin et al., 2009). It is hyporesponsive when subjects with PTSD are shown emotional or trauma-related stimuli; hyporesponsivity in the rACC may also be related to higher symptom severity in PTSD (Shin et al., 2009). The dorsal ACC (dACC), however, appears to be hyperresponsive in PTSD and may be involved in fear learning (Shin et al., 2009; Milad et al., 2007).

One may expect functional differences to accompany structural differences; however, in a study examining functional connectivity in patients with PTSD versus healthy controls, Gilboa et al. (2004) found no evidence of failure of the ACC to inhibit a hyperresponsive amygdala. Conversely, one study by Shin et al. (2009) found abnormally increased resting metabolic activity in the dACC and mid cingulate cortex (MCC) in twin pairs discordant for PTSD; veterans with PTSD and their twins showed significantly higher resting metabolic activity in the dACC/MCC than twin pairs without PTSD,

suggesting that functional differences in the cingulate may constitute a familial risk factor. In addition, Lanius et al. (2001) found lowered activation in the ACC during script-driven imagery in subjects with PTSD compared to trauma-exposed controls.

Hippocampus:

The hippocampus is a medial temporal lobe structure that is involved in memory encoding and the processing of contextual cues during fear conditioning (Alvarez et al. 2008; Yoon et al., 2011) and retrieval of fear memories after extinction (Corcoran and Maren, 2001; Gilbertson et al., 2007). Several studies have examined the role of the hippocampus in fear memories, indicating a possible role for it in PTSD. In an fMRI study, Alvarez et al. (2008) found hippocampal activation in contextual fear conditioning. Yoon et al. (2011) found that hippocampal lesions in rats were associated with deficits in distinguishing between conditioned and unconditioned contexts. Corcoran and Maren (2001) used reversible inactivation of the hippocampus to demonstrate its role in the context-specific expression of fear memories.

Hippocampus Structure in PTSD:

Several studies have found smaller hippocampal volumes in PTSD compared to controls. A meta-analysis by Woon et al. (2010) found that PTSD patients and trauma exposed controls had smaller hippocampal volumes than unexposed subjects, suggesting that smaller hippocampal volume may be related to trauma exposure. They also concluded, however, that abnormalities in the hippocampus were more severe in the

PTSD group than in the trauma-exposed group. This study did not take into account trauma that may have occurred before combat exposure. Felmingham et al. (2009) found smaller hippocampi in subjects with PTSD versus trauma-exposed controls. They also found a negative association between time elapsed since trauma and grey matter volume in the right hippocampus.

Among the regions of interest presented here, the hippocampus has been most demonstrated to be linked with symptom severity. In addition to finding smaller ACC and amygdala volumes, a meta-analysis by Karl et al. (2006) showed that hippocampal volumes covaried inversely with symptom severity. Bonne et al. (2001) did not find differences in hippocampal volume in subjects with PTSD and concluded that reduced hippocampal size may be a feature of severe, unremitting PTSD.

Freeman et al. (2006) did not find smaller hippocampal volumes in former POWs with PTSD. This study was unique in that there was very little comorbidity among participants, indicating that this sample may have been made up of exceptionally resilient individuals. Jatzko et al. (2006) also did not find differences in hippocampal grey matter or white matter volume. They concluded that, where the mean time elapsed since trauma was 15 years, hippocampal changes had either disappeared or had not yet developed.

Abnormalities in the hippocampus may be linked to problems with fear extinction in PTSD (Milad et al., 2009). In an MRI study, Pohlack et al. (2011) demonstrated an association between smaller hippocampal volumes and decreased ability to distinguish between conditioned contexts in fear learning. Results of a study by Gilbertson et al. (2007) suggested that the hippocampus is involved in allocentric processing of contextual cues. Deficits in the hippocampus may help explain problems with fear extinction outside

of the original traumatic context; problems decoding contextual cues may inhibit the ability of people with PTSD to inhibit fear responses associated with individual cues such as the sound of a helicopter (Gilbertson et al., 2007).

Differences in hippocampal volume may be related to some of the cognitive difficulties that can accompany PTSD. Ramon et al. (2006) examined the link between cognitive difficulties and hippocampal volume in PTSD, but did not find a significant relationship. However, they did find smaller hippocampal volumes in the group with PTSD compared to trauma-exposed controls. Gilbertson et al. (2007) assessed subjects with PTSD used spatial processing tasks that may be related to the hippocampus. Monozygotic twin pairs in which the trauma exposed twin had PTSD performed lower on allocentric spatial processing tasks than twin pairs where the trauma exposed brother did not develop PTSD. These differences corresponded to differences in hippocampal volume, indicating that difficulties in spatial processing may accompany PTSD; that the differences appeared between twin pairs discordant for PTSD may indicate that differences in hippocampal volume and allocentric spatial processing may represent a familial risk factor.

Although there are several possible explanations for findings of decreased hippocampal volume in PTSD (Pitman, 2001), two competing suggestions about abnormalities in the hippocampus appear most in the literature: Smaller hippocampi may represent a familial predisposition to PTSD, or neurotoxicity related to the symptoms of PTSD may be associated with smaller hippocampi. In a voxel-based morphometry study, Kasai et al. (2008) found that subjects with PTSD showed reduced grey matter density in the hippocampus compared to their unexposed cotwins; they concluded that these

differences between twins pointed to smaller hippocampi as an acquired sign of PTSD. In a study of monozygotic twins discordant for combat exposure, Gilbertson et al. (2002) not only found smaller hippocampal volumes in twin pairs with one twin with PTSD, but also that the hippocampal volume of the unexposed cotwin in PTSD pairs predicted the symptom severity of the brother with PTSD. This indicated that smaller hippocampi may constitute a familial predisposition to PTSD. Differences in methods may account for the differing findings between Kasai et al. (2008) and Gilbertson et al. (2002); Kasai et al. (2008) used voxel-based morphometry whereas Gilbertson et al. (2002) did not. Woodward et al. (2006) found smaller hippocampal volumes only with comorbid alcoholism but could not rule out smaller hippocampus size as a predispositional factor for PTSD.

Hippocampus Function in PTSD:

The direction of findings on hippocampal activation in PTSD is not found as consistently as the direction of structural findings. In an fMRI study on amygdala and hippocampal function during encoding of negative and neutral pictures, Brohawn et al. (2010) found no differences in hippocampal activation between PTSD subjects and controls in response to negative words. They did find that PTSD subjects but not trauma-exposed control subjects showed a significant positive correlation between hippocampal and amygdala activation when presented negative words. Finally, the PTSD group showed greater hippocampal activation when assessing negative pictures that they remembered. A study by Shin et al. (2004) showed elevated regional cerebral blood flow

(rCBF) in subjects with PTSD and a positive association between symptom severity and rCBF.

In an MRI study on women with a history of childhood sexual abuse and current PTSD, Bremner et al. (2003) found both structural and functional deficits in the hippocampus in subjects with PTSD. In an fMRI study, Hayes et al. (2010) found that reduced hippocampal activity during presentation of trauma-related stimuli may be associated with abnormal encoding of trauma reminders to help maintain the disorder.

The current study:

With some exceptions, reduced volumes in the cingulate, amygdala, and hippocampus have been well established. The current investigation compares monozygotic twin pairs discordant for combat-exposure and PTSD to determine if volumetric differences are present in these three brain regions; if differences are present, this twin design will allow us to determine whether these differences represent a familial predisposition to PTSD or if they arise from the disorder itself. Results from Kasai et al. (2008) suggest that smaller rACC volumes in PTSD may represent an acquired characteristic of the disorder. Evidence from Gilbertson et al. (2002) suggests that smaller hippocampal volumes in PTSD may be the result of a familial predisposition.

We hypothesized that reduced volume in the rACC will represent an acquired characteristic of PTSD in line with Kasai et al. (2008). There will be volume reductions in the dACC associated with PTSD (Woodward et al., 2005; Kitayama et al., 2006). In line with Rauch et al (2003), there will be significant volume reductions in the SCC associated with PTSD. In line with Nardo et al (2010), there will be volume reductions in

the PCC in PTSD. Interconnectivity between the ACC and the amygdala suggests that, if differences in amygdala volumes are found, they also will represent an acquired characteristic of the disorder. Differences in hippocampal volume, however, have been associated with a familial predisposition to PTSD (Gilbertson et al., 2002), and we may expect to see similar results in the present study. In addition, we hypothesized that hippocampal volume will be inversely related to CAPS scores; the hippocampal volume of combat unexposed twins will be related to the symptom severity of their combat-exposed co-twins with PTSD in line with Gilbertson et al (2002).

METHODS

PARTICIPANTS:

Most subjects were originally recruited via the Vietnam Era Twin Registry (VETR) using the methods from Orr et al. (2003). Besides those subjects recruited via the VETR, additional twin pairs were recruited through letters sent to Vietnam veterans receiving disability compensation for PTSD from the Veterans Benefits Administration in Washington, DC. The letters asked recipients whether they had twin brothers who had not been exposed to combat in Vietnam and if they were willing to participate in the study. (Orr et al., 2003). Eighteen participants recruited using this method were included in our study. Overall, 90 participants were included in the current analyses.

Subjects were divided into four groups: Combat exposed veterans with current PTSD (P), their identical cotwins who were unexposed to combat and did not develop

PTSD (PC), combat exposed veterans who never had PTSD (C), and their identical cotwins who were unexposed to combat and did not have PTSD (CC). The twin pair in which the combat exposed twin developed PTSD is referred to as the "PTSD pair," and the twin pair in which the combat exposed twin did not develop PTSD is referred to as the "Control pair."

DEMOGRAPHICS:

The Clinician-Administered PTSD scale (CAPS) (Weathers et al., 2001) was used to determine PTSD diagnoses and to assess severity of symptoms of PTSD. Of subjects included in our cingulate analyses, 1 was ambidextrous (PC), 5 were left-handed (1 P, 2 PC, 2 CC), and 80 were right-handed. Of subjects included in our hippocampus and amygdala analyses, 1 was ambidextrous (PC), 5 were left-handed (1 P, 2 PC 2 CC) and 84 were right-handed.

Of the subjects included in our cingulate analyses (P n = 21, PC n = 21, C n= 22, CC n = 22), current comorbid disorders included panic disorder (2 P), simple phobia (2 P, 2 PC, 1 C), social phobia (3 P, 1 PC), GAD (1 P), MDD (5 P, 1 PC), dysthymia (4 P, 1 C, 2 CC), bipolar disorder (1 PC), alcohol abuse/dependence (1 P, 3 PC, 1 CC), substance abuse/dependence (3 P), and eating disorders (1 P). Data on diagnoses were not obtained for three subjects (1 PC, 1 C, 1 CC). Of the subjects included in the analyses of the hippocampus and amygdala (P n = 21, PC n = 21, C n = 24, CC n = 24), current comorbid disorders included panic disorder (2 P), simple phobia (2 P, 2 PC, 1 C), social phobia (3 P, 1 PC), GAD (1 P), MDD (5 P, 1 PC), dysthymia (4 P, 1 C, 2 CC), bipolar disorder (1 PC), alcohol dependence/abuse (1 P, 3 PC, 1 CC), substance dependence/abuse (3 P), and

eating disorders (1 P, 1 C). Three participants were missing data on other diagnoses (1 PC, 1 C, 1 CC).

Potentially confounding drugs or medications included antidepressants, benzodiazepenes, neuroleptics, anticonvulsants, sedatives, narcotics, antihistamines, sympathomimetics, sympatholytics, parasympathomimetics, parasympatholytics, skeletalmuscle relaxants, hypotensive agents, vasodilating agents, pressor agents, beta-blockers, antiarrhythmics, calcium channel blockers, other psychotherapeutic agents, cerebral stimulants, and hypnotics. (Shin et al., 2009).

Of the subjects included in our cingulate analyses, 15 were on SSRIs (9 P, 1 PC, 2 C, 3 CC), 10 were on other antidepressants (4 P, 2 PC, 1 C, 3 CC), 6 were on benzodiazepenes (3 P, 1 PC, 1 C, 1 CC), 4 were on antipsychotics (3 P, 1 PC), 3 were on anticonvulsants (1 P, 1 PC, 1 CC), two subjects were on sleep medication (1 P, 1 C), 6 were on opiates (4 P, 1 PC, 1 C), one subject was on a sympathomimetic (1 PC), 10 subjects were on sympatholytics (4 P, 1 PC, 2 C, 3 CC), 3 subjects were on skeletalmuscle relaxants (1 P, 1 PC, 1 CC), 13 were on ACE inhibitors (4 P, 5 PC, 2 C, 2 CC), 2 were on calcium channel blockers (cingulate analyses: 1 PC; hippocampus/amygdala analyses: 1 PC, 1 C), 2 were on antihypothyroid meds (1 P, 1 CC), 1 was on medication for HIV (1 PC), 4 were on antibiotics (1 P, 1 PC, 1 C, 1 CC), 23 were on antihyperlipidemia meds (5 P, 7 PC, 5 C, 6 CC), 20 were on NSAIDs (cingulate analyses: 6 P, 3 PC, 4 C, 6 CC; hippocampus/amygdala analyses: 6 P, 3 PC, 5 C, 6 CC), 6 were on diuretics (2 P, 2 PC, 1 C, 1 CC), 7 were on hypoglycemic (3 P, 2 PC, 2 CC), and 31 were on other medications.

Table 1a. Demographics Cingulate Analyses

	PTSD Pairs		Control Pairs	
	P (n= 21)	PC (n= 21)	C (n= 22)	CC (n= 22)
Age	56.7 (SD= 3.5)		56.7 (SD= 2.3)	
Education	13.4 (SD= 2.4)	13.7 (SD= 2.4)	13.8 (SD= 2.0)	13.8 (SD= 2.0)
CAPS	65.0 (SD= 21.7)	7.6 (SD= 13.7)	6.0 (SD= 8.6)	1.6 (SD= 3.8)
BDI	13.4 (SD= 10.9)	3.2 (SD= 4.3)	4.2 (SD= 4.5)	4.0 (SD= 5.2)
MAST	9.5 (SD= 5.4)	8.2 (SD= 5.2)	5.9 (SD= 2.0)	5.3 (SD= 1.4)

Table 1b. Demographics Hippocampus/Amygdala Analyses

	PTSD Pairs		Control Pairs	
	P (n= 21)	PC (n= 21)	C (n= 24)	CC (n= 24)
Age	56.7 (SD= 3.5)		57.0 (SD= 2.5)	
Education	13.4 (SD= 2.4)	13.7 (SD= 2.4)	14.0 (SD= 2.1)	13.9 (SD= 2.0)
CAPS	65.0 (SD= 21.7)	7.6 (SD= 13.7)	6.0 (SD= 8.4)	1.9 (SD= 4.0)
BDI	13.4 (SD= 10.9)	3.2 (SD= 4.3)	4.0 (SD= 4.4)	3.9 (SD= 5.0)
MAST	9.5 (SD= 5.4)	8.2 (SD= 5.2)	5.8 (SD= 2.0)	5.1 (SD= 1.5)

MAGNETIC RESONANCE IMAGING PROCEDURES:

We used a Siemens Medical Systems Symphony/Sonata 1.5-T whole-body high speed magnetic resonance imaging (MRI) scanner with a 3-axis gradient head coil (Shin et al., 2009). Expandable foam cushions limited head movement. We obtained high resolution structural MRI images (3- dimensional magnetization prepared rapid gradient-echo; repetition time/echo time/flip angle = 2.73 seconds/3.31 milliseconds/7°) with a 1.33-mm slice thickness after acquiring an automated scout image and completing shimming procedures to optimize field homogeneity (Shin et al., 2009).

Using the images obtained from the MRI, we examined the thickness, area, and volume of the rACC, subcallosal cingulate, dACC, and posterior cingulate . We also measured volumes in the hippocampus and the amygdala. The Center for Morphometric Analysis (CMA) conducted the segmentation and cortical parcellation. These methods differ from the voxel-based morphometry used by Kasai et al (2008) and have been described elsewhere (Rauch et al., 2003).

STATISTICAL ANALYSIS:

Analyses examining cingulate thickness, area, and volume included subjects in the P group (n = 21), the PC group (n= 21), the C group (n= 22) and the CC group (n= 22). Further analyses of the cingulate excluding pairs with CAPS scores below the mean P CAPS score (65) included 10 PTSD pairs and 22 control pairs. The final sample examining hippocampal and amygdala volume included 21 pairs in which the combat-exposed twin had PTSD and 24 control pairs in which the combat-exposed twin did not have PTSD. We ran additional analyses excluding participants with relatively low CAPS scores to reveal any effects that may have been masked by low symptom severity. Participants with CAPS scores below the mean P CAPS score (65) were excluded from these analyses. Analyses including only high CAPS participants included 10 PTSD pairs and 24 control pairs. In addition, we calculated brain volume in the cingulate, hippocampus, and amygdala as a percentage of whole brain volume. We used left and right cerebral exterior as our measure of whole brain volume, which included the cerebrum, cortex, subcortical nuclei, and lateral ventricles.

We used SPSS to run separate ANOVAs on each dependent variable, treating cotwins as repeated measures to find the main effect of PTSD Diagnosis, the main effect of Exposure and any interactions between PTSD Diagnosis and Exposure. A main effect of PTSD Diagnosis will indicate a familial predisposition to PTSD and a main effect of Exposure will indicate an association between brain abnormalities and trauma. PTSD Diagnosis x Exposure interactions will highlight differences both within twin pairs and between PTSD pairs and Control pairs. An interaction effect could indicate an acquired characteristic of the disorder. We ran additional repeated measures ANOVAs with cingulate, hippocampus and amygdala volumes calculated as percentages of cerebral exterior volumes. Where indicated, follow-up t-tests compared area, thickness, and volume of subdivisions of the cingulate in P vs C groups, PC vs. CC groups, P vs CC groups, and C vs PC groups. Follow-up t-tests also examined differences in these regions within pairs (P vs PC and C vs CC).

RESULTS

rACC Volume:

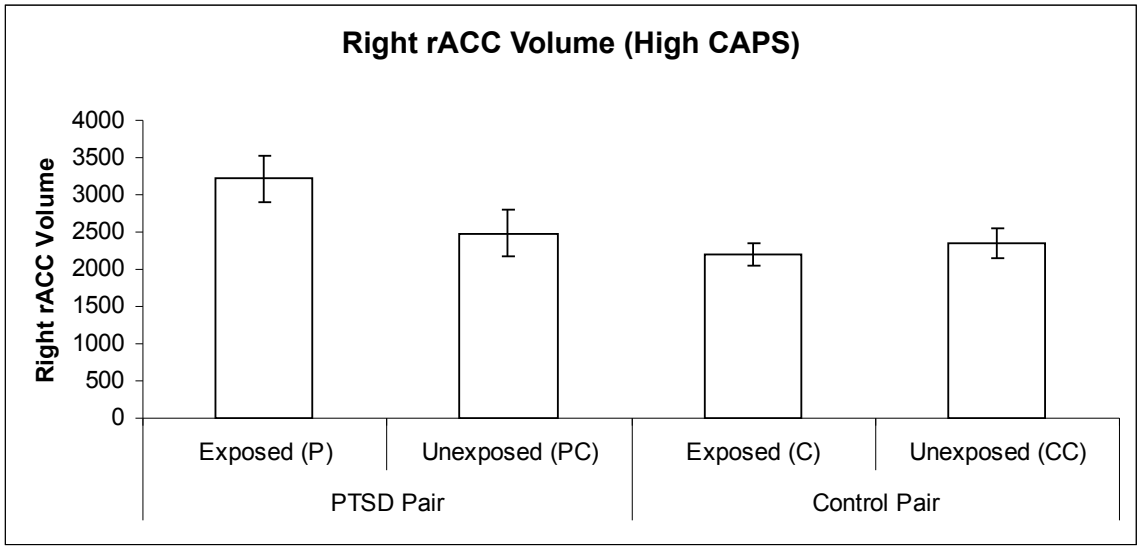
Including all participants:

A 2 (PTSD Diagnosis: PTSD, Control) x 2 (Exposure: Exposed, Unexposed) repeated measures ANOVA did not show significant differences in right rACC volume (all ps > 0.082). Results in the left rACC were also nonsignificant (all ps > 0.16). After adjusting for whole brain volume, there were no significant differences in the right rACC (all ps > 0.11) or left rACC (all ps > 0.16).

High CAPS participants:

A 2 (PTSD Diagnosis: PTSD, Control) x 2 (Exposure: Exposed, Unexposed) repeated measures ANOVA (Fig 1) showed a main effect of PTSD Diagnosis; means showed that right rACC volume was greater in PTSD pairs than control pairs (Table 2), ($F(1,30) = 4.812$, $p = .036$). An independent samples T test (Fig 1) showed that P subjects showed greater right rACC volume than C subjects (Table 2) ($t(30) = 3.316$, $p = .002$). Results of an independent samples T test comparing PC subjects with CC subjects showed no significant differences ($p = .11$).

Fig 1.



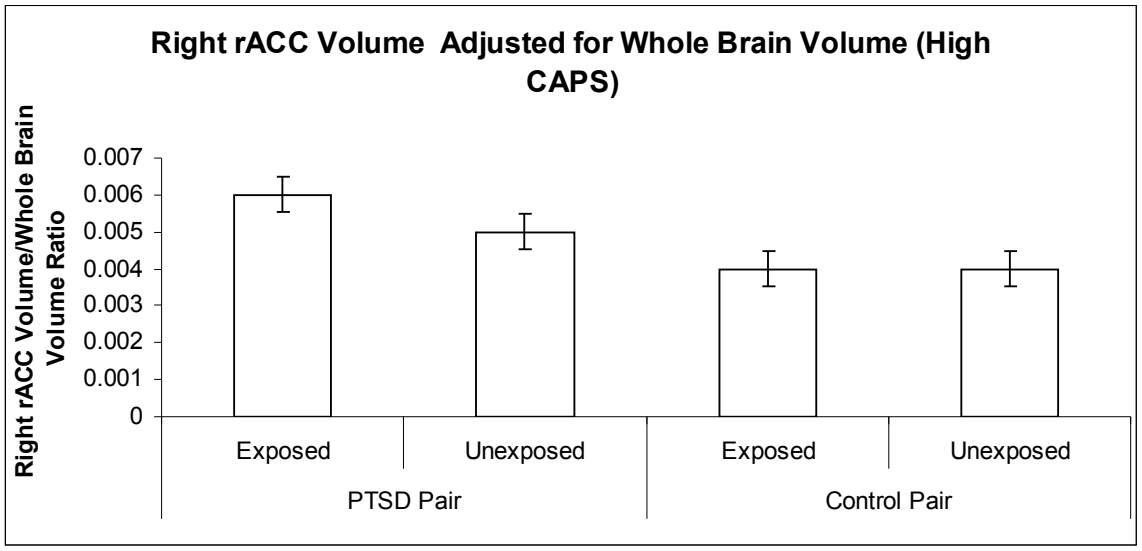
A 2 (PTSD Diagnosis: PTSD, Control) x 2 (Exposure: Exposed, Unexposed) repeated measures ANOVA (Fig 1) also showed an PTSD Diagnosis x Exposure interaction in the right rACC (Table 4), ($F(1, 30) = 4.573$, $p = .041$). Independent samples t tests showed that P subjects showed significantly greater right rACC volumes than C subjects (Table 1) ($t(30) = 3.316$, $p = 0.002$), and than CC subjects ($t(30) = 2.46$, $p = 0.02$). Independent samples t tests comparing P subjects vs PC subjects, C subjects vs CC

subjects, PC subjects vs CC subjects, and PC vs C subjects were all nonsignificant (all ps > 0.12). There was no significant main effect of Exposure (p = 0.16).

A 2 (PTSD Diagnosis: PTSD, Control) x 2 (Exposure: Exposed, Unexposed) repeated measures ANOVA showed no significant effects in the left rACC (all ps > 0.34).

For further analyses we calculated rACC volume as a percentage of whole brain volume. A 2 (PTSD Diagnosis: PTSD, Control) x 2 (Exposure: Exposed, Unexposed) repeated measures ANOVA (Fig 2) showed greater right rACC/whole brain ratio in PTSD groups than in control groups (Table 5) ($F(1, 30) = 4.322$, $p = .028$). An independent samples t test showed that P subjects showed significantly higher right rACC volumes than C subjects (Table 5) ($t(30) = 3.45$, $p = 0.002$). PC subjects' right rACC volumes were not significantly different from CC subjects (p= 0.582).

Fig 2.



There was a PTSD Diagnosis x Exposure interaction (Table 7) in right rACC volume after adjusting for whole brain volume (Fig 2) ($F(1, 30) = 4.322$, $p = 0.046$). Independent samples t tests (Table 5) showed that P subjects had significantly larger right rACC volume than C subjects ($t(30) = 3.45$, $p = .002$) and than CC subjects ($t(30) = 2.41$, $p = 0.022$). Independent samples t tests showed no significant differences between P subjects and PC subjects, between C subjects and CC subjects, between PC and CC subjects, or between C subjects and CC subjects (all ps > 0.16). There was no main effect of Exposure ($p = 0.22$).

No significant differences were found in the left rACC after adjusting for whole brain volume (all ps > 0.46).

rACC Thickness:

Including all participants:

A 2 (PTSD Diagnosis: PTSD, Control) x 2 (Exposure: Exposed, Unexposed) repeated measures ANOVA showed no significant results in the right rACC (all ps > 0.15) or in the left rACC (all ps > 0.71).

High CAPS participants:

A 2 (PTSD Diagnosis: PTSD, Control) x 2 (Exposure: Exposed, Unexposed) repeated measures ANOVA showed no significant differences in cortical thickness of the right rACC (all ps > .086). Results in the left rACC were also nonsignificant (all ps > . 28).

rACC Area:

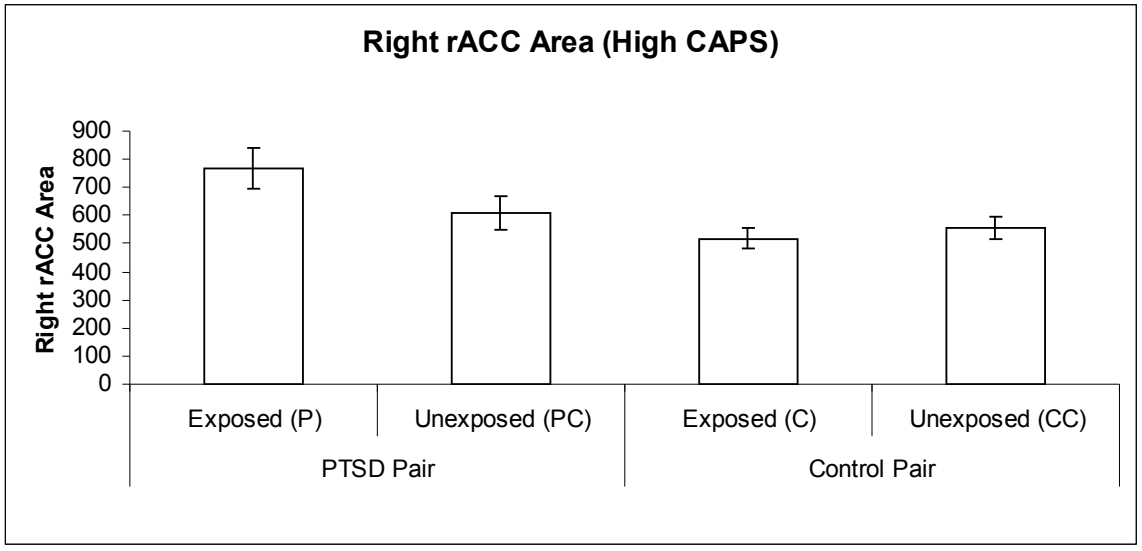
Including all participants:

A 2 (PTSD Diagnosis: PTSD, Control) x 2 (Exposure: Exposed, Unexposed) repeated measures ANOVA showed no significant differences in right rACC area (all ps > 0.09) or in left rACC area (all ps > 0.15).

High CAPS participants:

A 2 (PTSD Diagnosis: PTSD, Control) x 2 (Exposure: Exposed, Unexposed) repeated measures ANOVA (Fig 3) treating cotwins as repeated measures showed that PTSD pairs had greater right rACC area than control pairs, (Table 2) ($F(1, 30) = 7.829$, $p = .009$). An independent samples t test showed that P subjects had significantly larger right rACC area than C subjects (Table 2) ($t(30) = 3.54$, $p = 0.001$). Right rACC area was not significantly different between PC and CC subjects ($p = 0.49$).

Fig 3.



There was also an PTSD Diagnosis x Exposure interaction in the right rACC (Fig 3), ($F(1, 30) = 4.288$, $p = .047$). that showed a significant difference between P subjects and C subjects ($t(30)= 3.54$, $p = 0.001$) and between P subjects and CC subjects (Table 4) ($t(30) = 2.73$, $p = 0.01$). Independent samples t tests showed no significant differences between P subjects and PC subjects, between C subjects and PC subjects, between C subjects and CC subjects, or between CC subjects and PC subjects (all ps > 0.10). There was no main effect of Exposure ($p = 0.20$).

A 2 (PTSD Diagnosis: PTSD, Control) x 2 (Exposure: Exposed, Unexposed) repeated measures ANOVA showed no significant differences in area in the left rACC (all ps > 0.51).

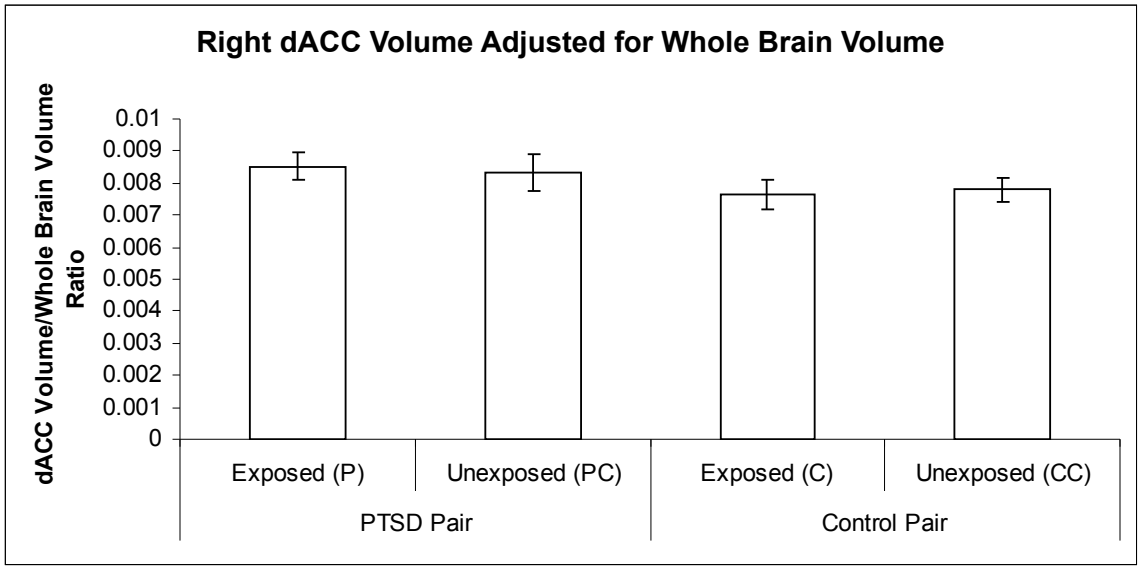
dACC Volume:

Including all participants:

There were no significant differences in the right dACC volume (all ps > 0.14) or left dACC volume (all ps > 0.38). After adjusting for whole brain volume, there was a trend toward a main effect of PTSD diagnosis in the right dACC (Table 5, Fig 4); PTSD pairs showed larger right dACC volumes than Control pairs, ($F(1, 41) = 2.96$, $p = 0.093$). A t test comparing P vs C right dACC/whole brain volume ratio was nonsignificant ($p = 0.73$). A t test comparing PC vs CC right dACC/whole brain volume ratio was also nonsignificant ($p = 0.18$). There was no main effect of Exposure ($p = 0.97$) or PTSD Diagnosis x Exposure interaction ($p = 0.73$) in the right dACC after adjusting for whole

brain volume. There were no significant differences in the left dACC (all ps > 0.30) after adjusting for whole brain volume.

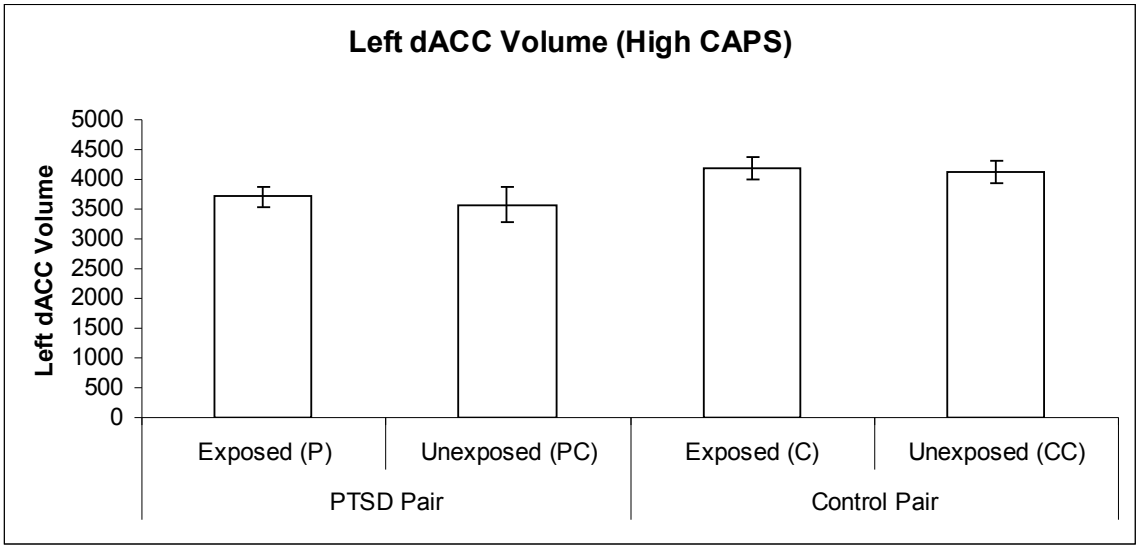
Fig 4



High CAPS participants:

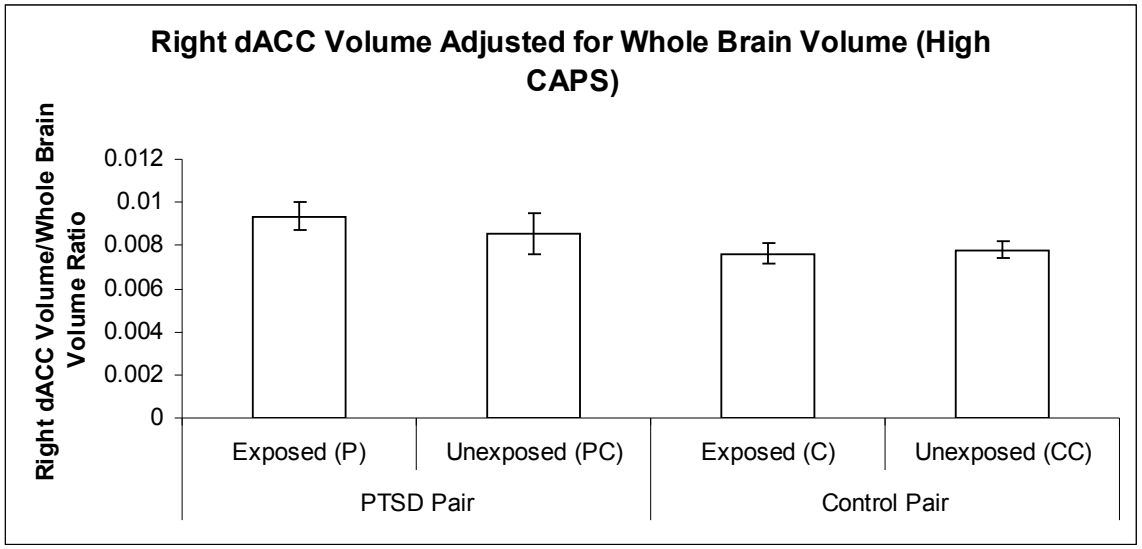
A 2 (PTSD Diagnosis: PTSD, Control) x 2 (Exposure: Exposed, Unexposed) repeated measures ANOVA showed no significant effects regarding right dACC volume (all ps > 0.08). For left dACC volume, a 2x2 repeated measures ANOVA (Fig 5) showed a main effect of PTSD Diagnosis in left dACC volume (Table 2): PTSD pairs showed smaller left dACC volumes than control pairs ($F(1, 30) = 4.580$, $p = .041$). T tests comparing left dACC volume in P subjects vs C subjects showed no significant results ($p = 0.13$). There were also no significant differences between PC subjects and CC subjects ($p = 0.12$). There was no main effect of Exposure ($p = 0.63$) or PTSD Diagnosis x Exposure interaction ($p = 0.91$).

Fig 5.



Additional analyses after adjusting for whole brain volume showed that PTSD pairs showed significantly larger right dACC volumes than Control pairs (Table 5, Fig 6) ($F(1,30) = 5.077$, $p = 0.034$). An independent samples t test showed that P subjects had greater right dACC volume than C subjects (Table 5) ($t(30) = 2.10$, $p = 0.044$). There was no significant difference in right dACC area between PC and CC subjects ($p = 0.38$). There was no main effect of Exposure ($p = 0.62$) or PTSD Diagnosis x Exposure interaction ($p = 0.46$).

Fig 6.



Analyses of left dACC volume adjusted for whole brain volume did not yield significant results (all ps > .13).

<u>dACC Thickness:</u>

Including all participants:

There were no significant differences in thickness in the right dACC (all ps > 0.34) or in the left dACC (all ps > 0.61).

High CAPS participants:

There were no significant differences in thickness in the right dACC (all ps > 0.39) or in the left dACC (all ps > 0.18).

<u>dACC Area:</u>

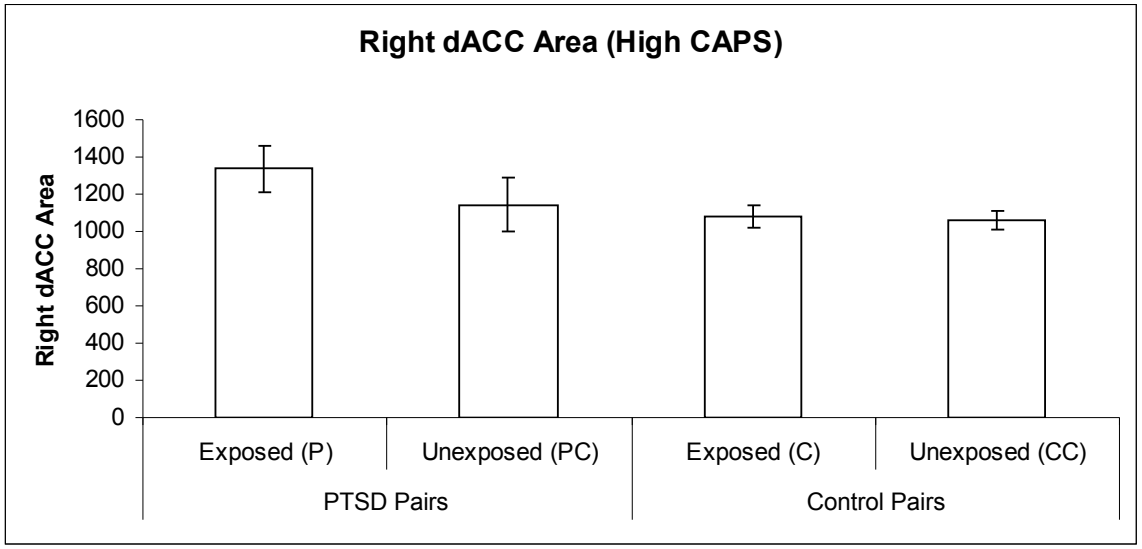
Including all participants:

A 2 (PTSD Diagnosis: PTSD, Control) x 2 (Exposure: Exposed, Unexposed) repeated measures ANOVA showed no significant differences in area in the right dACC (all ps > 0.16) or in the left dACC (all ps > 0.66).

High CAPS participants:

A 2 (PTSD Diagnosis: PTSD, Control) x 2 (Exposure: Exposed, Unexposed) repeated measures ANOVA (Fig 7) showed a trend in main effect of PTSD Diagnosis in the right dACC area that did not reach significance (Table 2): PTSD pairs' mean right dACC area was larger than that of Control pairs ($F(1, 30) = 3.799$, $p = .061$). An independent samples t test (Table 2, Fig 7) showed that the area of the right dACC was greater in P subjects than in C subjects, ($t(30) = 2.111$, $p = .043$).An independent samples t test showed no significant difference in right dACC area between PC subjects and CC subjects ($p = 0.49$). There was no main effect of Exposure ($p = 0.22$) or PTSD Diagnosis x Exposure interaction ($p = 0.30$).

Fig 7.



A 2 (PTSD Diagnosis: PTSD, Control) x 2 (Exposure: Exposed, Unexposed) repeated measures ANOVA did not show significant results in left dACC area (all ps > 0.14).

SCC Volume:

Including all participants:

2 (PTSD Diagnosis: PTSD, Control) x 2 (Exposure: Exposed, Unexposed) repeated measures ANOVAs showed no significant differences in volume in the right SCC (all ps > 0.53) or the left SCC (all ps > 0.22). After adjusting for whole brain volume there were no significant differences in the right SCC (all ps > 0.39) or the left SCC (all ps > 0.29).

High CAPS participants:

2 (PTSD Diagnosis: PTSD, Control) x 2 (Exposure: Exposed, Unexposed) repeated measures ANOVAs showed no significant differences in volume in the right SCC (all ps > 0.31) or the left scACC (all ps > 0.62). After adjusting for whole brain volume there were no significant differences in the right SCC (all ps > 0.48) or the left SCC (all ps > 0.71).

SCC Thickness:

Including all participants:

2 (PTSD Diagnosis: PTSD, Control) x 2 (Exposure: Exposed, Unexposed) repeated measures ANOVAS showed no significant differences in thickness in the right SCC (all ps > 0.46) or in the left SCC (all ps > 0.68).

High CAPS participants:

2 (PTSD Diagnosis: PTSD, Control) x 2 (Exposure: Exposed, Unexposed) repeated measures ANOVAs showed no significant differences in thickness in the right SCC (all ps > 0.20) or in the left SCC (all ps > 0.54).

SCC Area:

Including all participants:

2 (PTSD Diagnosis: PTSD, Control) x 2 (Exposure: Exposed, Unexposed) repeated measures ANOVAs showed no significant differences in area in the right SCC (all ps > 0.55) or left SCC (all ps > 0.18).

High CAPS participants:

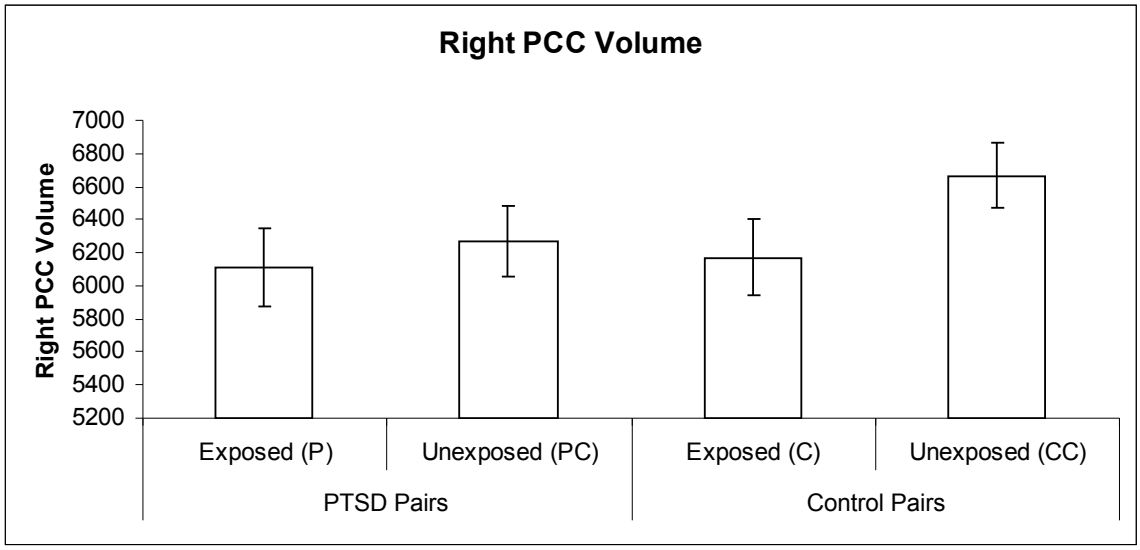
2 (PTSD Diagnosis: PTSD, Control) x 2 (Exposure: Exposed, Unexposed) repeated measures ANOVAs showed no significant differences in area in the right SCC (all ps > 0.50) or left SCC (all ps > 0.41).

PCC Volume:

Including all participants:

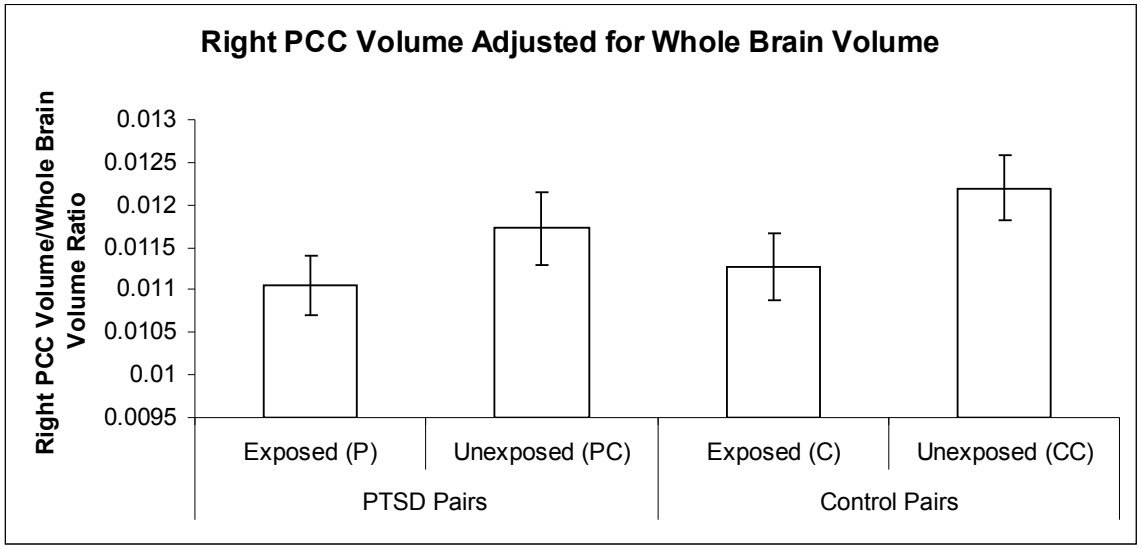
A 2 (PTSD Diagnosis: PTSD, Control) x 2 (Exposure: Exposed, Unexposed) repeated measures ANOVA (Fig 8) showed a difference in right PCC volume that did not reach significance (Table 2) ($F(1, 41) = 3.645$, $p = 0.063$). Combat-exposed subjects showed smaller right PCC volumes than combat-unexposed subjects. Independent samples t tests showed no significant differences between P and PC subjects ($p = 0.96$), and C and CC ($p = 0.12$). There was no main effect of PTSD Diagnosis ($p = 0.38$) or PTSD Diagnosis x Exposure interaction ($p = 0.33$). There were no significant differences in left PCC volume (all ps > 0.25).

Fig 8.



After adjusting for whole brain volume, a 2 (PTSD Diagnosis: PTSD, Control) x 2 (Exposure: Exposed, Unexposed) repeated measures ANOVA (Fig 13) showed that combat-exposed subjects had smaller right PCC volumes than combat-unexposed subjects (Table 6) ($F(1, 41) = 6.609$, $p = 0.014$). Independent samples t tests showed no significant differences in right PCC volume between P subjects vs PC subjects ($p = 0.23$) or between C subjects vs CC subjects ($p = 0.10$). There was no main effect of PTSD Diagnosis ($p = 0.45$) or PTSD Diagnosis x Exposure interaction ($p = 0.68$).

Fig 9.

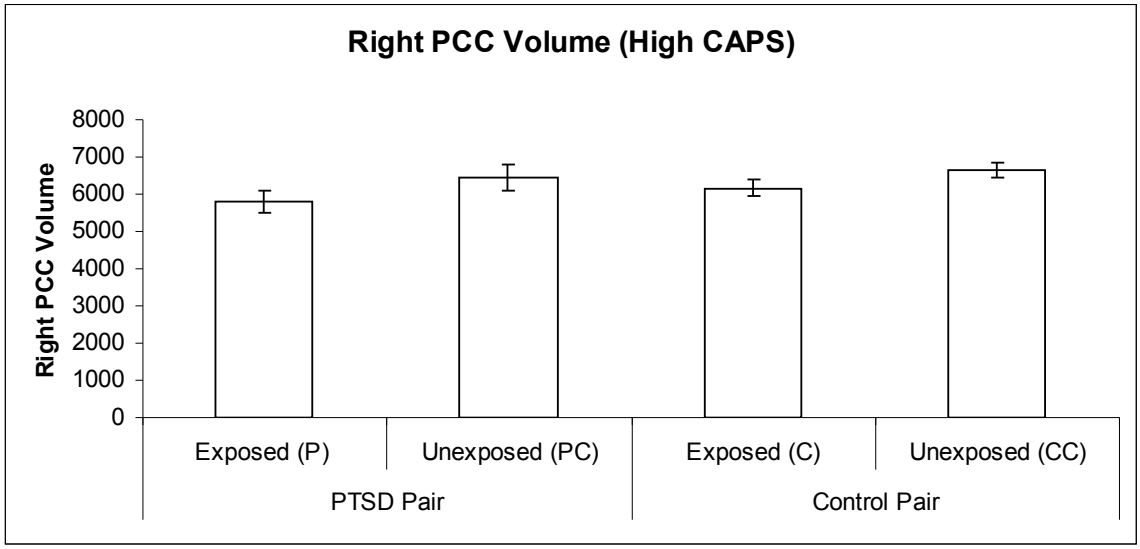


Results for the left PCC remained nonsignificant after adjusting for whole brain volume (all ps > 0.27).

High CAPS participants:

A 2 (PTSD Diagnosis: PTSD, Control) x 2 (Exposure: Exposed, Unexposed) repeated measures ANOVA (Fig 10) showed that subjects exposed to combat had smaller right PCC volume than subjects unexposed to combat (Table 3) ($F(1, 30) = 7.527$, $p = .01$). Independent samples t tests showed no significant differences in right PCC volumes between P subjects and PC subjects ($p = 0.17$) or between C subjects and CC subjects ($p = 0.11$). There was no main effect of PTSD Diagnosis ($p = 0.36$) or PTSD Diagnosis x Exposure interaction ($p = 0.69$).

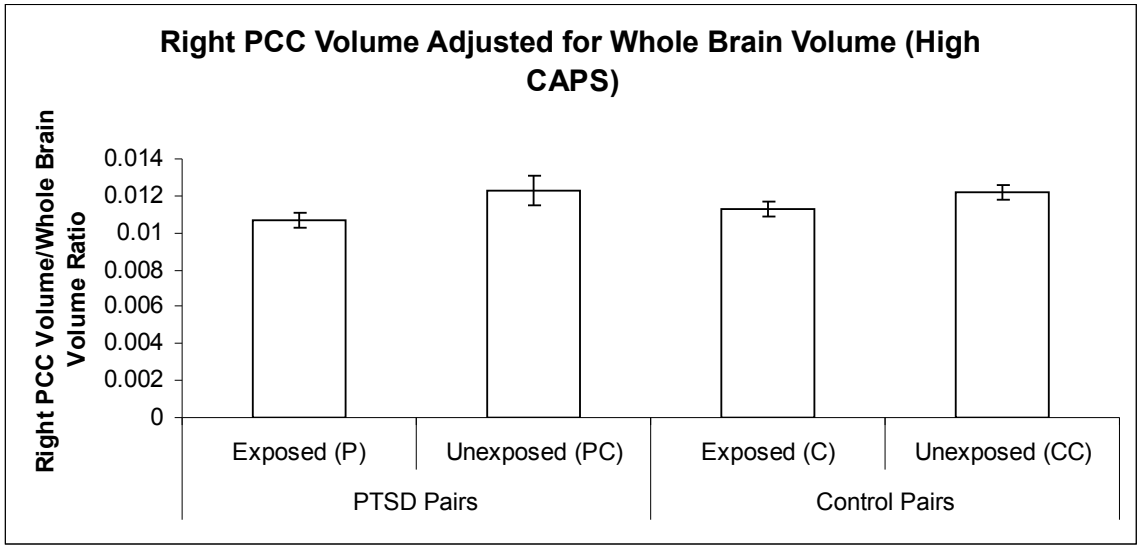
Fig 10.



A 2 (PTSD Diagnosis: PTSD, Control) x 2 (Exposure: Exposed, Unexposed) repeated measures ANOVA showed no significant differences in left PCC volume (all ps > 0.31).

After adjusting for whole brain volume, a 2 (PTSD Diagnosis: PTSD, Control) x 2 (Exposure: Exposed, Unexposed) repeated measures ANOVA (Fig 11) showed that combat-exposed subjects had smaller right PCC volumes than combat-unexposed subjects (Table 6) ($F(1, 30) = 9.833$, $p = 0.004$). Independent samples t tests showed no significant differences in right PCC volume between P subjects and PC subjects ($p = .09$) or between C subjects and CC subjects ($p = 0.10$). There was no main effect of PTSD Diagnosis ($p = 0.68$) or PTSD Diagnosis x Exposure interaction ($p = 0.41$).

Fig 11.



A 2 (PTSD Diagnosis: PTSD, Control) x 2 (Exposure: Exposed, Unexposed) repeated measures ANOVA showed no significant differences in left PCC volume after adjusting for whole brain volume (all ps > 0.35).

PCC Thickness:

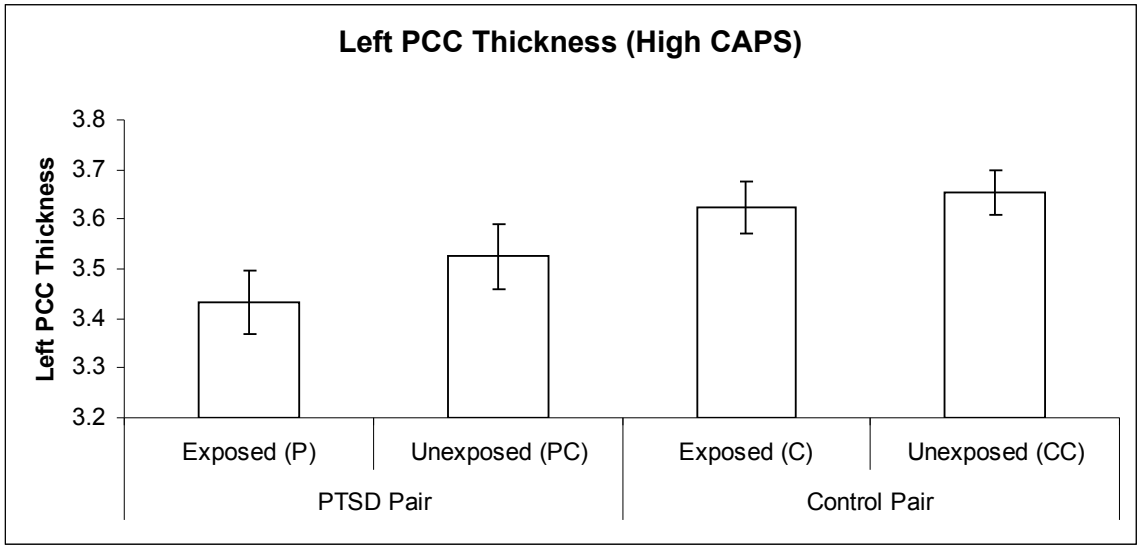
Including all participants:

A 2 (PTSD Diagnosis: PTSD, Control) x 2 (Exposure: Exposed, Unexposed) repeated measures ANOVA showed no significant differences in right PCC thickness (all ps > .26) or left PCC thickness (all ps > 0.10).

High CAPS participants:

A 2 (PTSD Diagnosis: PTSD, Control) x 2 (Exposure: Exposed, Unexposed) repeated measures ANOVA showed no significant effects in right PCC thickness (all ps > 0.13), however PTSD pairs had thinner cortex in the left PCC than Control pairs (Table 2, Fig 12), ($F(1, 30) = 5.43$, $p = .027$). Follow-up t-tests (Fig 12) showed that P subjects had thinner cortex in the left PCC than C subjects, ($t(30) = -2.191$, $p = 0.036$). There were no significant differences between PC subjects and CC subjects ($p = 0.11$). There was no main effect of Exposure ($p = 0.21$) or PTSD Diagnosis x Exposure interaction ($p = 0.52$).

Fig 12.



PCC Area:

Including all participants:

A 2 (PTSD Diagnosis: PTSD, Control) x 2 (Exposure: Exposed, Unexposed) repeated measures ANOVA showed no significant differences in right PCC area (all ps > 0.13) or left PCC area (all ps > 0.15).

High CAPS participants:

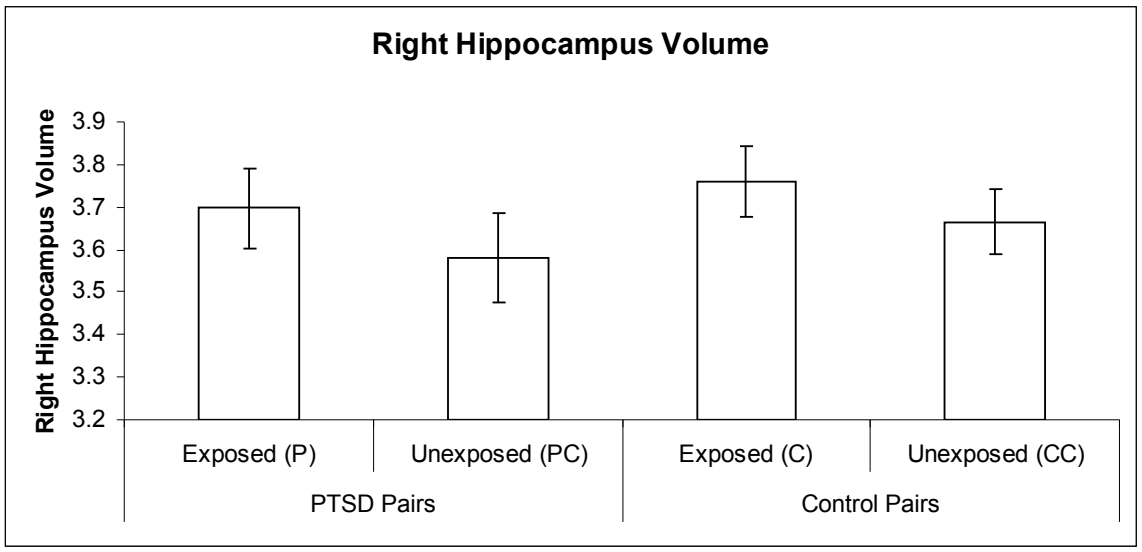
A 2 (PTSD Diagnosis: PTSD, Control) x 2 (Exposure: Exposed, Unexposed) repeated measures ANOVA showed no significant differences in right PCC area (all ps > 0.07) or left PCC area (all ps > 0.19).

Hippocampus Volume:

Including all participants:

A 2 (PTSD Diagnosis: PTSD, Control) x 2 (Exposure: Exposed, Unexposed) repeated measures ANOVA (Fig 13) showed a main effect of Exposure in hippocampal volumes. Subjects exposed to combat showed greater volume in the right hippocampus (Table 3) than subjects unexposed to combat, ($F(1, 43) = 6.277$, $p = .016$). This effect held after removing subjects taking SSRI's ($F(1, 28) = 5.476$, $p = 0.027$). Independent samples t tests showed no significant differences between P subjects and PC subjects ($p = 0.41$) or between C subjects and CC subjects ($p = 0.41$). There was no main effect of PTSD Diagnosis ($p = 0.53$) or PTSD Diagnosis x Exposure interaction ($p = 0.79$). This held after removing subjects taking SSRI's (all p's > 0.24).

Fig 13.



A 2 (PTSD Diagnosis: PTSD, Control) x 2 (Exposure: Exposed, Unexposed) repeated measures ANOVA showed no significant differences in left hippocampus volume (all ps > 0.19). This held after removing subjects taking SSRI's (all p's > 0.08).

After adjusting for whole brain volume, 2 (PTSD Diagnosis: PTSD, Control) x 2 (Exposure: Exposed, Unexposed) repeated measures ANOVAS showed no significant differences in right hippocampus volume (all ps > 0.30) or left hippocampus volume (all ps > 0.61). This held after removing subjects taking SSRI's in the right (all p's > 0.19) and left (all p's > 0.43) hippocampi.

High CAPS participants:

2 (PTSD Diagnosis: PTSD, Control) x 2 (Exposure: Exposed, Unexposed) repeated measures ANOVAS showed no significant differences in right hippocampus volume (all ps > 0.07) or left hippocampus volume (all ps > 0.43).

After adjusting for brain volume, 2 (PTSD Diagnosis: PTSD, Control) x 2 (Exposure: Exposed, Unexposed) repeated measures ANOVAs showed no significant differences in right hippocampus volume (all ps > 0.40) or left hippocampus volume (all ps > 0.42).

Amygdala Volume:

Including all participants:

2 (PTSD Diagnosis: PTSD, Control) x 2 (Exposure: Exposed, Unexposed) repeated measures ANOVAs showed no significant volumetric differences in the right amygdala (all ps > 0.30) or left amygdala (all ps > 0.57).

After adjusting for whole brain volume, 2 (PTSD Diagnosis: PTSD, Control) x 2 (Exposure: Exposed, Unexposed) repeated measures ANOVAS showed no significant differences in the right amygdala (all ps > 0.16) or left amygdala (all ps > 0.34).

High CAPS participants:

2 (PTSD Diagnosis: PTSD, Control) x 2 (Exposure: Exposed, Unexposed) repeated measures ANOVAs showed no significant volumetric differences in the right amygdala (all ps > 0.37) or left amygdala (all ps > 0.60).

After adjusting for whole brain volume, 2 (PTSD Diagnosis: PTSD, Control) x 2 (Exposure: Exposed, Unexposed) repeated measures ANOVAS showed no significant

volumetric differences in the right amygdala (all ps > 0.16) or left amygdala (all ps > 0.37).

Anterior Amygdala Volume:

Including all participants:

2 (PTSD Diagnosis: PTSD, Control) x 2 (Exposure: Exposed, Unexposed) repeated measures ANOVAs showed no significant volumetric differences in the anterior portion of the right amygdala (all ps > 0.32) or the anterior portion of the left amygdala (all ps > 0.25).

After adjusting for whole brain volume, 2 (PTSD Diagnosis: PTSD, Control) x 2 (Exposure: Exposed, Unexposed) repeated measures ANOVAS showed no significant differences in the anterior portion of the right amygdala (all ps > 0.28) or the anterior portion of the left amygdala (all ps > 0.25).

High CAPS participants:

2 (PTSD Diagnosis: PTSD, Control) x 2 (Exposure: Exposed, Unexposed) repeated measures ANOVAs showed no significant volumetric differences in the anterior portion of the right amygdala (all ps > 0.30) or the anterior portion of the left amygdala (all ps > 0.24).

After adjusting for whole brain volume, 2 (PTSD Diagnosis: PTSD, Control) x 2 (Exposure: Exposed, Unexposed) repeated measures ANOVAS showed no significant differences in the anterior portion of the right amygdala (all ps > 0.35) or the anterior portion of the left amygdala (all ps > 0.26).

Table 2

	Main effect of PTSD Diagnosis		
	Region	**M of PTSD Pairs**	**M of Non PTSD Pairs**
PTSD Pairs > Non PTSD Pairs	Right rACC Volume (High CAPS)	2849.5	2278.818
	Right rACC Area (High CAPS)	686.5	536.523
	Right dACC Area (High CAPS) Trend	1239.6	1069.318
Non PTSD Pairs > PTSD Pairs	Left PCC Thickness (High CAPS)	3.48	3.64
	Left dACC Volume (High CAPS)	3641.45	4156.409
	Within Unexposed Twins		
	Region	**M of PTSD**	**M of Non PTSD**
PTSD > Non PTSD	None	None	None
Non PTSD > PTSD	None	None	None
	Within Exposed Twins		
	Region	**M of PTSD**	**M of Non PTSD**
PTSD > Non PTSD	Right rACC Volume (High CAPS)	3214	2207.64
	Right rACC Area (High CAPS)	767.1	518.5
	Right dACC Area (High CAPS) Trend	1336	1078.18
Non PTSD > PTSD	Left PCC Thickness (High CAPS)	3.43	3.62

Table 3

	Main Effect of Exposure Means		
	Region	**M of Exposed**	**M of Unexposed**
Exposed > Unexposed	Right Hippocampus Volume	3.73	3.63
Unexposed > Exposed	Right PCC Volume (High CAPS)	5979.641	6560.114
	Right PCC Volume Trend	6141.317	6468.197

Table 4

PTSD Diagnosis x Exposure Interaction Means

	PTSD		Control	
Region	**Exposed**	**Unexposed**	**Exposed**	**Unexposed**
Right rACC Volume (High CAPS)	3214	2485	2207.636	2350
Right rACC Area (High CAPS)	767.1	605.9	518.5	554.545

Table 5

Main Effect of Diagnosis after Adjustment for Whole Brain Volume

	Region	M of PTSD Pairs	M of Non PTSD Pairs
PTSD Pairs > Non PTSD Pairs	right rACC (High CAPS)	0.005	0.004
	right dACC (High CAPS)	0.009	0.008
	right dACC Trend	0.008	0.008
Non PTSD Pairs > PTSD Pairs	None	None	None
	Within Unexposed Twins		
		M of PTSD	M of Non PTSD
PTSD > Non PTSD	None	None	None
Non PTSD > PTSD	None	None	None
	Within Exposed Twins		
		M of PTSD	M of Non PTSD
PTSD > Non PTSD	right rACC (High CAPS)	0.006	0.004
	right dACC (High CAPS)	0.009	0.008
Non PTSD > PTSD	None	None	None

Table 6

Main Effect of Exposure after Adjustment for Whole Brain Volume

	Region	M of Exposed	M of Unexposed
Exposed > Unexposed	None	None	None
Unexposed > Exposed	right PCC (High CAPS)	0.011	0.012
	right PCC	0.011	0.012

Table 7

PTSD Diagnosis x Exposure Interaction after Adjustment for Whole Brain Volume

		PTSD		Control	
	Region	**Exposed**	**Unexposed**	**Exposed**	**Unexposed**
Exposed > Unexposed	right rACC (High CAPS)	0.006	0.005	0.004	0.004
Unexposed > Exposed	None	None	None	None	None

ADDITIONAL ANALYSES RESULTS

Additional Analyses Excluding Two Ineligible Subject Pairs:

We ran additional analyses excluding two ineligible pairs. These included one PTSD Pair in which participants were dizygotic and one Control pair in which the unexposed cotwin had non-military related PTSD. These analyses yielded some results that differed from analyses including these pairs.

rACC Volume:

Including all participants:

Results in rACC volume and rACC volume/whole brain volume ratio were similar before and after excluding ineligible subjects.

High CAPS participants:

Two significant effects from the original analyses became trends after excluding ineligible subjects. Results of a repeated measures ANOVA showed a trend toward a main effect of PTSD Diagnosis. PTSD pairs (M = 2828.611) showed significantly greater right rACC volume than Control pairs (M = 2343.143), $F(1, 28) = 3.46$, $p = 0.073$. There was also a trend toward a PTSD Diagnosis x Exposure Interaction (P M = 3209.889, PC M = 2447.333, C M = 2277.476, CC M = 2408.810) ($F(1, 28) = 4.14$, $p = 0.052$). Independent samples t tests showed that P subjects (M = 3209.89) showed greater right rACC volume than C subjects (M = 2277.48) ($t(28) = 3.03$, $p = 0.005$) and than CC subjects (M = 2408.81) ($t(28) = 2.18$, $p = 0.04$). There were no significant differences between P and PC groups, between PC and C groups, between PC and CC groups, and between C and CC groups (all p's >0.14).

Results in the left rACC were similar to results from the original analyses.

A significant effect in the right rACC from the original analyses after adjusting for whole brain volume became a trend after excluding ineligible subjects. There was a trend toward a PTSD Diagnosis x Exposure interaction (P M = -.006, PC M = 0.005, C M = 0.04, CC = 0.004), ($F(1,28) = 3.97$, $p = 0.056$). Follow-up t tests showed that P subjects showed greater right rACC volume than C subjects ($t(28) = 3.22$, $p = 0.003$) and than CC subjects ($t(28) = 2.19$, $p = 0.037$). Independent samples t tests comparing P and PC groups, PC and C groups, PC and CC groups, and between C and CC groups were nonsignificant (all p's > 0.18).

Results in the left rACC adjusted for whole brain volume were similar before and after excluding ineligible subjects.

rACC Thickness:

Including all participants:

Results in the rACC were similar before and after excluding ineligible subjects.

High CAPS participants:

After excluding ineligible subjects, a trend toward a main effect of exposure in the right rACC appeared. Unexposed subjects (M = 3.977) showed smaller rACC thickness than exposed subjects (M = 4.132) ($F(1,28) = 3.182$, $p = 0.085$). Follow-up t tests showed no significant differences between P and PC groups ($p = 0.25$) or between C and CC groups ($p = 0.58$).

Results in the left rACC were similar before and after excluding ineligible participants.

rACC Area:

Including all participants:

Results in the rACC were similar before and after excluding ineligible subjects.

High CAPS participants:

Results in the rACC were similar before and after excluding ineligible subjects.

dACC Volume:

Including all participants:

A main effect of PTSD diagnosis in which PTSD pairs showed a greater dACC/whole brain volume ratio than Control pairs disappeared after excluding ineligible subjects. All other results were similar before and after excluding ineligible subjects.

High CAPS participants:

A significant main effect of PTSD diagnosis adjusted for whole brain volume became a trend after excluding ineligible subjects. PTSD pairs (M = 0.009) showed a greater right dACC/whole brain volume ratio than Control pairs (M = 0.008) ($F(1, 28) = 3.94$, $p = 0.057$). Follow-up t tests showed no significant differences between P and C subjects ($p = 0.57$) or between PC and CC subjects ($p = 0.41$). Other results were similar before and after excluding ineligible subjects.

dACC Thickness:

Including all participants:

Results in dACC thickness were similar before and after excluding ineligible subjects.

High CAPS participants:

Results in dACC thickness were similar before and after excluding ineligible subjects.

dACC Area:

Including all participants:

Results in dACC area were similar before and after excluding ineligible subjects.

High CAPS participants:

Results in dACC area were similar before and after excluding ineligible subjects.

SCC Volume:

Including all participants:

Results in SCC volume and SCC volume/whole brain volume ratio were similar before and after excluding ineligible subjects.

High CAPS participants:

Results in SCC volume and SCC volume/whole brain volume ratio were similar before and after excluding ineligible subjects.

SCC Thickness:

Including all participants:

Results in SCC thickness were similar before and after excluding ineligible subjects.

High CAPS participants:

Results in SCC thickness were similar before and after excluding ineligible subjects.

SCC Area:

Including all participants:

Results in SCC area were similar before and after excluding ineligible subjects.

High CAPS participants:

Results in SCC area were similar before and after excluding ineligible subjects.

PCC Volume:

Including all participants:

Results in PCC volume and PCC volume/whole brain volume ratio were similar before and after excluding ineligible subjects.

High CAPS participants:

Results in PCC volume and PCC volume/whole brain volume ratio were similar before and after excluding ineligible subjects.

PCC Thickness:

Including all participants:

After excluding ineligible subjects, a trend toward a main effect of PTSD Diagnosis appeared in the left PCC; PTSD pairs (M = 3.523) showed thinner cortex than Control pairs (M = 3.652) ($F(1, 39) = 3.986$, $p = 0.053$). A follow-up t test comparing P and C groups was not significant ($p = 0.121$). A follow-up t test comparing PC (M = 3.53) and CC (M = 3.66) groups showed a trend toward thicker left PCC in the CC group ($t(39) = -1.772$, $p = 0.084$).

High CAPS participants:

After excluding ineligible subjects, a trend toward a main effect of PTSD Diagnosis appeared in the right PCC; PTSD pairs (M = 3.328) showed thinner cortex than Control pairs (M = 3.468) ($f(1, 28) = 3.877$, $p = 0.059$). Follow-up t tests showed that P subjects (M = 3.27) showed thinner cortex than C subjects (M = 3.46) ($t(28) = -2.074$, $p = 0.047$). There were no significant differences betweeen PC and CC groups (p

= 0.32). Other PCC volume and PCC volume/whole brain volume ratio were similar before and after excluding ineligible subjects.

PCC Area:

Including all participants:

Results in PCC area were similar before and after excluding ineligible participants.

High CAPS participants:

After excluding ineligible subjects, a main effect of Exposure appeared in which unexposed participants (M = 1839.230) showed larger right PCC area than exposed subjects (M = 1694.000) ($F(1, 28) = 6.119$, $p = 0.02$). Follow-up t tests showed no significant differences between P and C groups ($p = 0.375$) or between PC and CC groups ($p = 0.654$). Other results in PCC area were similar before and after excluding ineligible subjects.

Hippocampus Volume:

Including all participants:

Results in the hippocampus were similar before and after excluding ineligible participants.

High CAPS participants:

After excluding ineligible participants, a trend toward a main effect of Exposure emerged; unexposed subjects (M = 3.648) showed smaller right hippocampus volumes than exposed subjects (M = 3.748) ($F(1, 30) = 3.696$, $p = 0.064$). Follow-up t tests

showed no significant differences between P and PC groups ($p = 0.71$) or between C and CC groups ($p = 0.34$). Other results in the hippocampus were similar before and after excluding ineligible participants.

Amygdala Volume:

Including all participants:

Results in the amygdala were similar before and after excluding ineligible participants.

High CAPS participants:

Results in the amygdala were similar before and after excluding ineligible participants.

Anterior Amygdala:

Including all participants:

Results in the anterior portion of the amygdala were similar before and after excluding ineligible participants.

High CAPS participants:

Results in the anterior portion of the amygdala were similar before and after excluding ineligible participants.

Correlations with CAPS Scores:

We ran additional correlations in each region of interest to determine whether symptom severity was associated with structural differences.

Hippocampus Correlations:

All Exposed (P and C subjects) vs All Unexposed (PC and CC subjects):

There was no significant correlation between CAPS scores of all exposed subjects and right hippocampal volume of unexposed subjects ($r(41) = -0.1$, $p = 0.30$). This held after removing subjects taking SSRI's ($r(28) = -0.27$, $p = 0.16$). There was no significant correlation between CAPS scores of all exposed subjects and left hippocampal volume of unexposed subjects ($r(41) = -0.15$ $p = 0.13$). This held after removing subjects taking SSRI's ($r(28) = -0.22$, $p = 0.23$).

After adjusting for whole brain volume, there was no significant correlation between CAPS scores of exposed subjects overall and right hippocampal volume in unexposed subjects ($r(41) = -0.04$ $p = 0.98$). This held after removing subjects taking SSRI's ($r(28) = -0.19$, $p = 0.33$). There was also no significant correlation between CAPS scores of exposed subjects overall and left hippocampal volume in unexposed subjects ($r = 0.03$, $p = 0.86$). This held after removing subjects taking SSRI's ($r(28) = -0.15$, $p = 0.43$).

Across All Subjects:

There was no significant correlation between right hippocampus volume and CAPS total ($r(84) = -0.06$, $p = 0.58$) or between left hippocampus and CAPS total ($r(84) = 0.07$, $p = 0.53$).

After adjusting for whole brain volume, there was no significant correlation between CAPS total and right hippocampus volume ($r(84) = 0.004$, $p = 0.97$) or left hippocampus volume ($r(86) = 0.001$, $p = 0.99$).

Amygdala:

All Exposed (P and C subjects) vs All Unexposed (PC and CC subjects):

There were no significant correlations between CAPS total and right amygdala ($r(41) = 0.098$, $p = 0.53$) or between CAPS total and left amygdala ($r(41)= 0.04$, $p = 0.80$).

After adjusting for whole brain volume, there were no significant correlations between CAPS total and right amygdala ($r(41) = 0.21$, $p = 0.17$) or left amygdala ($r(41) = 0.05$, $p = 0.76$).

Across All Subjects: There were no significant correlations between CAPS total and right amygdala volume ($r(84) = 0.03$, $p = 0.81$) or left amygdala volume ($r(84) = 0.03$, $p = 0.81$).

After adjusting for whole brain volume, there were no significant correlations between CAPS total and right amygdala volume ($r(84) = 0.04$, $p = 0.74$) or left amygdala volume ($r(84) = 0.03$, $p = 0.81$).

Anterior Amygdala:

All Exposed (P and C subjects) vs All Unexposed (PC and CC subjects):

There was no significant correlation between CAPS total and right anterior amygdala

volume (r(41) = -0.13, p = 0.39) or left anterior amygdala volume (r(41) = -0.08, p = 0.61).

After adjusting for whole brain volume, there was no significant correlation between CAPS total and right anterior amygdala volume (r(41) = 0.21, p = 0.42) or left anterior amygdala volume (r(41) = 0.06, p = 0.68).

Across All Subjects:

There was no significant correlation between CAPS total and right anterior amygdala volume (r(84) = -0.14, p = 0.21). There was a trend toward a significant negative correlation between CAPS total (M = 18.5) and left anterior amygdala volume (M = 0.106) (r(84) = 0.19, p = 0.08).

After adjusting for whole brain volume, there was no significant correlation between CAPS total and right anterior amygdala volume (r(84) = -0.13, p = 0.22). There was a trend toward a significant negative correlation between CAPS total (M = 18.5) and left anterior amygdala volume (M = 0.0002) (r(84) = -.20, p = 0.08).

rACC Volume:

All Exposed (P and C subjects) vs All Unexposed (PC and CC subjects):

There was no significant correlation between CAPS total and right rACC volume (r(39) = -0.057, p = 0.72) or left rACC volume (r(39) = -0.19, p = 0.25).

After adjusting for whole brain volume, there was no significant correlation between CAPS total and right rACC volume (r(39) = -0.02, p = 0.90) or left rACC volume (r(39) = -0.16, p = 0.32).

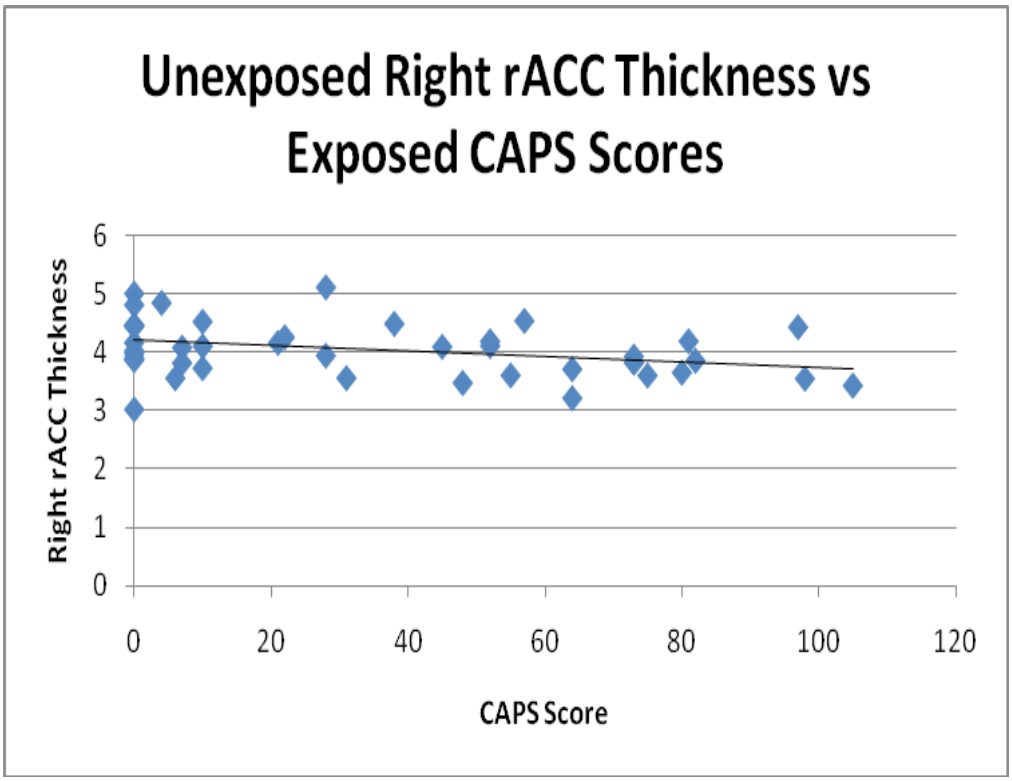
Across All Subjects: There was no significant correlation between CAPS total and right rACC volume (r(80) = 0.18, p = -.10) or left rACC volume (r(80) = -0.01, p = 0.92).

After adjusting for whole brain volume, there was a trend toward a positive correlation between CAPS total (M = 19.1) and right rACC volume (M = 0.004) (r(80)= 0.19, p = 0.08). There was no significant correlation between CAPS total and left rACC volume (r(80) = 0.003, p = 0.98).

rACC Thickness:

All Exposed (P and C subjects) vs All Unexposed (PC and CC subjects): There was a significant negative correlation (Fig 14) between CAPS totals in exposed subjects (M = 34.7) and right rACC thickness in unexposed subjects (M = 4.04) (r (39) = -0.33, p = 0.034).

Fig. 14



There was no significant correlation between CAPS totals in unexposed subjects and left rACC thickness in unexposed subjects ($r(39) = -0.19$, $p = 0.24$).

Across All Subjects:

There was no significant correlation between CAPS total and right rACC thickness ($r(80) = -0.03$, $p = 0.52$) or left rACC thickness ($r(80) = -0.03$, $p = 0.77$).

rACC Area:

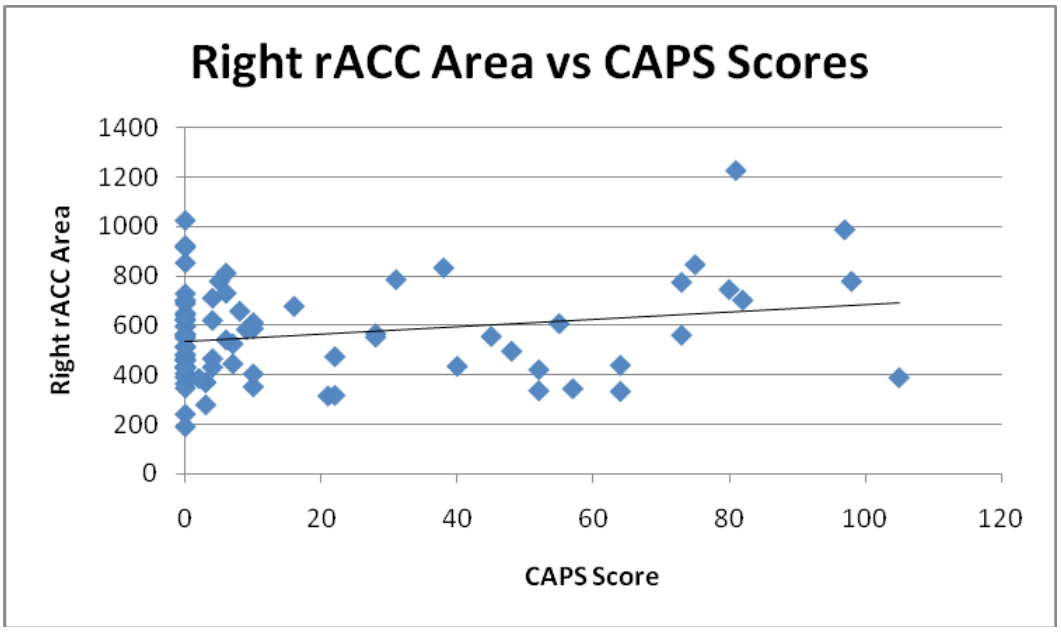
All Exposed (P and C subjects) vs All Unexposed (PC and CC subjects):

There was no significant correlation between CAPS totals of exposed subjects and right rACC area ($r(39) = 0.02$, $p = 0.92$) or left rACC area ($r(39) = -0.13$, $p = 0.41$).

Across All Subjects:

There was a significant positive correlation between CAPS total (M = 19.1) and right rACC area (M = 561.78) (r(80) = 0.23, p = 0.039).

Fig 17



There was no significant correlation between CAPS total and left rACC area (r(80) = -0.001, p = 0.99).

<u>dACC Volume:</u>

All Exposed (P and C subjects) vs All Unexposed (PC and CC subjects):

There were no significant correlations between exposed subjects' CAPS totals and unexposed subjects' right dACC volume (r(39) = 0.08, p = 0.62) or left dACC volume (r(39) = -0.10, p = 0.53).

After adjusting for whole brain volume, there were no significant correlations between exposed subjects' CAPS totals and unexposed subjects' right dACC volume ($r(39) = 0.12$, $p = 0.43$) or left dACC volume ($r(39) = -0.11$, $p = 0.77$).

Across All Subjects:

There was no significant correlation between CAPS total and right dACC volume ($r(80) = 0.12$, $p = 0.31$) or left dACC ($r(80) = -0.14$, $p = 0.21$).

After adjusting for whole brain volume, there was no significant correlation between CAPS total and right dACC volume ($r(80) = 0.12$, $p = 0.28$) or left dACC volume ($r(80) = 0.02$, $p = 0.32$).

dACC Thickness:

All Exposed (P and C subjects) vs All Unexposed (PC and CC subjects):

There were no significant correlations between exposed subjects' CAPS scores and unexposed subjects right dACC thickness ($r(39) = -0.12$, $p = 0.46$) or left dACC thickness ($r(39) = -0.11$, $p = 0.49$).

Across All Subjects:

There were no significant correlations between CAPS totals and right dACC thickness ($r(80) = 0.19$, $p = 0.26$) or left dACC thickness ($r(80) = -0.08$, $p = 0.39$).

dACC Area:

All Exposed (P and C subjects) vs All Unexposed (PC and CC subjects):

There were no significant correlations between exposed subjects' CAPS scores and unexposed subjects right dACC area ($r(39) = 0.11$, $p = 0.50$) or left dACC area ($r(39) = 0.09$, $p = 0.60$).

Across All Subjects:

There was a trend toward a significant positive correlation between CAPS totals ($M = 19.1$) and right dACC area ($M = 1124.10$) ($r(80) = 0.19$, $p = 0.09$). There was no significant correlation between CAPS totals and left dACC area ($r(80) = 0.08$, $p = 0.49$).

SCC Volume:

All Exposed (P and C subjects) vs All Unexposed (PC and CC subjects

There was no significant correlation between exposed subjects' CAPS scores and unexposed subjects' right SCC volume ($r(39) = 0.05$, $p = 0.77$) or left SCC volume ($r(39) = -0.04$, $p = 0.80$).

After adjusting for whole brain volume, there was no significant correlation between exposed subjects' CAPS scores and unexposed subjects' right SCC volume ($r(39) = -0.004$, $p = 0.98$) or left SCC volume ($r(39) = 0.02$, $p = 0.89$).

Across All Subjects:

There was no significant correlation between CAPS totals and right SCC volume ($r(80) = -0.07$, $p = 0.55$) or left SCC volume ($r(80) = 0.07$, $p = 0.54$).

After adjusting for whole brain volume, there was no significant correlation between CAPS totals and right SCC volume ($r(80) = -0.05$, $p = 0.68$) or left SCC volume ($r(80) = 0.07$, $p = 0.36$).

SCC Thickness:

All Exposed (P and C subjects) vs All Unexposed (PC and CC subjects):

There was no significant correlation between exposed subjects' CAPS scores and unexposed subjects right SCC thickness ($r(39) = -0.04$, $p = 0.81$) or left SCC thickness ($r(39) = -0.01$, $p = 0.95$).

Across All Subjects:

There was no significant correlation between CAPS totals and right SCC thickness ($r(80) = -0.08$, $p = 0.50$) or left SCC thickness ($r(80) = -0.09$, $p = 0.40$).

SCC Area:

All Exposed (P and C subjects) vs All Unexposed (PC and CC subjects):

There was no significant correlation between exposed subjects' CAPS scores and unexposed subjects right SCC area ($R(39) = 0.02$, $p = 0.89$) or left SCC area ($R(39) = 0.03$, $p = 0.84$).

Across All Subjects:

There was no significant correlation between CAPS totals and right SCC area ($R(80) = 002$, $p = 0.98$) or left SCC area ($R(80) = 015$, $p = 0.17$).

PCC Volume:

All Exposed (P and C subjects) vs All Unexposed (PC and CC subjects):

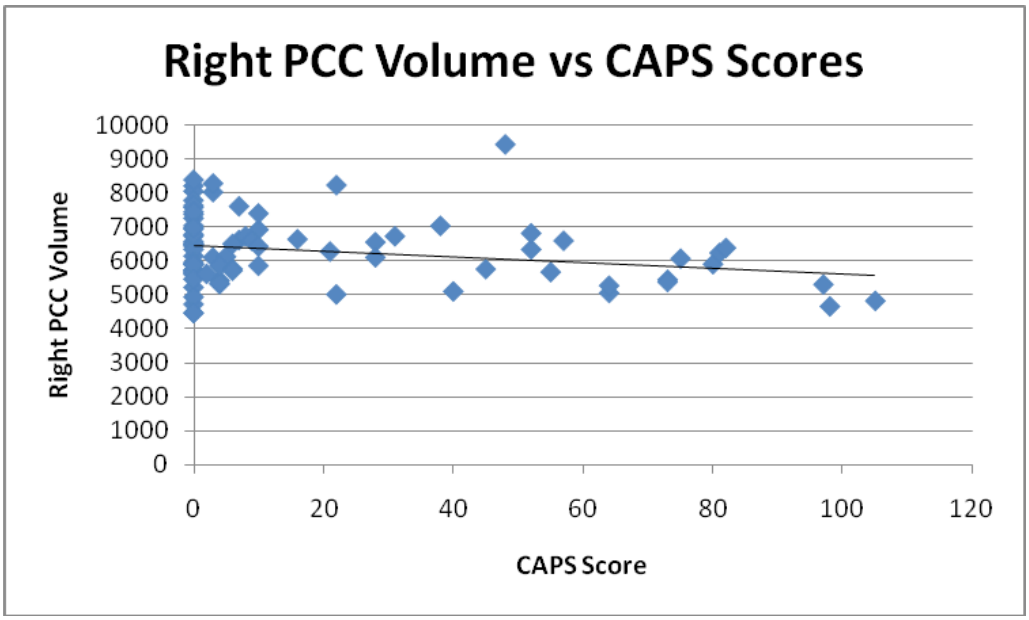
There were no significant correlations between exposed subjects' CAPS scores and unexposed subjects right PCC volume (R(39) = 0.14, p = 0.37) or left PCC volume (R(39) = -.01, p = 0.52).

After adjusting for whole brain volume, there were no significant correlations between exposed subjects' CAPS scores and unexposed subjects right PCC volume (R(39) = -0.02, p = 0.90) or left PCC volume (R(39) = 0.03, p = 0.84).

Across All Subjects:

There was a significant negative correlation between CAPS totals (M = 19.12) and right PCC volume (M = 6297.63) (r(80) = -0.25, p = 0.025).

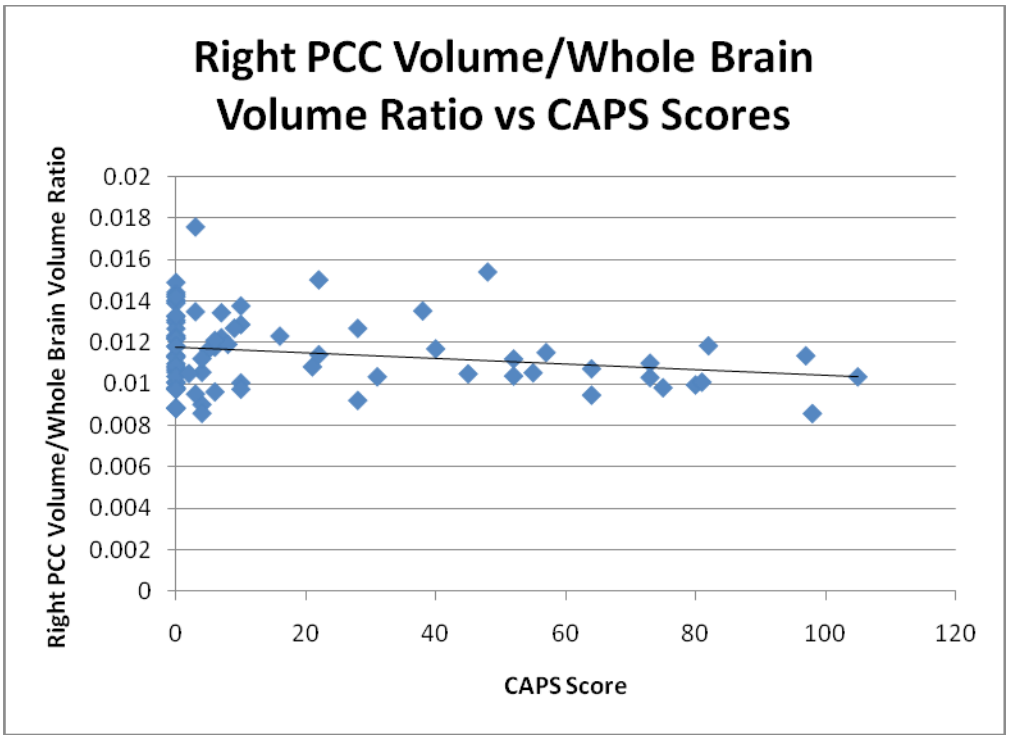
Fig 18



There was no significant correlation between CAPS totals and left PCC volume (r(80)= -0.02, p = 0.88).

After adjusting for whole brain volume, there was a significant negative correlation between CAPS totals (M = 19.12) and right PCC volume (M = 0.012) (r(80) = -0.23, p = 0.042).

Fig 19



There was no significant correlation between CAPS totals and right PCC volume (r(80) = 0.02, p = 0.86).

PCC Thickness:

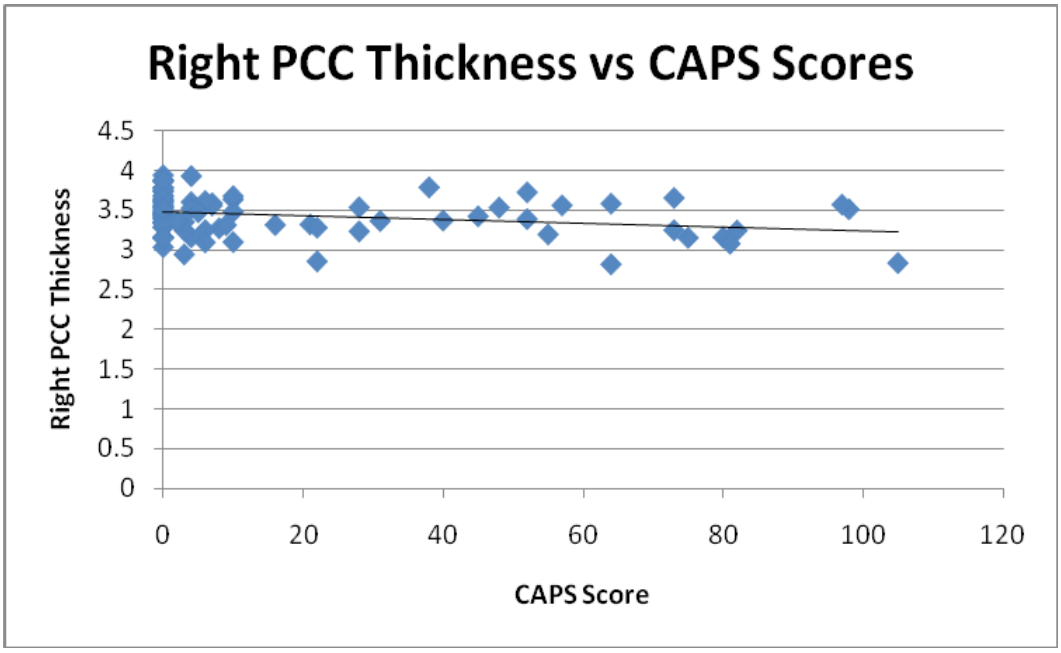
All Exposed (P and C subjects) vs All Unexposed (PC and CC subjects):

There were no significant correlations between exposed subjects' CAPS scores and unexposed subjects right PCC thickness (r(39) = -0.17, p = 0.30) or left PCC thickness (r(39) = -0.24, p = 0.14).

Across All Subjects:

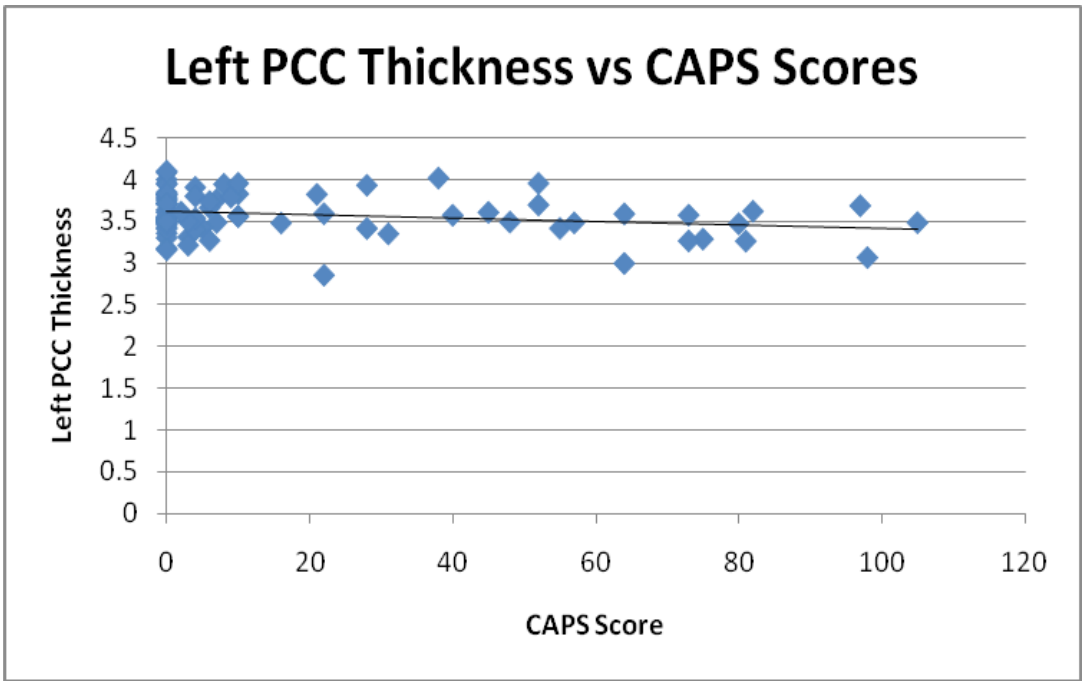
There was a significant negative correlation between CAPS totals (M = 19.1) and right PCC thickness (M = 3.43) (r(80) = -0.26, p = 0.017).

Fig 20



There was also a significant negative correlation between CAPS totals (M = 19.1) and left PCC thickness (M = 3.59) (r(80) = -0.26, p = 0.019).

Fig 21



PCC Area:

All Exposed (P and C subjects) vs All Unexposed (PC and CC subjects):

There were no significant correlations between exposed subjects' CAPS scores and unexposed subjects' right PCC area ($r(39) = -0.11$, $p = 0.48$) or left PCC area ($r(39) = -0.01$, $p = 0.94$).

Across All Subjects:

There were no significant correlations between CAPS totals and right PCC area ($r(80) = -0.14$, $p = 0.21$) or left PCC ($r(80) = -0.09$, $p = 0.45$).

Correlations with Age

Hippocampus:

There were no significant correlations between age and right hippocampus volume ($r(84) = -0.05$, $p = 0.66$) or left hippocampus volume ($r(84) = -0.05$, $p = 0.62$).

After adjusting for whole brain volume, there were no significant correlations between age and right hippocampus volume ($r(84)= 0.10$, $p = 0.37$) or left hippocampus volume ($r(84) = 0.11$, $p = 0.30$).

Amygdala:

There were no significant correlations between age and right amygdala volume ($r(84) = 0.07$, $p = 0.53$) or left amygdala volume ($r(84) = 0.05$, $p= 0.66$).

After adjusting for whole brain volume, there were no significant correlations between age and right amygdala volume ($r(84)= 0.14$, $p = 0.21$) or left amygdala volume ($r(84) = 0.12$, $p = 0.28$).

Anterior Amygdala:

There were no significant correlations between age and right anterior amygdala volume ($r(84) = -0.03$, $p = 0.77$) or left anterior amygdala volume ($r(84) = -0.03$, $p = 0.80$).

After adjusting for whole brain volume, there were no significant correlations between age and right anterior amygdala volume ($r(84) = -0.01$, $p = 0.90$) or left anterior amygdala volume ($r(84) = -0.02$, $p = 0.88$).

rACC Volume:

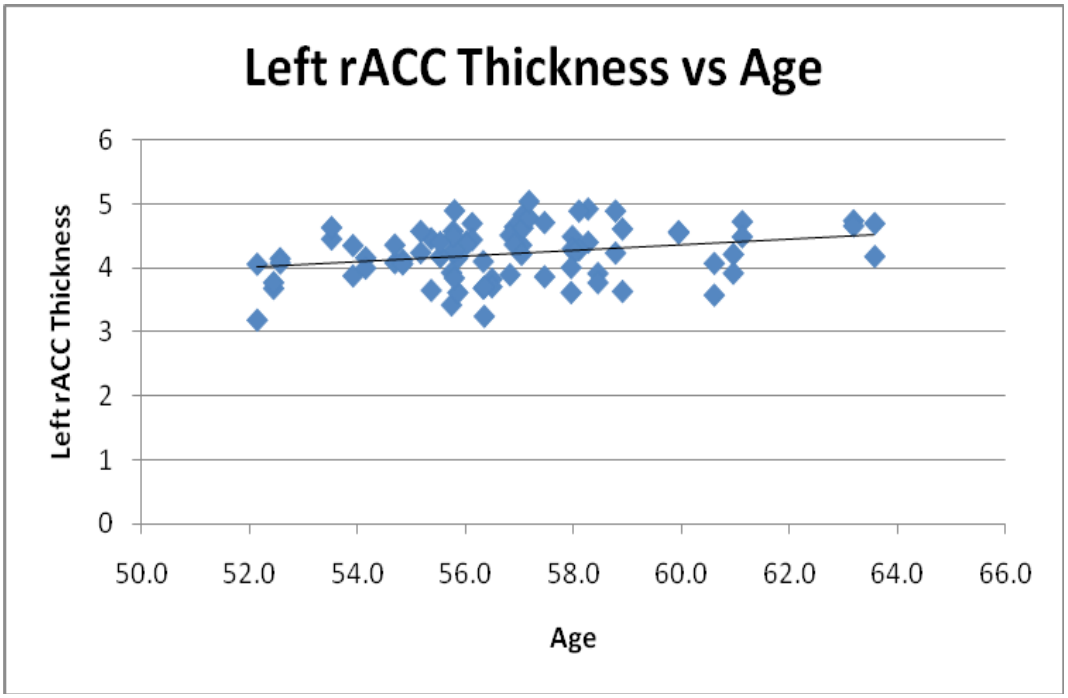
There were no significant correlations between age and right rACC volume ($r(80) = -0.04$, $p = 0.70$) or left rACC volume ($r(80) = -0.04$, $p = 0.73$).

After adjusting for whole brain volume, there were no significant correlations between age and right rACC volume (r(80) = 0.01, p = 0.91) or left rACC volume (r(80) = 0.02, p = 0.89).

rACC Thickness:

There was no significant correlation between age and right rACC thickness (r(80) = 0.09, p = 0.45). There was a significant positive correlation between age (M = 56.9) and left rACC thickness (M = 4.23) (r(80) = 0.28, p = 0.011).

Fig 22



rACC Area:

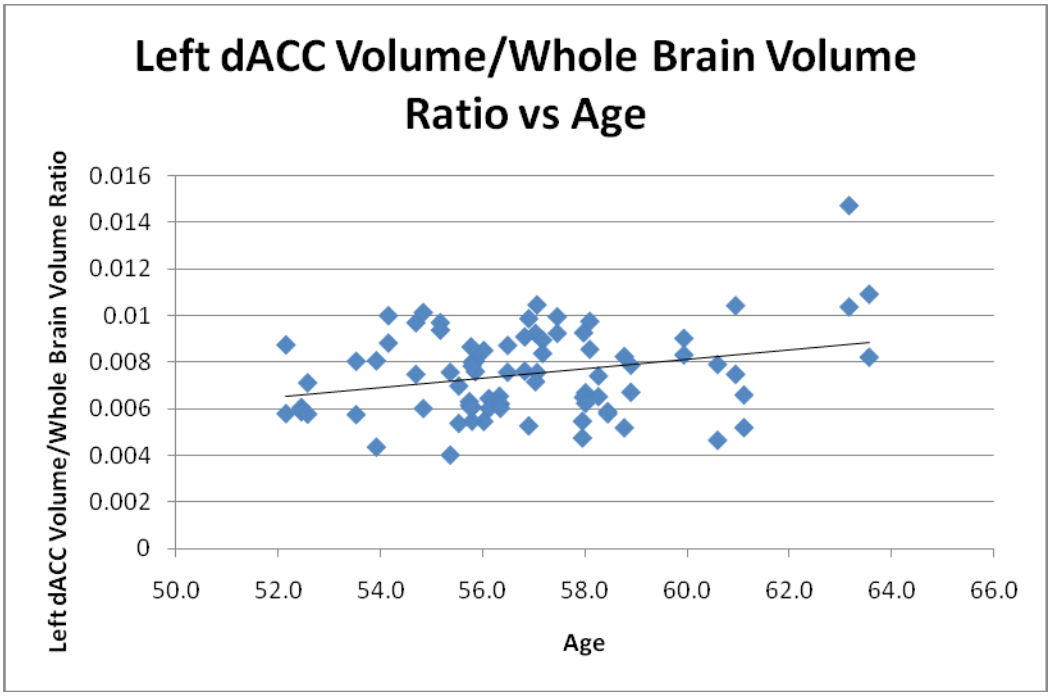
There were no significant correlations between age and right rACC area (r(80) = -0.07, p = 0.51) or left rACC area (r(80) = -0.11, p = 0.33).

dACC Volume:

There was no significant correlation between age and right dACC volume (r(80) = -0.04, p = 0.42). There was a trend toward a significant positive correlation between age (M = 56.9) and left dACC volume (M = 4110.60) (r(80) = 0.19, p = 0.089).

After adjusting for whole brain volume, there was no significant correlation between age and right dACC volume (r(80) = -0.02, p = 0.89). There was a significant positive correlation between age (M = 56.9) and left dACC volume (M = 0.008) (r(80) = 0.28, p = 0.010).

Fig 23



dACC Thickness:

There was no significant correlation between age and right dACC thickness (r(80) = 0.17, p = 0.14) or left dACC thickness (r(80) = 0.17, p = 0.12).

dACC Area:

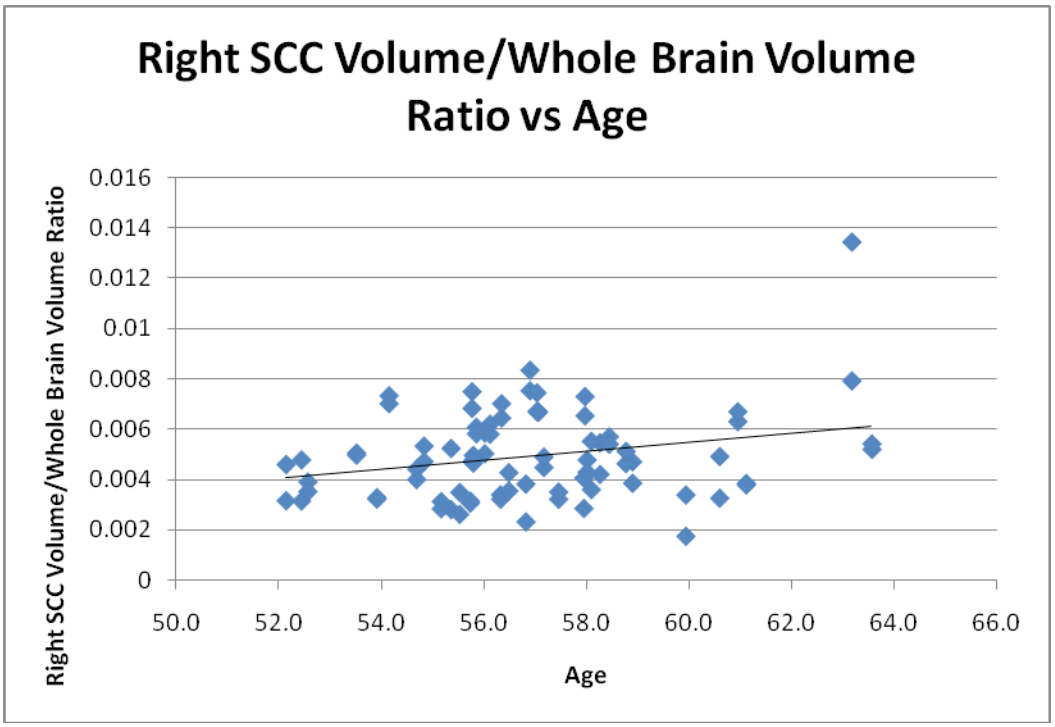
There was a trend toward a significant negative correlation between age (M = 56.9) and right dACC area (M = 1124.10) (r(80) = - 0.19, p = 0.087). There was no significant correlation between age and left dACC area (r(80) = 0.09, p = 0.42).

SCC Volume:

There was a trend toward a significant positive correlation between age (M = 56.9) and right SCC volume (M = 2706.20) (r(80) = -.20, p = 0.077). There was no significant correlation between age and left SCC volume (r(80) = 0.10, p = 0.38).

After adjusting for whole brain volume, there was a significant positive correlation between age (M = 56.9) and right SCC volume (M = 0.005) (r(80) = 0.26, p = 0.017).

Fig 24



There was no significant correlation between age and left SCC volume (r(80) = 0.18, p = 0.11).

SCC Thickness:

There were no significant correlations between age and right SCC thickness (r(80)= 0.05, p = 0.64) or left SCC thickness (r(80) = 0.02, p = 0.87).

SCC Area:

There was a trend toward a significant positive correlation between age (M = 56.9) and right SCC area (M = 624.01) (r(80) = 0.20, p = 0.071). There was no significant correlation between age and left SCC area (r(80) = 0.07, p = 0.53).

PCC Volume:

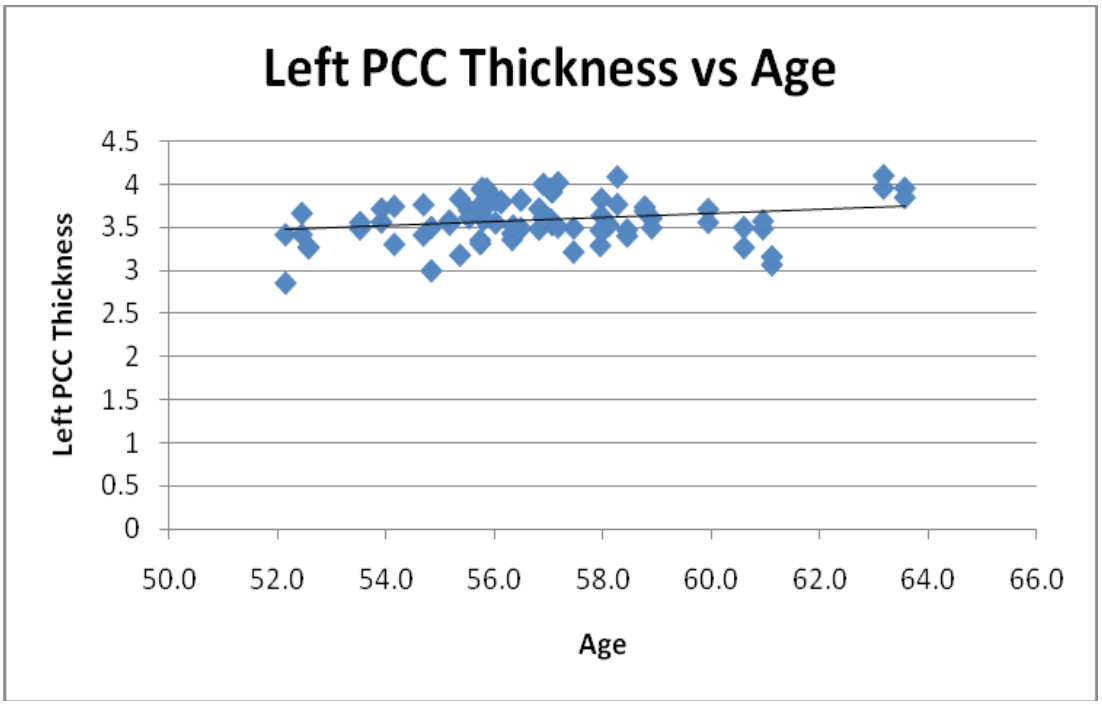
There were no significant correlations between age and right PCC volume (r(80) = 0.20, p = 0.51) or left PCC volume (r(80) = 0.10, p = 0.95).

After adjusting for whole brain volume, there were no significant correlations between age and right PCC volume (r(80) = 0.06, p = 0.61) or left PCC volume (r(80) = 0.17, p = 0.14).

PCC Thickness:

There was a trend toward a significant positive correlation between age (M = 56.9) and right PCC thickness (M = 3.43) (r(80) = 0.19, p = 0.087). There was a significant positive correlation between age (M = 56.9) and left PCC thickness (M = 3.59) (r(80)= 0.24, p = 0.034).

Fig 25



PCC Area:

There were no significant correlations between age and right PCC area ($r(80) = -0.12$, $p = 0.27$) or left PCC area ($r(80) = -0.08$, $p = 0.49$).

DISCUSSION

This investigation sought to determine whether brain abnormalities exist in the cingulate, hippocampus, and amygdala in PTSD and, if such abnormalities are present, to determine whether they are due to a familial predisposition or are acquired characteristics of the disorder. We hypothesized that reduced volume, area, and thickness in the rACC would represent an acquired characteristic of PTSD. If abnormalities were found in the amygdala, they also would represent an acquired characteristic of PTSD. We would see significant volume reductions in the dACC, SCC, and PCC. We also hypothesized that

reduced volumes in the hippocampus would represent a familial predisposition to PTSD and would be inversely related to PTSD symptom severity.

Kremen et al. (2011) point out that most twin studies on PTSD have drawn from the VETR and that findings may not be generalizable to females, younger participants, or to individuals' non-military related trauma exposure. Despite similarities between the sample included in the present study and other twin studies on PTSD, findings differ between this study and past studies of morphological differences in PTSD. Results in the present study differ from those of a previous cycle of data from this study (Gilbertson et al., 2002, Kasai et al., 2008). The inconsistent findings between cycles may be due to decreased symptom severity: in the first-cycle sample, the P group had a mean CAPS score of 72.2 and the C group had a mean CAPS score of 6.2. In the current sample, the P group has a mean CAPS score of 65.0 and the C group has a mean CAPS score of 6.0. Differences between the previous cycle of data and the current sample may also be due to aging of our participants, however correlations between age and ROI volumes overall do not support this. There were some significant correlations between age and ROI volume, thickness, and area that do not explain our findings. Also, the interaction between PTSD and aging is not well understood (Lapp et al, 2011). Differences between the present study and previous cycles of data may be attributed to differing samples: the first cycle of data and the current cycle only share 6 P participants, 7 PC participants, 15 C and 15 CC participants.

High rates of alcoholism and substance abuse in addition to high rates of MDD in our sample may have confounded results. However, while these may have presented a

confound, it is likely that substance abuse and MDD may be more likely to have lower volumes in these regions of interest (Woodward et al, 2006).

Anterior, Posterior, and Subcallosal Cingulate:

Results in the cingulate cortex were mixed; findings in the rACC, dACC, and SCC did not support our hypothesis that we would find reduced area, thickness and volume in these regions of interest. Some abnormalities in the rACC, however, appeared to be acquired characteristics of PTSD. Our findings in the PCC were more in line with our hypotheses, although fewer studies have connected differences in PCC volume with PTSD (Nardo et al, 2010).

All significant structural differences in subdivisions of the anterior cingulate were found only after subjects with CAPS scores below 65 were excluded from the analyses. In some subdivisions of the cingulate, our results showed larger volumes in twin pairs with PTSD, which does not support previous research on structural differences in this region of interest. Because we found significant results in the ACC only after excluding low CAPS participants, it is possible that this restricted sample represents a more chronic (but not necessarily more severe) form of PTSD. Our small n in the PTSD group may have prevented us from finding significant differences in participants with CAPS scores below 65; additionally, some of the lack of significant findings in ACC area and thickness where we found volumetric differences may be attributed to a small n. Unexpectedly larger volumes in subdivisions of the cingulate may represent a chronic form of the disorder or could be due to effects of aging, decreased symptom severity from previous cycles of data, or effects of treatment.

Studies examining the ACC as a whole have consistently found smaller volumes in PTSD than controls. Yamasue et al. (2003) found overall left ACC volume reductions in PTSD as well as smaller left ACC volume in subjects with more severe symptoms. In a meta-analysis of morphological abnormalities in regions of interest in both combat related and non-combat related PTSD, Karl et al. (2006) found that PTSD subjects had smaller overall ACC volume than trauma-exposed controls. In a study of combat-exposed veterans with and without PTSD, Woodward et al. (2005) found reduced ACC volumes in PTSD.

rACC:

Our findings on the right rACC volume are not in line with previous research on twin pairs with and without PTSD. After including only subjects with high CAPS, there was a PTSD Diagnosis x Exposure interaction in which subjects with PTSD showed greater rACC volume than either C or CC participants but were not significantly different from their own cotwins. Additionally, the PC group was not significantly different from either the C or CC participants. Results were similar in right rACC area. This interaction indicates that larger right rACC volume and area may be acquired characteristics of PTSD. However, the lack of significant difference between the P and PC groups is evidence against this. Our small sample of P and PC subjects may have impaired our ability to find a significant difference within PTSD pairs. If so, these findings may support the findings of Kasai et al. (2008) that rACC abnormalities are an acquired

characteristic of PTSD; our findings, however, are in the opposite direction of those of Kasai et al. (2008).

Additional analyses excluded two subject pairs who were ineligible for the study. The significant main effect of PTSD Diagnosis and the PTSD Diagnosis x Exposure interaction in right rACC volume became trends. These results may provide evidence for an absence of structural differences in the rACC in PTSD. Additionally, removal of ineligible pairs yielded a trend towards a main effect of Exposure in right rACC thickness in which unexposed participants showed thinner cortex than exposed participants. Symptom severity was negatively associated with right rACC thickness but positively associated with right rACC area. The inconsistent effects found in the rACC render the present results inconclusive.

dACC:

There was a main effect of Exposure in which control pairs showed greater left dACC volume than PTSD pairs, however this effect disappeared after adjusting for whole brain volume. This greater volume in control pairs is inconsistent with the rest of our cingulate results, including results in the right dACC: Adjustment for whole brain volume yielded a main effect of PTSD Diagnosis in the right dACC in which PTSD pairs showed greater volumes than control pairs. Specifically, P subjects showed greater volumes than C subjects, however additional analyses excluding ineligible participants reduced this effect to a trend. There was a similar main effect of PTSD Diagnosis in right dACC area. These conflicting results within the dACC show that further research should examine morphology in individual subdivisions in PTSD. Symptom severity does not appear to be related to structural differences in the dACC, however there was a trend toward a positive

correlation in right dACC area. Inconsistencies within brain regions could be due to differing volumes between hemispheres.

Although little research has examined dACC volume in PTSD (Thomaes et al., 2010; Rauch et al., 2003), this subdivision has been shown to be hyperresponsive in PTSD (Shin 2009). It may have a role in conditioned fear responses and may be related to lack of extinction recall in PTSD (Milad et al. 2007, 2009). Shin et al. (2009) found enhanced resting metabolic activity in the dACC in PTSD pairs compared to control pairs. This could help account for the increased volumes in PTSD pairs in the present study, however the relationship between dACC structure and function is not clear. It may be that enhanced activity affected volume and area through Hebbian learning in the dACC to produce the increased dACC volume and area found here. Rauch et al. (2003), however, did not find any significant volumetric difference in the dACC. The relationship between dACC structure and function in PTSD requires further study.

SCC:

The lack of significant findings in the SCC does not support a previous study by Rauch et al. (2003), which found decreased SCC volumes in PTSD, suggesting that a dysfunctional SCC may fail to mitigate amygdala responses. Symptom severity was not related to SCC volume, thickness, or area. While we hypothesized that we would find reduced volumes, thickness, and areas in all subdivisions of the cingulate, fewer studies have connected volume reductions in the SCC with PTSD.

PCC:

Our results showed decreased right PCC volumes in combat-exposed subjects versus combat-unexposed subjects but no main effect of PTSD Diagnosis. This finding became more robust after adjusting for whole brain volume. This was also the only significant difference that arose in the cingulate before including only high CAPS participants. These results are not consistent with those of Nardo et al. (2010), who found decreased PCC volumes in PTSD subjects versus trauma-exposed controls. This investigation, however, found differences in the right PCC whereas Nardo et al. (2010) found differences in the left PCC. The role of the PCC has been examined less often than the ACC, but may have a role in the development of PTSD symptoms (Eckart 2011). Results of the present study showed significantly thinner left PCC in PTSD pairs versus Control pairs but did not find significantly different volume or area. Results in the right PCC seem to point to decreased PCC volumes as an effect of trauma exposure, however results in the left PCC seem to point to decreased PCC thickness as a familial predisposition.

Right PCC volume and thickness were negatively associated with overall symptom severity. Additional analyses excluding ineligible participants yielded a trend toward a main effect of PTSD Diagnosis with PTSD pairs showing thinner PCC than Control pairs. We also found a main effect of Exposure in which unexposed participants showed greater right PCC area than exposed participants. The mixed main effects of PTSD Diagnosis and Exposure in the absence of a significant interaction prevents us from drawing strong conclusions about the origins of these differences.The PCC has been

shown to have memory-related functions and to be activated during the processing of threat-related stimuli and may be related to emotion-related memory enhancement (Maddock et al. 2003). Dysregulation of these functions may help explain some PTSD symptoms, although further study of the role of the PCC in PTSD is needed. Previous studies have found that during resting state, subjects with PTSD show decreased activation between the PCC and other regions of the "default network" (Bluhm et al., 2009, Lanius et al., 2009). These findings suggest that alterations in the PCC's role in the "default network" may be associated with problems evaluating self-relevant information, including the relationship between past information and current environmental events (Bluhm et al., 2009). These findings may help explain the hyperarousal symptoms of PTSD: abnormal connectivity between the default network and the amygdala and hippocampus could explain the emotional responses to trauma reminders. While the relationship between structure and function in the present ROIs remains unclear, this abnormal connectivity may help explain structural abnormalities in the PCC.

Hippocampus:

We found a main effect of Exposure in the right hippocampus with exposed subjects showing larger hippocampi than unexposed subjects, but this finding only arose when we included subjects with CAPS scores below 65. These differences disappeared when only high CAPS participants were included, however the decrease in sample size after excluding low CAPS subjects may account for this. Additionally, this effect persisted after excluding low CAPS subjects when we ran additional analyses excluding two ineligible pairs. These differences also disappeared after adjusting for whole brain

volume. These findings do not fall in line with those of Karl et al. (2006), who found smaller bilateral hippocampi only in subjects with severe PTSD. The present study, conversely, found differences in hippocampus volume only when subjects with CAPS scores below 65 were included. It is possible that a larger n would have yielded more robust results, although to date there have been mixed results on structural differences in the hippocampus in PTSD.

Using a previous cycle of data from this study, Gilbertson et al. (2002) concluded that smaller hippocampi in PTSD constitute a familial predisposition to PTSD. Apfel et al. (2011) found that current but not lifetime PTSD was associated with smaller hippocampal volume, suggesting that it may constitute a risk factor for unremitting PTSD, a hypothesis that the current findings do not support. Felmingham et al. (2009) found that especially severe PTSD was related to smaller hippocampi. Findings from Jatzko et al. (2006), however, did not find significantly different gray or white matter volumes in PTSD. The possibility that alcoholism may play a part in hippocampal volumes in veterans is supported by Woodward et al. (2006), who found greater differences between PTSD subjects and controls with whom alcoholism was a factor than between nonalcoholic PTSD subjects and controls. Jatzko et al. (2006) suggested that their sample had not yet developed or had recovered from hippocampal differences that other studies were able to detect, but this explanation does not account for the findings in the current sample. In a study of Holocaust survivors with and without PTSD, Golier et al. (2005) attributed a lack of significant differences in hippocampal volume to an aged sample; this may help account for differences between findings of the present study and the findings of Gilbertson et al. (2002). It is possible that a floor effect occurred in which

reduced hippocampal volumes associated with normal aging obscured differences between control and PTSD subjects (Stoub et al., 2011) .

Results of the current study suggest rather that hippocampus size is related to trauma exposure and not to a diagnosis of PTSD. These results do not support our hypothesis that hippocampal volumes would represent a familial predisposition to PTSD but rather that trauma exposure is related to larger hippocampal volumes.

However, it is possible that these results represent volume recovery after many years that exceeded volumes before exposure. In an investigation examining elderly Holocaust survivors, Golier et al. (2006) found slightly increased hippocampal volumes in subjects with PTSD compared to unexposed controls and compared to trauma-exposed controls. This does not support our finding of a main effect of trauma exposure but does support an association between increased hippocampal volume and PTSD. This same study, however, investigated a sample of veterans and found age-associated hippocampal atrophy in PTSD subjects compared to trauma-exposed and trauma-unexposed controls. A meta-analysis by Woon et al. (2010) supported our finding of a main effect of Exposure but in the opposite direction: trauma-exposed subjects both with and without PTSD showed smaller hippocampal volumes than unexposed subjects. Findings from previous studies point to the need for the replication of these results.

Additionally, these results do not support our hypothesis that PC hippocampus volumes would predict P symptom severity. Larger hippocampus volumes in CC subjects were, however, associated with lower CAPS scores in C subjects. It is unclear why this relationship did not arise in the groups with PTSD symptoms, however that there was a relationship between CAPS scores and hippocampal volume in our control group does

point to a potential confounding variable in our sample; PTSD symptoms in our control group may have affected our results across all regions of interest, however symptom severity in PC, C, and CC groups was not severe (Table 1). Correlations between all unexposed subjects' left and right hippocampal volumes and all exposed subjects CAPS scores were not significant. These correlations remained nonsignificant after removing subjects taking SSRI's, ruling out SSRI use as a potential confound.

Amygdala:

Previous studies using this sample and samples from a previous cycle of this data set have focused less on the amygdala as a region of interest; however previous research has found smaller amygdala volumes in PTSD (Nardo et al. 2010). The lack of significant structural differences in the amygdala in this investigation does not support the findings of these studies. These studies examined younger samples with non-military related PTSD that may have been systematically different from the sample in the present study, however our findings suggest that failure to regulate fear responses rather than the fear responses in the amygdala are associated with PTSD symptoms. Furthermore, amygdala volume was not related to symptom severity. Trends in the left anterior portion of the amygdala suggest an inverse relationship between the anterior amygdala and symptom severity, but these results were not robust and need replication. These results support the

findings of a meta-analysis of 9 studies by Woon and Hedges (2009), who found no significant decrease in amygdala volume in PTSD.

CONCLUSION

Many of the results of this investigation conflict with previous findings and need to be replicated. The stark differences between our results and those of previous studies may be accounted for by the presence of significance bias in the literature; according to Iaonnidis (2011), selective outcome reported and selective analyses reporting may contribute to an overabundance of "positive" results in brain morphology studies. Larger volumes found in PTSD pairs in the present study may be accounted for by differences in age and symptom severity from previous studies. Our limited sample size may have affected our ability to find more robust effects. Differences in age and symptom severity may help explain differences between findings of previous cycles of this study and the current study, however there were few significant correlations between age and ROI volumes. Presence of mild PTSD symptoms in control subjects may have confounded results. Future research may compare volumes in these regions of interest within subjects with PTSD over time, for symptom severity, and for effects of treatment.

REFERENCES

American Psychiatric Association. Diagnostic and statistical manual of mental disorders 4th edition, text revision. Washington, DC: Author; 2000.

Alvarez RP, Biggs A, Chen G, Pine DS, Grillon C (2008). Contextual fear conditioning in humans: Cortical-hippocampal and amygdala contributions. *Journal of Neuroscience, 28*(24) 6211-6219.

Apfel BA, Ross J, Hlaven J, Meyerhoff DJ, Metzler TJ, Marmar CR, Weiner MW, Schuff N, Neylan TC. (2011). Hippocampal volume differences in Gulf War veterans with current versus lifetime posttraumatic stress disorder symptoms. *Biol Psychiatry, 69*(6) 541-548.

Bluhm RL, Williamson PC, Osuch EA, Frewen PA, Stevens TK, Boksman K, Neufeld RWJ, Theberge J, Lanius RA. (2009). Alterations in default network connectivity in posttraumatic stress disorder related to early-life trauma. *J. Psychiatry Neurosci, 34*(3), 187-194.

Bonne O, Brandes D, Gilboa A, Gomori JM, Shenton ME, Pitman RK, Shalev AY. (2001). Longitudinal MRI study of hippocampal volume in trauma survivors with PTSD. *Am J Psychiatry, 158*(8) 1248-1251.

Bremner JD, Vythilingam M, Vermetten E, Southwick SM, McGlashanT, Nazeer A, Khan S, Vaccarino LV, Soufer R, Garg PK, Ng CK, Staib LH, Duncan JS, Charney DS (2003). MRI and PET study of deficits in hippocampal structure and function in women with childhood sexual abuse and posttraumatic stress disorder. *Arch Gen Psychiatry, 160*(5) 924-932.

Bremner JD, Randall P, Verrnetten E, Staib L, Bronen RA, Mazure C, Capelli S, MCarthy G, Innis RB, Charney DS. (1997). Magnetic resonance imaging-based measurement of hippocampal volume in posttraumatic stress disorder related to childhood physical and sexual abuse- A preliminary report. *Society of Biological Psychiatry, 41* 23-32.

Corcoran KA and Maren S. (2001). Hippocampal inactivation disrupts retrieval of fear memory after extinction. *Journal of Neuroscience, 21*(5) 1720-1726.

Felmingham K, Williams LM, Whitford TJ, Falconer E, Kemp AH. (2009). Duration of posttraumatic stress disorder predicts hippocampal grey matter loss. *Behavioral, Integrative, and Clinical Neuroscience*, *20*(16), 1402-1406.

Fennema-Notestine, C., Stein, M.B., Kennedy, C.M., Archibald, S.L., Jernigan, T.L., (2002). Brain morphometry in female victims of intimate partner violence with and without posttraumatic stress disorder. *Biological Psychiatry 52,* 1089–1101.

Freeman, T, Kimbrell T, Booe L, Myers M, Cardwell D, Lindquist DM, Hart J, Komoroski RA. (2006). Evidence of resilience: neuroimaging in former prisoners of war. *Psychiatry Research: Neuroimaging*, *146*, 59-64.

Gilbertson MW, Shenton ME, Ciszewski A, Kasai K, Lasko NB, Orr SP, Pitman RK. (2002). Smaller hippocampal volume predicts pathologic vulnerability to psychological trauma. *Nature Neuroscience*, *5*(11), 1242-1247.

Gilbertson MW, Williston SK, Paulus LA, Lasko NB, Gurvits TV, Shenton ME, Pitman RK, Orr, SP. (2007). Configural cue performance in identical twins discordant for posttraumatic stress disorder: theoretical implications for the role of hippocampal function. *Biol Psychiatry*, *62*, 513-520.

Gilboa A, Shalev AY, Laor L, Lester H, Louzoun Y, Chisin R, Bonne O. (2004). Functional connectivity of the prefrontal cortex and the amygdala in posttraumatic stress disorder. *Biol Psychiatry*, *55*, 263-272.

Golier JA, Harve PD, Legge J. (2006). Memory performance in older trauma survivors: Implications for the longitudinal course of PTSD. *Annals of the New York Academy of Sciences, 1071*(1), 54-66.

Golier JA, Yehuda R, De Santi S, Segal S, Dolan S, de Leon MJ. (2005). Absence of hippocampal volume differences in survivors of the Nazi Holocaust with and without posttraumatic stress disorder. *Psychiatry Research: Neuroimaging, 139* 53-64.

Hara E, Matsuoka Y, Hakamata Y, Nagamine M, Inagaki M, Imoto S, Murakami K, Kim Y, Uchitomi Y. (2008). Hippocampal and amygdalar volumes in breast cancer survivors with posttraumatic stress disorder. *Journal of Neuropsychiatry, 20*(3), 302-308.

Hayes JP, LaBar KS, McCarthy G, Selgrade E, Nasser J, Dolcos F, VISN 6 Mid-Atlantic MIRECC workgroup, Morey RA. (2010). Reduced hippocampal and amygdala activity predicts memory distortions for trauma reminders in combat-related PTSD. *Journal of Psychiatric Research,* In Press.

Iaonnidis JPA. Excess significance bias in the literature on brain volume abnormalities. (2011). *Arch Gen Psychiatry,* doi:10.1001/archgenpsychiatry.2011.28

Jatzko A, Rothenhofer S, Schmitt A, Gaser C, Demirakca T, Weber-Fahrv W, Wessa M, Magnotta V, Braus DF. (2006). Hippocampal volume in chronic posttraumatic stress disorder (ptsd): mri study using two different evaluation methods. *Journal of Affective Disorders*, *94*, 121-126.

Karl A, Schaefer M, Malta LS, Dorfel D, Rohleder N, Werner A. (2006) A Meta-analysis of structural brain abnormalities in ptsd. *Neuroscience and Neurobehavioral Reviews*, *30*, 1004-1031.

Kasai K, Yamasue H, Gilbertson MW, Shenton ME, Rauch SL, Pitman RK. (2008). Evidence for acquired pregenual anterior cingulate gray matter loss from a twin

study of combat-related posttraumatic stress disorder. *Biol Psychiatry*, *63*, 550-556

Kitayama N, Quinn S, Bremner JD. (2006). Smaller volume of anterior cingulate cortex in abuse-related posttraumatic stress disorder. *Journal of Affective Disorders, 90* 171-174.

Koenigs M, & Grafman, J. (2009). Posttraumatic stress disorder: the role of medial prefrontal cortex and amygdala. *Neuroscientist*, *15*, 540-549.

Kremen WS, Koenen KC, Afari N, Lyons MJ. (2011). Twin studies of posttraumatic stress disorder: Differentiating vulnerability factors from sequelae. *Neuropharmacology, In Press.*

Lanius RA, Bluhm RL, Coupland NJ, Hegadoren KM, Rowe B, The´berge J, Neufeld WJ, Williamson PC, Brimson M. (2009). Default mode network connectivity as a redictor of post-traumatic stress disorder symptom severity in acutely traumatized ubjects. *Acta Psychiatrica Scandinavica,* 1-8. DOI: 10.1111/j.1600-447.2009.01391

Lanius RA Williamson, PC, Densmore, MB, Boksman, Gupta, MA, Neufeld, RW, Gati, JS, Menon, RS. (2001). Neural correlates of traumatic memories in posttraumatic stress disorder: a functional mri investigation. *The American Journal of Psychiatry*, *158*(11), 1920-1922.

Lapp LK, Agbokou C, Ferreri F. (2011). PTSD in the elderly: The interaction between trauma and aging. *International Psychogeriatrics,* 1-11. DOI: 10.1017/S1041610211000366

Lindauer RJL, Booij J, Habraken JBA, Uylings HBM, Olff M, Carlier IVE, den Heeten GJ, van Eck-Smit BLF, Gersons BPR (2004). Cerebral blood flow changes during script-driven imagery in police officers with posttraumatic stress disorder. *Biol Psy 56,* 853-861.

Maddock RJ, Garrett AS, Buonocore MH (2003). Posterior cingulate cortex activation by emotional words: fMRI evidence form a valence decision task. *Human Brain Mapping 18* 30-41.

Milad MR, Pitman RK, Ellis CB, Gold AL, Shin LM, Lasko NB, Zeidan MA, Handwerger K, Orr SP, Rauch SL. (2009). Neurobiological basis of failure to recall extinction memory in posttraumatic stress disorder. *Biol Psy 66* 1075-1082.

Milad MR, Quirk GJ, Pitman RK, Orr SP, Fischl B, Rauch SL. (2007). A role for the human dorsal anterior cingulate cortex in fear expression. *Biol Psy 62* 1191-1194

Orr SP, Metzger LJ, Lasko NB, Macklin ML, Hu FB, Shalev AY, Pitman RK. (2003). Physiologic responses to sudden, loud tones in monozygotic twin pairs discordant for combat exposure. *Arch Gen Psy, 60,* 283-288.

Pitman, RK. (2001). Hippocampal diminution in PTSD:More (or less?) than meets the eye. *Hippocampus. 11* 73-74.

Pohlack, S. T., Nees, F., Liebscher, C., Cacciaglia, R., Diener, S. J., Ridder, S., Woermann, F. G. and Flor, H. (2011), Hippocampal but not amygdalar volume affects contextual fear conditioning in humans. Human Brain Mapping, 32: n/a. doi: 10.1002/hbm.21224

Ramon, JLL, Olff M, van Meijel EPM, Carlier IVE, Gersons BPR. (2006). Cortisol, learning, memory, and attention in relation to smaller hippocampal volume in police officers with posttraumatic stress disorder. *Biol Psychiatry*, *59*, 171-177.

Rauch SL, Shin LM, Segal E, Pitman RK, Carson MA, McMullin K, Whalen PJ, Makris N. (2003). Selectively reduced regional cortical volumes in post-traumatic stress disorder. *NeuroReport, 14(7),* 913-916.

Shin, LM, & Handwerger, K. (2009). Is Posttraumatic stress disorder a stress-induced fear circuitry disorder?. *Journal of Traumatic Stress*, *22*(5), 409-415.

Shin, LM, Lasko NB, Macklin ML, Karpf RD, Milad MR, Orr SP, Goetz JM, Fischman AJ, Rauch SL, Pitman RK, (2009). Resting metabolic activity in the cingulate cortex and vulnerability to posttraumatic stress disorder. *Arch Gen Psychology*, *66*(10), 1099-1107.

Shin, LM, Rauch, SL, & Pitman, RK. (2006). Amygdala, prefrontal cortex, and hippocampal function in ptsd. *Annals New York Academy of Sciences*, *1071*, 67- 79.

Shin, LM, Wright CI, Cannistraro PA, Wedig MM, McMullin K, Martis B, Macklin ML, Lasko NB, Cavanagh SR, Krangel TS, Orr SP, Pitman RK, Whalen PJ, & Rauch SL. (2005). A Functional magnetic resonance imaging study of amygdala and medial prefrontal cortex responses to overtly presented fearful faces in posttraumatic stress disorder. *Arch Gen Psychology*, *62*, 273-281.

Shin LM, Shin PS, Heckers S, Krangel TS, Macklin ML, Orr SP, Lasko N, Segal E, Makris N, Richert K, Levering J, Schacter DL, Alpert NM, Fischman AJ, Pitman RK, Rauch SL. (2004). Hippocampal function in posttraumatic stress disorder. *Hippocampus, 204* 292-300.

Shin LM, McNally RJ, Kosslyn SM, Thompson WL, Rauch SL, Alpert NM, Metzger LJ, Lasko NB, Orr SP, Pitman RK. (1999). Regional cerebral blood flow during

script-driven imagery in childhood sexual abuse-related PTSD: A PET investigation. *Am J Psy, 156*(4), 575-584.

Stoub T, Barnes CA, Shah RC, Stebbins GT, Ferrari C, Detoledo-Morrell L. (2011). Age-related changes in the mesial-temporal lobe: the parahippocampal white matter region. *Neurobiol. Aging* (In Press).

Thomaes K, **Dorrepaal E**, **Draijer N**, **de Ruiter MB**, **van Balkom AJ**, **Smit JH**, **Veltman DJ**. (2010). Reduced anterior cingulate and orbitofrontal volumes in child abuse-related complex PTSD. *J Clin Psychiatry, 71*(12).

Weathers FW, Keane TM, & Davidson JR. (2001). Clinician-administered PTSD scale: a reviewof the first ten years of research. *Depress Anxiety*. *13(3),* 132-156.

Woon, FL, & Hedges, DW. (2009). Amygdala volume in adults with posttraumatic stress disorder: a meta-analysis. *J Neuropsychiatry Clin Neurosci*, *21*(1), 5-12.

Woon FL, et al, Hippocampal volume deficits associated with exposure to psychological trauma and posttraumatic stress disorder in adults: A meta-analysis, Prog Neuro-Psychopharmacol Biol Psychiatry (2010), doi:10.1016/j.pnpbp.2010.06.016

Woodward, SH, Kaloupek DG, Streeter CC, Kimble MO, Reiss AL, Eliez S,Wald LL, Renshaw PF, Frederick BB, Lane B, Sheikh JI, Stegman WK, Kutter CJ, Stewart LP, Prestel RS, Aresnault NJ. (2006). Hippocampal volume, ptsd, and alcoholism in combat veterans. *Am J of Psychiatry*, *163*, 674-681.

Yamasue H, Kasai K, Iwanami A, OTAN T, Yamada H, Abe O, Kuroki N, Fukida R Tochigi M, Furukawa S, Sadamatsu M, Sasaki T, Auki S, Ohtomo K, Asukai N, Kato N. (2003) Voxel-based analysis of MRI reveals anterior cingulate gray-matter volume reduction in posttraumatic stress disorder due to terrorism. *PNAS, 100(15),* 9039-9043.

Yoon T, Graham LK, Kim JJ. (2011). Hippocampal lesion effects on occasion setting by contextual and discrete stimuli. *Neurobiology of Learning and Memory 95* 176-184.