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Shivangi Sogani April 1st, 2021

Brain structure within the fear circuit early post-trauma as a mediator of sex differences in future post-traumatic stress disorder

by

Shivangi Sogani

Dr. Jennifer Stevens

Adviser

Neuroscience and Behavioral Biology: Grady Trauma Project

Dr. Jennifer Stevens

Adviser

Dr. Kristen E. Frenzel

Committee Member

Dr. Mar Sanchez

Committee Member

Alyssa Roeckner

Committee Member

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Abstract

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The purpose of this thesis was to measure the extent to which hippocampal, amygdala, dorsal anterior cingulate cortex (dACC), and rostral anterior cingulate cortex (rACC) volumes may serve as a biomarker for future PTSD onset and symptom severity. This work analyzed potential correlations between brain volume taken early post-trauma and later PTSD symptom severity as measured by PTSD symptom scale (PSS) scores over the course of 1-year post-trauma. Additionally, women are at a higher risk for developing PTSD after experiencing a traumatic incident as compared to men, so we assessed how sex differences may influence these correlations. As part of our analysis, we also tested for potential correlations between PTSD symptom trajectories and gender. We found a significant positive correlation between the left dACC and 12 month PSS scores. With gender as an interaction effect, we found significant positive correlations between 12 month PSS scores and the left hippocampus, left amygdala, and left dACC. When separated out by gender, the only significant correlation present was between the left dACC with 12 month PSS scores for women.

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Brain structure within the fear circuit early post-trauma as a mediator of sex differences in future post-traumatic stress disorder

Shivangi Sogani

Fall 2020-Spring 2021 Honors Thesis

Principal Investigator: Dr. Jennifer Stevens

Graduate student mentor: Alyssa Roeckner

Introduction

Background and Rationale

Post-traumatic stress disorder (PTSD) is a debilitating psychiatric disorder that can occur in people of any ethnicity, nationality, culture, gender, and age. Approximately 10% of individuals who experience traumatic events will develop PTSD (Sparta et al., 2014), and PTSD currently affects approximately 3.5% of total U.S. adults, which only includes those individuals who have been officially diagnosed (Parekh, 2017). Additionally, women are twice as likely as men to develop PTSD after experiencing a traumatic event (Glover et al., 2012). With debilitating symptoms including intrusive memories regarding the associated traumatic event, severe anxiety, exaggerated startle response, hypervigilance, and dissociation, it is important to be able to diagnose and treat this disorder as early as possible. Studying potential risk factors that may lead to a greater chance of developing PTSD is important for targeted treatment and early intervention, and it may lead to better-quality care for the patient and decreased symptoms in the future. In this study, we hoped to analyze how different brain volume regions in the immediate aftermath of a traumatic event can provide insight into later PTSD onset and symptom severity, and measure how sex differences may affect potential correlations.

Longstanding theories of PTSD posit the involvement of three primary brain regions of interest: the amygdala, hippocampus, and anterior cingulate cortex (ACC). The amygdala is involved in regulating fear processing and learned fear (Shin et al., 2006). It receives neural projections from both the ventromedial prefrontal cortex (vmPFC), which overlaps partially with the ACC, and the hippocampus (Stevenson, 2011). PTSD patients show reduced activation of the hippocampus and prefrontal cortex which may result in reduced top-down amygdala control (Zotev et al., 2018). This reduction in top-down control may result in a hyper-responsive

amygdala in response to fear stimuli and fear conditioning and lead to disordered fear regulation in PTSD (Forster et al., 2017). In anxiety circuitry, the amygdala receives information from both thalamic and cortical sensory inputs, and produces anxiety and fear-related behavioral outputs (Babaev et al., 2018). The hippocampus provides the contextual information that is needed for regulation of fear responses (van Rooij et al., 2018). Additionally, the hippocampus is important for modulating behavior when given new contextual and sensory stimuli, and previous studies have shown that increased hippocampal recruitment may help with the coping of traumatic stress (van Rooij et al., 2018). PTSD is characterized by increased and pathological forms of fear and stress that are invariant to context (appearing even in safe contexts), and this contextual fear conditioning is largely mediated by the hippocampus in combination with the amygdala (Garfinkel et al., 2014). The ACC is involved in adaptive responses to changing stimuli and conditions, and it plays an important role in emotion, cognition, and stress response (Jatzko et al., 2013). It also plays a key role in processing and monitoring behavioral prediction error, and it is critical for the safety and modulation of fear-conditioned responses as well as for reinforcement learning (Alexander et al., 2019). Reduced activation of the ACC impairs fear reaction control when faced with traumatic stimuli, and decreased control of fear response mediated by the amygdala and ACC plays a major role in the development and continued symptoms of PTSD (Jatzko et al., 2013).

Functional alterations in PTSD may be accompanied by differences in brain structure. Prior research has examined the correlation between brain region volume and PTSD; however, most of these studies focus on data of participants already diagnosed with PTSD. Reduced volume in the left amygdala compared to the right amygdala is observed in patients diagnosed with PTSD (Starcevic et al., 2014). Additionally, in a study measuring amygdala volume in

military veterans with PTSD, thinner and more concave shapes of the anterior left and right amygdala, as well as reduced volume within the left and right posterior amygdala were associated with individuals diagnosed with PTSD compared to those who were not diagnosed with PTSD (Morey et al., 2020). Furthermore, structural changes in the amygdala for patients with PTSD have been found to be nuclei-specific (Morey et al., 2020). Smaller volumes of bilateral paralaminar nuclei in the amygdala, but larger volumes of central, medial, and cortical nuclei, are associated with PTSD diagnosis in military veterans (Morey et al., 2020). While these structural analyses can help researchers and clinicians better understand the correlation between brain region volume and certain behaviors, it does not allow them to predict PTSD prognosis or how changes in brain region volume may affect the susceptibility of certain symptoms. Thus, it is important to deepen our understanding of how brain region volume can serve as an early biomarker for predicting future PTSD symptoms to allow for earlier intervention and more specific symptom-focused treatment modalities.

The hippocampus is another primary brain region affected by trauma, and it is involved in the formation of contextual and intrusive memories in individuals with PTSD (Maren et al., 2013). Hippocampal volume has previously predicted both the onset and severity of PTSD-related symptoms. Decreased bilateral hippocampal volume has been found in patients who showed greater PTSD-related symptoms (Admon et al., 2013; Fenster et al., 2018; Logue et al., 2018; Starcevic et al., 2014). Furthermore, hippocampal volume is negatively correlated with positive outcomes following treatment for PTSD, thus smaller hippocampal volumes are associated with increased risk for developing PTSD after experiencing a traumatic event (Admon et al., 2013; Fenster et al., 2018; Neria, 2016) and smaller left hippocampal volume, specifically, serves as a risk factor for the persistence of PTSD (van Rooij et al., 2015).

The ACC, which encompasses both the rostral and dorsal anterior cingulate cortices (rACC and dACC, respectively), is a key structure involved in top-down and bottom-up processing (Young et al., 2018). It has both afferent and efferent neural connections to both the amygdala and hippocampus, and it inhibits amygdala activation to threat and fear stimuli. The dACC contains neural projections to the prefrontal cortex and is consequently more associated with cognitive processing as compared to the rACC (Young et al., 2018). On the other hand, the rACC has greater functional connectivity to limbic structures including the amygdala and hippocampus and is consequently more associated with emotional processing as compared to the dACC (Young et al., 2018). In previous studies, researchers have often interchanged the terminology of the rACC and the ventromedial prefrontal cortex (vmPFC); however, in the context of this study, the rACC is defined as the subcomponent of the vmPFC rather than interchangeably with the vmPFC. The vmPFC, which includes the rACC, mediates fear extinction and the voluntary regulation of negative emotion, via inhibition of amygdala fear responses (Motzkin et al., 2015). A partial loss of the inhibition of the amygdala may account for PTSD-related symptoms such as diminished and deficient fear extinction. ACC circuitry is also involved in threat response, and particularly in the context of its connectivity with the insula (Fenster et al., 2018). The cingulate cortex is associated with arousal, emotional regulation, and attention (Stein et al., 2020). rACC volumes (Bromis et al., 2018) and vmPFC volumes have been found to be reduced in PTSD patients (Bromis et al., 2018; Shin et al., 2006). Furthermore, smaller dACC volumes were measured in adult combat veterans with PTSD and lifetime alcohol dependence (Woodward et al., 2006). This reduced ACC volume for patients with PTSD was also found in subjects without any history of alcoholism (Woodward et al., 2006). However, another study found that, while reduced rACC volume was predictive of PTSD symptoms at 3

months post-trauma, it was not predictive of those same PTSD symptoms at 6 months post-trauma (Stein et al., 2020).

Overall, these studies highlight the importance of measuring specific brain region volumes early-on after trauma exposure because they can serve as a key indicator of treatment responsiveness and PTSD onset and symptom severity. In previous literature, reduced cortical volume and reduced cortical thickness in the hippocampus, prefrontal cortex, and ACC were risk factors for developing future PTSD, and increased volumes in the above three subcortical regions were found to have a protective effect and lead to greater resilience against PTSD symptoms (Stein et al., 2020). Some of the observed weaknesses and research gaps are a lack of investigating early post-trauma risk factors for PTSD and chronic symptom trajectory.

Sexual differences in brain region volume may influence differences in PTSD onset, symptom type, and symptom severity between men and women. Although women suffer from higher rates of PTSD than men, little is known about whether there are similar neural correlates across gender or sex. The volumetric findings cited above were not gender-specific, and there is an overall lack of findings regarding the influence of gender differences on brain volume as a predictive biomarker of PTSD.

A limited number of studies have looked at relationships between brain volume and sex differences in PTSD. One study compared early childhood trauma exposure to recent trauma exposure in both male and female adults. Male patients scanned post-trauma who were diagnosed with PTSD were found to have decreased grey matter in regions including the prefrontal cortex, amygdala, and hippocampus (Helpman et al., 2017). In female patients with PTSD, early (childhood) trauma exposure was correlated with increased amygdala volume (Helpman et al., 2017). However, another study found a decrease in amygdala volume in female

patients who were trauma-exposed and diagnosed with PTSD as compared to males (Starcevic et al., 2014). These differences between studies may be impacted by analyses focusing on differing sets of amygdala nuclei including the basal, lateral, accessory basal, anterior amygdaloid, and central, medial, cortical, and paralaminar nuclei, which have been shown to have varying correlations with PTSD onset, although we cannot confirm this as the primary limitation (Morey et al., 2020). Females with PTSD have also been found to have lower hippocampal volume as compared to men with PTSD; however, the difference in volumes did not correlate with the higher prevalence of PTSD in females as compared to males (Woon et al., 2011). It is possible that volumes of other cortical regions, such as the ACC or amygdala, play a larger role in the correlation between sex differences, trauma exposure, and PTSD symptom presence and severity, but only in context of patients already diagnosed with PTSD. To combat this limitation, longitudinal studies are important to measure how certain factors such as brain region volume may serve as early biomarkers of future PTSD onset. Thus, rather than only focusing on these measures in patients who have already been diagnosed, we may be able to predict the risk of future diagnosis and the likelihood of certain symptom presentations, which can help inform treatment modalities and early intervention.

Three PTSD symptom trajectories have been utilized in prior research to evaluate general trauma populations: chronic, resilient, and recovery (Hinrichs et al., 2019; Dikmen-Yildiz et al., 2018), in work using latent growth mixture modeling to assign individuals to the trajectory class membership that had the highest posterior probability (Michopoulos et al., 2019). The chronic PTSD trajectory is characterized by persistently elevated symptoms throughout the 1-year time frame post-trauma (Galatzer-Levy et al., 2013; Michopoulos et al., 2019). Additionally, participants assigned to the chronic PTSD class were found to be exposed to significantly higher

rates of IPV (interpersonal violence) before the most recent trauma as compared to individuals in the resilient and recovery trajectories (Michopoulos et al., 2019). The resilient PTSD trajectory is characterized by a progressive decrease in symptoms over the course of the 12-15 months post-trauma. The recovery PTSD trajectory is characterized by a rapid decrease in symptoms between the five months after the traumatic event that stay decreased or completely stop after the 6 months post-trauma time point. It is therefore the chronic PTSD trajectory which includes individuals who do not recover naturally from PTSD symptoms and likely require treatment or intervention for their symptoms. Neuroimaging biomarker studies of the early-post-trauma period can provide very early information about which individuals may be at risk to end up on the chronic PTSD symptom trajectory.

In order to evaluate the correlation as well as study possible prediction models between brain volume and PTSD severity across multiple time points post-trauma, and evaluate the influence of gender on these correlations, brain volumes and PTSD scores across multiple time points post trauma were analyzed from a longitudinal emergency department (ED) study. Participants were recruited from the trauma center within 24 hours of an event that met DSM-5 Criterion A for trauma exposure. Brain volumes were collected via MRI 1-month post-trauma, and PTSD symptom scores were collected at 1 month, 3 months, 6 months, and 12 months post-trauma. By looking at brain volumes collected early post-trauma, before the diagnosis of PTSD, we may identify early brain volume biomarkers as a trait that is predictive of PTSD severity. While brain volumes may change after a trauma, there is limited research on the timeline of structural plasticity post-trauma, and thus for the purposes of this study, early brain volume is considered as a trait that may predispose an individual to certain symptoms of PTSD if they undergo a traumatic experience such as the patients evaluated in our study. Furthermore, we

can assess how the relationship between brain volumes and PTSD may be influenced by gender. Together, this data may help strengthen the field by providing greater insight into brain region volume as an early biomarker of PTSD and how potential correlations may present differently between women and men.

Research Questions

This thesis primarily evaluated the extent to which brain volume of the amygdala, hippocampus, dACC, and rACC collected shortly after the traumatic incident can serve as an early biomarker of future PTSD symptom severity (as measured by PTSD Symptom Scale scores within one-year post-trauma) and how sex differences may influence these correlations. As a secondary analysis, class variables (chronic, resilient, recovery) were analyzed to determine if there is a potential correlation between class and gender for PTSD Symptom Scale (PSS) scores.

Hypotheses

We predicted that hippocampal, dACC, and rACC volumes will negatively correlate with PSS scores, so as volume is reduced, PSS scores should increase, based on results highlighted in the Introduction above from the Admon et al., 2013, Fenster et al., 2018, Woodward et al., 2006 and Bromis et al., 2018 publications. Predicting the directionality of the relationship between amygdala volume and PSS scores was a little more difficult due to the contrasting results from previous literature based largely on measuring different nuclei within the amygdala region (Morey et al., 2020 ; Starcevic et al., 2014). However, since the amygdala and hippocampus both play a role in fear extinction, and PTSD may also result in impaired fear extinction, we predicted that higher PSS scores will also be correlated with lower amygdala volume. When considering gender as an interaction effect on the correlation between PSS scores and subcortical volume regions and consequently separating out by gender, we hypothesized to find a significant

difference between women and men in the ACC regions, rather than the hippocampal or amygdala regions (Helpman et al., 2017; Morey et al., 2020). In addressing our secondary research question, we predicted that women would be more susceptible to being in the chronic PTSD trajectory as compared to men.

Methods

Participants

Ninety-four participants were included in the statistical data analysis for this paper, and all participants were recruited from a larger study consisting of 504 participants from a PTSD biomarkers study. All subjects were Emergency Department (ED) patients at Grady Memorial Hospital in Atlanta, GA, who had experienced a traumatic event within the past twenty-four hours of arrival to the ED. Out of the 94 subjects, 54 identified as male and 40 identified as female. All subjects spoke English, were between the ages of 18-65 years, and experienced a criterion A trauma. Criterion A trauma refers to the type of stressor, and its characterization has evolved with each new Diagnostic and Statistical Manual. Currently, it is characterized by the individual being exposed to either death or threatened death, actual or threatened sexual violence, or actual or threatened serious injury, through direct exposure, witnessing the trauma, or indirect exposure (Friedman et al., 2011). In our study, trauma types included 3 non-sexual assaults, 54 motor vehicle collisions, 4 motorcycle crashes, 15 pedestrian versus automobile accidents, 3 gunshot wounds, 1 stabbing, 4 industrial or home accidents, 1 animal attack, 4 bike or bike versus automobile accidents, and 5 sexual assaults. Participant exclusion criteria included previous hospitalization for mental health reasons, current intoxication, suicidal ideation, attempted suicide in the past 3 months, or altered mental states at the time of the ED visit. The

study procedures were approved by Emory University's and Grady Memorial Hospital's Institutional Review Boards (IRB). Interviews were conducted by researchers who were trained by the Grady Trauma Project in trauma-informed care, and all patients provided written consent for each part of the study.

Table 1. Clinical and demographic features of the sample

	Total	Males	Females
	N=94	N=54	N=40
Age, mean (SD)	36 (12.70)	37 (12.82)	34 (12.46)
Race (%)			
Hispanic	4 (4.26%)	2 (3.70%)	2 (5.00%)
Non-Hispanic White	14 (14.89%)	10 (18.52%)	4 (10.00%)
Non-Hispanic Black	72 (76.60%)	38 (70.37%)	34 (85.00%)
Non-Hispanic Other	4 (4.26%)	4 (7.41%)	0 (0.00%)
Education level (%)			
Doctoral Degree	1 (1.06%)	1 (1.85%)	0 (0.00%)
Master's Degree	3 (3.19%)	2 (3.70%)	1 (2.50%)
Some graduate school	1 (1.06%)	1 (1.85%)	0 (0.00%)
Bachelor's Degree	11 (11.70%)	9 (16.67%)	2 (5.00%)
Associate's/some college	43 (45.74%)	21 (38.89%)	22 (55.00%)
High School Degree	25 (26.60%)	15 (27.78%)	10 (25.00%)
Some High School	10 (10.64%)	5 (9.26%)	5 (12.50%)

Trauma Type *Non-Sexual Assault* *Motor Vehicle Collision* *Motorcycle Crash (MCC)* *Ped v. Auto* *Gunshot Wound* *Stabbing* *Industrial/Home Accident* *Animal Bite/Attack* *Bike Accident/Bike v. Auto* *Sexual Assault*	3 (3.19%) 54 (57.45%) 4 (4.26%) 15 (15.96%) 3 (3.19%) 1 (1.06%) 4 (4.26%) 1 (1.06%) 4 (4.26%) 5 (5.32%)	3 (5.56%) 27 (50.00%) 4 (7.41%) 10 (18.52%) 3 (5.56%) 0 (0.00%) 4 (7.41%) 1 (1.85%) 2 (3.70%) 0 (0.00%)	0 (0.00%) 27 (67.50%) 0 (0.00%) 5 (12.50%) 0 (0.00%) 1 (2.50%) 0 (0.00%) 0 (0.00%) 2 (5.00%) 5 (12.50%)
1-month PSS, mean (SD)	16.11 (11.53)	13.72 (11.36)	19.33 (11.09)
3-month PSS, mean (SD)	11.37 (9.83)	10.60 (10.55)	12.48 (8.70)
6-month PSS, mean (SD)	9.23 (9.62)	8.02 (9.41)	11.14 (9.82)
12-month PSS, mean (SD)	7.63 (9.27)	6.45 (8.81)	9.25 (9.82)
Brain Volume (denoted as % intracranial volume or %ICV for hippocampus and amygdala, mm			

for dACC and rACC)			
Total intracranial volume, mean (SD) in microliters	1390000.00 (14142.14)	1290000.00 (127279.22)	1325000.00 (106066.02)
Right amygdala volume, mean (SD)	0.0011 (0.0002)	0.0011 (0.0001)	0.0011 (0.0002)
Left Amygdala volume, mean (SD)	0.0011 (0.0002)	0.0011 (0.0002)	0.0011 (0.0002)
Right hippocampus volume, mean (SD)	0.0028 (0.0003)	0.0027 (0.0003)	0.0029 (0.0003)
Left hippocampus volume, mean (SD)	0.0027 (0.0003)	0.0026 (0.0003)	0.0029 (0.0003)
Right dACC thickness, mean (SD)	2.6169 (0.3348)	2.5528 (0.2477)	2.7018 (0.4119)
Left dACC thickness, mean (SD)	2.6531 (0.2707)	2.6070 (0.2548)	2.7141(0.2821)
Right rACC thickness, mean (SD)	2.7183 (0.2882)	2.6639 (0.2573)	2.7903 (0.3135)
Left rACC thickness, mean (SD)	2.6974 (0.2572)	2.6533 (0.2752)	2.7557 (0.2212)

PTSD Symptom Scale (PSS), Scale: 0-40, dACC = dorsal anterior cingulate cortex, rACC = rostral anterior cingulate cortex, SD = standard deviation. Brain volume was measured in percent intracranial volume (%ICV) for the hippocampus and amygdala regions due to normalized data, and in millimeters for the dorsal and rostral anterior cingulate cortex thickness).

Emergency Department (ED) Assessment and Follow-Up Assessments

A primary measure of PTSD severity is the PTSD Symptom Scale (PSS), a 17-item semi-structured interview that assesses the presence and severity of DSM-V PTSD symptoms. These PSS scores can change longitudinally across different timepoints following trauma and can provide insight into the likelihood of the onset of PTSD itself, developing chronic versus acute PTSD, and help to identify the class trajectory of PTSD (resilient, recovery, chronic). PSS scores were determined at four different timepoints following the ED visit: at 1 month (baseline), 3 months, 6 months, and 12 months (chronic PTSD outcome) post-trauma. During the follow-up

interviews, patients were also asked if they had been going to therapy or undergoing other potential treatments.

Structural Brain Imaging

Structural brain magnetic resonance imaging (MRI) data were collected approximately 1 month after exposure to civilian trauma in these 94 participants to measure subcortical volumes, cortical region thickness, and intracranial volume. The MRI data includes brain volume data for the right and left hippocampus, right and left amygdala, and right and left dorsal and rostral anterior cingulate cortex. The volumes of these regions will be compared to PSS scores collected across the four timepoints. Three scanner sites were used over the course of the data collection part of the study due to upgrades in two of the scanners mid-study. The structural brain imaging data were acquired on three Siemens 3.0-Tesla Magnetom Trio Total Imaging Matrix (TIM) MRI scanners differentiated by Center for Systems Imaging (CSI: Siemens, n=12), Biomedical Imaging Technology Center (BITC: Malvern, n=26), and Center for Advanced Brain Imaging (CABI: PA, n=56), using a 12-channel head coil. Due to the use of different scanners, separate acquisition parameters were used for each scanner to determine brain region volume. For all statistical analyses, a scanner covariate was used to account for the three different scanners. Structural images were acquired using multi-echo T1-weighted images. Bilateral hippocampal volume, bilateral amygdala volume, thickness and volume of the bilateral dorsal and rostral anterior cingulate cortex, and total intracranial volume which includes the cerebrospinal fluid, grey matter, and white matter, were all measured. Any outliers (due to brain abnormalities, falx calcifications, motion during scan, or improper scans) were identified and left out the statistical analyses, and consequently only patients with neurotypical brain scans were included in the analysis. After quality checks were performed using the ENIGMA 2 and ENIGMA 3 protocols

for cortical thickness and surface area, one brain scan was identified as having automated segmentation errors and was omitted from analysis.

For the bilateral hippocampi and amygdala volume data, data was normalized by regressing regional volumes against intracranial volume and extracting the standardized residuals to ensure consistency across participants who may have differing head size and total ICV, which correlated with raw hippocampal and amygdala volume data. Rostral ACC and dorsal ACC thickness were analyzed for possible correlations with total intracranial volume. Overall intracranial volume did not correlate with left rACC thickness ($r(92)= -0.08$, $p > 0.05$), right rACC thickness ($r(92)= -0.04$, $p > 0.05$), left dACC thickness ($r(92)= -0.09$, $p > 0.05$), or right dACC thickness ($r(92)= -0.17$, $p >0.05$), and thus were not normalized.

Statistical Analysis Overview

We created Table 1 to obtain clinical and demographic features of the sample. To address the hypothesis that women have higher PSS scores than men overall, we coupled Table 1 findings with T-tests. We then ran T-tests to analyze the sex differences in volume or thickness for the 8 ROIs. 8 ROIs were defined at the beginning of the statistical analysis: left hippocampus, right hippocampus, left amygdala, right amygdala, left dACC, right dACC, left rACC, and right rACC. To determine whether hemisphere should be considered in the analysis, volumes were compared across hemispheres for each subcortical brain region. To address the research question of whether subcortical region volume serves as an early biomarker of PTSD onset and symptom severity, we ran a correlation test between total PSS scores and brain region volume without gender as an interaction effect. The analyses focused on the 1 month (baseline: concurrent with MRI scan) and 12 month (chronic PTSD outcome) timepoints. We then wanted to analyze how sex differences may affect these associations; thus, we ran regression models to analyze PTSD as

a function of gender and volume, thereby including gender as an interaction effect. We then conducted sensitivity analyses of these effects, including the following covariates: age, race, scanner type, and specifically baseline PSS scores (1 month) for the 12-month models. For the ROIs that had significant interaction effects for gender ($p<0.05$), data was split by gender and analyzed in separate regression models to directly test for potential differences between women and men. We tested our secondary research question of measuring for a potential correlation between PTSD trajectory (class) and gender by using a Fisher's Exact Test.

Results

The inter-hemisphere correlations indicated that the left and right hemisphere would provide unique variance, for most of the ROIs (except for hippocampus: $r = 0.90$), and we therefore determined to analyze the left and right hemispheres separately. The additional correlations were amygdala: $r = 0.78$, dACC: $r = 0.62$ and rACC: $r = 0.35$.

In assessing laterality, R-values were very low for the dACC ($r=0.62$) and rACC (0.35) and thus there were large volume differences between the left and right dACC & left and right rACC relative to the hippocampus ($r=0.90$) and amygdala ($r=0.78$) hemispheres, which displayed high similarity in volume between hemispheres.

Considering the 1 month baseline timepoint and 12 month chronic PTSD outcome timepoint, women had a higher mean PSS score at both timepoints compared to men (Table 1).

The only significant correlation between PSS and ROI without considering gender as an interaction effect is at the 12 month time point for the left dACC ($r=0.283$, $p=0.0345$*).

Table 2. Regression Analysis for 12 month PSS scores and Subcortical Volumes with Gender as an Interaction Effect

ROI	**Main effect (*p*-value)**	**gender x ROI (*p*-value)**
Right Hippocampus	0.233	0.322
Left Hippocampus	0.0478*	0.0430*
Right Amygdala	0.262	0.205
Left Amygdala	0.0289*	0.0171*
Right dorsal ACC	0.290	0.185
Left dorsal ACC	0.03430*	0.00641**
Right rostral ACC	0.451	0.393
Left rostral ACC	0.524	0.368

*** Uncorrected p<0.05.**
**** Uncorrected p<0.01. None of the effects met a corrected threshold of 0.00625.** Left dACC volume was significantly correlated with 12 month PSS scores (r(92)=0.283, p = 0.035).

Table 3a. Regression Sensitivity Analyses: Analyzing PTSD as function of gender and volume with covariates included.

	Beta	SE	T	p-value
PSS (12 month)				
PSS (1 month)	5.008e-01	8.933e-02	5.606	9.97e-07 ***
Scanner	-3.335e-01	1.560e+00	-0.214	0.8316
Age	-8.115e-02	8.429e-02	-0.963	0.3405
Race	6.937e-01	6.549e-01	1.059	0.2948
Gender	-4.452e+01	2.145e+01	-2.076	0.0433 *
Left Hippocampus Volume	-2.376e+04	1.170e+04	-2.031	0.0478 *
Gender:Left Hippocampus Volume	1.589e+04	7.645e+03	2.079	0.0430 *

Table 3b.

	Beta	SE	T	p-value
PSS (12 month)				
PSS (1 month)	5.041e-01	8.755e-02	5.758	5.87e-07 ***
Scanner	1.445e-01	1.527e+00	0.095	0.9250
Age	-8.036e-02	8.388e-02	-0.958	0.3429
Race	5.862e-01	6.432e-01	0.911	0.3666
Gender	-3.571e+01	1.442e+01	-2.476	0.0168 *
Left Amygdala Volume	-4.099e+04	1.819e+04	-2.253	0.0289 *
Gender:Left Amygdala Volume	3.161e+04	1.279e+04	2.471	0.0171 *

Table 3c.

	Beta	SE	T	p-value
PSS (12 month)				
PSS (1 month)	0.44948	0.08591	5.232	3.64e-06 ***
Scanner	0.58245	1.44137	0.404	0.68794
Age	-0.00148	0.08009	-0.018	0.98533
Race	0.42921	0.62355	0.688	0.49456
Gender	-49.55486	17.15692	-2.888	0.00579 **
Left dACC Volume	-21.46218	9.85115	-2.179	0.03430 *
Gender:Left dACC Volume	18.04950	6.33161	2.851	0.00641 **

***uncorrected p < 0.05; **uncorrected p < 0.01 (p=0.05 is the uncorrected significance threshold).**

Left hippocampus volume, left amygdala volume, and left dACC thickness were significantly correlated with 12 month PSS scores, when assessed with an uncorrected p-value of p=0.05 and with gender as an interaction effect. Rostral ACC thickness was non-significant when correlated with the 12 month PSS scores with gender as an interaction effect. However, left rACC thickness was significantly correlated with 1 month PSS scores when considering gender as an interaction effect. Regression sensitivity analysis were conducted with gender as an interaction effect for the three ROIs that showed significant correlations between ROI volume and 12 month PSS scores (left hippocampus, left amygdala, left dACC), with race, age, scanner type, and 1 month PSS scores as covariates. Additionally, the beta values also known as b coefficients showed flipped directionality in all 3 ROIs when looking at the volume and 12 month PSS score regressions compared to those with gender as an interaction effect, as it changes from a negative to a positive relationship when gender is taken into account as an interaction effect. There were no significant correlations for any individual covariate, supporting that with the given conditions of the study, age, race, scanner type and baseline PTSD symptoms are unrelated to the response variable: 12 month PSS scores. Thus, it strengthens the correlation directly between subcortical volume and 12 month PSS scores for the left hippocampus, left amygdala, and left dACC.

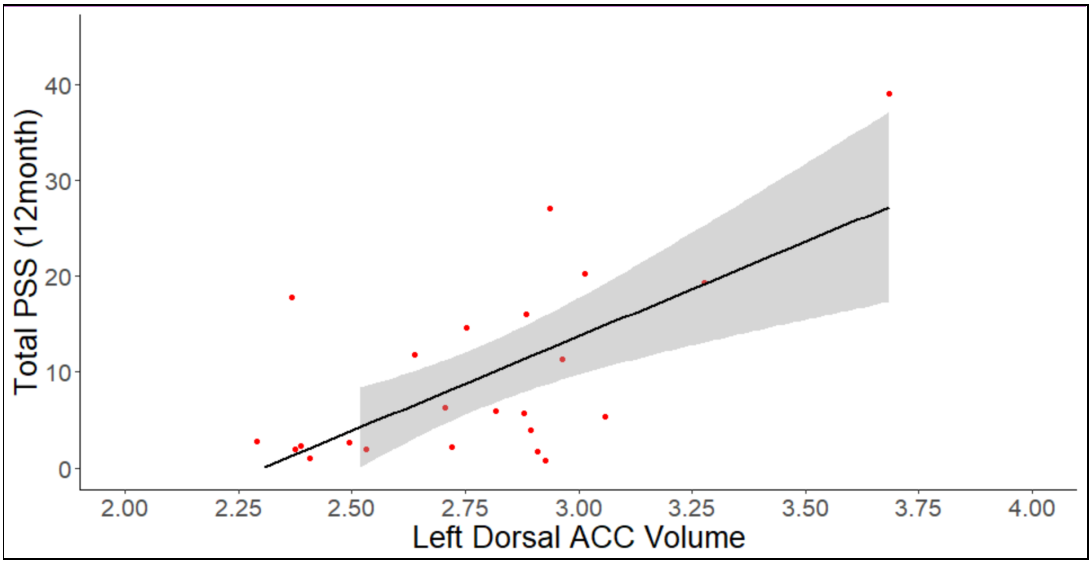

Figure 1a) Total PSS at 12 months Post-Trauma vs. Left dACC Volume (Women). Total 12 month PSS scores significantly correlated with left dorsal ACC volumes in women (r(39)=0.6477, p=0.00451*).[2]

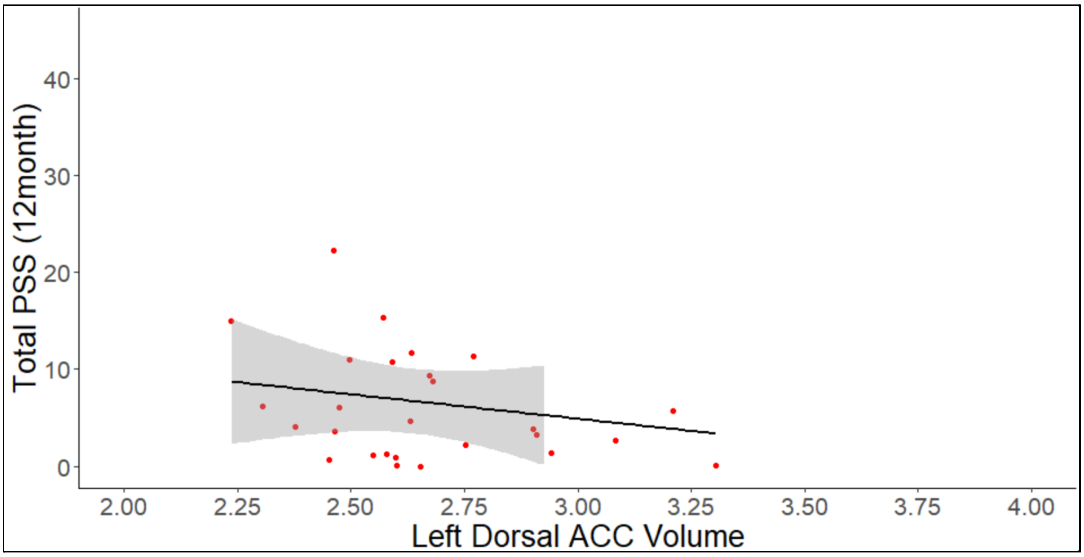

Fig 1b) Total PSS at 12 months Post-Trauma vs. Left dACC Volume (Men) There is a negative, non-significant relationship between total PSS scores and left dACC volumes in men at 12 months post-trauma, thus as left dACC volume increases, the total PSS scores decrease (r(52)= -0.1015 , p=0.395).

Based on values that are significant for Pr(>|t|) gender:ROI (Table 3 models that had significant gender interaction effects), we analyzed male and female data separately by running separate regressions. There was a significant positive correlation between the left dACC volume

and PSS score at 12 months for women (r(39)=0.6477, p=0.00451*) (Fig 1a), but not men (r(52)= -0.1015 , p=0.395) (Fig 1b). For the left hippocampus and amygdala, there were positive, non-significant associations for women (Hippocampus: r(39)=0.1566 , β=7628.3121 , p=0.2945 ; Amygdala: r(39)=0.3192 , β=23064.9727 , p=0.0797), and negative, non-significant associations for men (Hippocampus: r(52)= -0.2006 , β= -7.615e+03, p=0.1051, Amygdala: r(52)= -0.1527 , β= -8.338e+03, p=0.240). The effect sizes were large, suggesting that the analyses separating the gender groups were underpowered to detect a significant association.

Class distinctions (chronic, resilient, recovery) were also analyzed as a secondary factor in the correlation between PSS outcomes and gender. A Fisher's test was used to account for a smaller sample size in the chronic class. This modified chi-squared test compared the three classes with gender, and resulted in a p-value of 0.1331, thus being non-significant.

Discussion

Interpretation of Results

Overall, the purpose of this thesis was to analyze whether amygdala, hippocampus, rACC and dACC volumes collected early post-trauma via MRI predicted later PTSD symptom severity (PSS), in order to examine brain volume as a predictor of later PTSD. Sex differences were closely examined, and based on the results, it is likely that a combination of psychosocial and biological markers also contribute to differing onset and symptom severity of PTSD between patients. Women had a higher mean PSS score for all four timepoints (1 month, 3 months, 6 months, 12 months after traumatic event) compared to men. This supported that women are at a higher risk for developing PTSD and have higher PTSD symptom severity as compared to men.

We hypothesized that lower hippocampal, rACC and dACC volume will correlate with higher PSS scores. However, our correlation model does not support this hypothesis. Without gender as an interaction effect, the only significant correlation that was present was for the left dACC with 12 month PSS scores ($r=0.283$, $p=0.0345$*), and this was a positive significant correlation rather than the predicted negative significant correlation. When considering gender as an interaction effect, any correlation at the 1 month baseline or 12 month chronic timepoints (12 month: left hippocampus, left amygdala, left dACC ; 1 month: left rACC) also had positive significant correlations rather than negative significant correlations. Thus, as the ROI volume or thickness increased, the PSS score for that respective timepoint also increased. This change in the directionality of the association may be due to other predisposing factors or confounding variables that will be discussed in detail later. It may also be due to differing salience of certain ROIs in the fear and anxiety circuitry associated with PTSD when comparing women versus men. When separated out by gender, left dACC with 12 month PSS scores for women was the only significant correlation ($r(39)=0.6477$, $p=0.00451$*). This supports our hypothesis that the greatest difference between women and men would be in the ACC region as compared to the hippocampus or amygdala. More specifically, our results showed a difference in significance only between the dACC rather than for the rACC as well, where the dACC region had the significant correlation for women but not for men. All other ROIs had non-significant correlations and consequently minimal differences between women and men.

For the dACC, which is involved in cognitive processing and fear expression, significant gender differences were found when comparing women versus men for the left dACC at the 12 months time point. For women, there was a significant positive correlation: as the left dACC volume increased, the PSS score at 12 months increased as well. For men, there was no

significant relationship between left dACC volume and PSS scores at 12 months post-trauma. In contrast, prior studies found that the ACC volume is reduced during symptomatic states in PTSD patients (Bromis et al., 2018; Shin et al., 2006). While male left dACC volume has a slight negative trend, with volume increasing as PSS score decreases, in line with prior literature, this correlation is not significant. This distinction between positive versus negative trends for women versus men, respectively, highlights the importance of brain volume differences between men and women and how trauma affects women versus men differently.

Based on prior research, we expected to find minimal difference in overall amygdala volumes between men and women as correlated with PSS scores. Smaller volumes of lateral and paralaminar amygdala nuclei are present in patients with PTSD (Morey et al., 2020). However, in these same patients, there were larger central, medial, and cortical nuclei amygdala volumes. Amygdala volume is thus still debated as a predictor of PTSD-related symptoms, with results depending on which amygdala areas are analyzed. Results from the current study demonstrate a non-significant, positive trend between PSS scores and left amygdala volume at 12 months post-trauma for women, with some outliers that may be driving this. However, there was no significant association for this same ROI at 12 months for men. Again, different amygdala nuclei may account for these differences, and this highlights the importance of potential differences in research methods involving measuring the amygdala as a whole as opposed to specific amygdala nuclei, and how they may have varying implications on PSS scores. Some other predisposing factors such as differing gonadal hormone levels will be discussed in detail later in this discussion.

In previous studies, lower hippocampal volume has generally been associated with greater PTSD symptoms, but these studies aren’t separated by gender. Additionally, in one study,

a history of childhood maltreatment and abuse is associated with reduced hippocampal volumes in adult males, but not in adult females (Samplin et al., 2013). However, this study was only done on Caucasians, who only make a small subset of our sample size in the current study. The data from this study also showed that females may be more resilient to structural and neurological effects of childhood maltreatment, but that females are not more resilient to the psychiatric symptoms associated with this early trauma exposure (Samplin et al., 2013). Furthermore, childhood emotional and physical abuse was not measured separately or used as a covariate in our analysis. When interpreting the hippocampal volume results from our study, there were no significant associations found for either women or men when correlating PSS scores and left hippocampal volumes at 12 months post-trauma. Thus, similar results were not seen in our study when separated by gender. Confounding variables such as hormonal differences between genders, lack of considering some covariates such as previous trauma history and future social support, and measuring brain volume at different timepoints across studies (1 month post-trauma in our study versus after PTSD diagnosis in most other studies) are a few of the factors that may collectively be potential explanations to these differences between previous studies and our study.

For males, early trauma exposure during childhood is previously shown to involve long-term grey matter loss in the amygdala, hippocampus, and prefrontal cortex (Helpman et al., 2017). Contrary to this, in the current study, men do not have a significant relationship between hippocampus, amygdala, or ACC volumes and PSS scores at neither 1 month nor 12 months post-trauma. However, for women, early trauma exposure correlates with bigger amygdala volume (Helpman et al., 2017) which supports the generally positive, though non-significant, trend for women between 12 month PSS scores and left amygdala volume in the current study.

However, this study focused on early trauma exposure in adults who faced childhood maltreatment, as opposed to our current study, which focused on recent adult civilian trauma exposure.

PTSD symptom severity at 12 months did not significantly correlate with volumes of interest in the right hemisphere, when accounting for covariates and gender interaction effects. Laterality differences that may account for this include cortical gyrification and the resulting structural differences between the two hemispheres. In one study, researchers found that, after accounting for covariate effects of comorbid psychiatric symptoms, significant positive associations were found in the left hemisphere frontal cortex cluster between PTSD severity and the local gyrification index, that were not found in the right hemisphere (Gharehgazlou et al., 2021). The atypical gyrification between the left and right hemispheres, especially in the prefrontal cortex, including the ACC, may play a role in the psychopathology and pathogenesis of PTSD, and the results from the study support the role of prefrontal cortex structure in determining immediate and emergent severity of PTSD after trauma (Gharehgazlou et al., 2021).

Predisposing Factor: Differing Hormone Levels

There may be other predisposing factors that make women more vulnerable to developing PTSD after experiencing a traumatic event. Not only are women twice as likely as men to develop PTSD, they experience different symptoms and comorbidities associated with PTSD (Pooley et al., 2018). One key biomarker aside from brain volume that may account for sex differences in PTSD is differing gonadal hormone levels that may result from menstrual cycle phases and external factors such as hormonal birth control. E2 has been found to have a protective effect against developing PTSD symptoms, in regulating emotional processing, and

decreasing the negative response to stressful stimuli (Miedl et al., 2018). Exogenous estrogen treatment may also serve as a beneficial pharmacological addition to fear extinction therapies because of this protective effect (Glover et al., 2015), and current studies at Grady and other research labs are currently tracking fluctuating E2 levels in the menstrual cycle and noting potential effects. Tracking E2 levels may help determine ability to cope with fear and anxiety processing and it may serve as potential resilience markers. Testosterone (T) levels also have a protective effect and high T is associated with lower PTSD risk. Testosterone has anxiolytic and antidepressant properties, and when T undergoes aromatization to E2, this protective effect may decrease, and this may help explain why men experience decreased symptom severity and why men are less likely to be diagnosed with PTSD after experiencing a traumatic event as compared to women (McHenry et al., 2014).

For the purposes of this thesis, estrogen levels were not analyzed to measure potential correlation with PSS scores. These differing gonadal hormone levels (low estrogen in particular) may serve as cofactors that increase risk for developing PTSD and/or developing greater symptom severity especially of both anxiety and depression in women (Glover et al., 2012), and show potential activational effects of changing E2 levels. Additionally, one study showed an organizational effect (permanent and occurring in early development) as opposed to an activational effect (transient) where prenatal T exposure is correlated with decreased grey matter volume within the dACC and that reduced dACC gray matter mediates the association between the ratio of the second and fourth digit of the hand (2D:4D ratio) and increased trait aggression in women but not in men. With its role in cognitive reappraisal, dACC grey matter volume is correlated with a greater tendency to reinterpret the significance of emotionally triggering stimuli for women but not for men (Gorka et al., 2015). It is important to note that the effects of E2 on

emotional regulation are nuanced and complex, so further studies are needed to be able to help make these distinctions between potential effects on different symptoms. More studies measuring estrogen and testosterone levels as an interaction effect with the correlation between brain region volumes and PSS scores would hopefully shed more light to this area of study.

Another steroid hormone class that may explain differences between women and men other than brain volume for PTSD onset and severity is increased glucocorticoid release. If women are in the luteal phase of their menstrual cycle during the traumatic incident, increased glucocorticoid release alongside the hippocampus, may facilitate the consolidation of trauma memories (Bryant et al., 2011), and thus may lead to increased PSS scores in the months after the traumatic event.

As mentioned above, a better understanding of the role of endogenous hormone levels in PTSD psychopathology may help researchers develop a greater understanding of how PTSD affects women and men differently, and how brain volume structural imaging may aid in early intervention. Exogenous hormone administration may also provide greater insight on the impact of E2 and T to help a subset of the population: victims of sexual assault. Victims of sexual assault are particularly vulnerable to developing PTSD and exhibit multiple symptoms associated with the syndrome, including avoidance, hyperarousal, and intrusive re-experiencing symptoms (all subsets of the PSS analysis). One study analyzed the relationship between emergency contraception (EC) administration in the immediate aftermath of the sexual assault and PTSD symptoms in female survivors of sexual assault (Ferree et al., 2012). Ogestrel, an emergency contraceptive that contains a combination of estrogen ethinyl E2, progestin levonorgestrel, and progesterone has led to significantly lower total PTSD symptom levels in this population subset (Ferree et al., 2012). Thus, having an understanding of the role of synthetic E2 and progesterone

(similar protective effects) can help develop more effective treatments and decrease the severity of symptoms in the long-term due to more promising results in the immediate aftermath of the traumatic event.

Limitations

However, it is important to note that although all participants in our study identified with a binary gender, hormones can vary across gender identification, and future extensions of this work should be explored among non-binary, transgender, gender-fluid, and gender-nonconforming individuals. Additional limitations include the sheer number of confounding variables that may impact the relationship between brain volume, sex differences, and PTSD severity across timepoints, and how many of these variables are subjective such as other demographic data, previous medical history and/or trauma history, and future access to support resources and counseling. Furthermore, even though questions are standardized in the PSS semi-structured interview, the responses are still subjective and thus cannot be quantified in the exact same way across the 94 participants. Some examples of this include participants' responses to categorizing and rating the degree of physical and emotional trauma.

Due to the nature of this research, no pre-trauma data is available, and we cannot conclude how volume may change from pre- to post- trauma. A common limitation due to the lack of pre-trauma data in many PTSD studies including our study is being able to distinguish whether trauma leads to structural deficits and atrophy in individuals who become diagnosed with PTSD or whether having a specific subcortical volume predisposes that individual to PTSD after they experience a traumatic event(s). For our study, we considered the latter due to the lack of pre-trauma data and lack of MRI data at later time points such as the 12 month time point post-trauma, and thus considered early brain volume as a predisposing trait for future PTSD.

Future Studies and Implications

Exposure to psychotherapy and/or mental health counseling may mediate PTSD symptom scores. In our study there was only one interview question that addressed therapy exposure, which asked for a yes/no response as to whether or not the patient sought out therapy post-trauma. It was too general of a measure to accurately analyze for an effect on PSS scores, and was thus left out of the analysis. Previous literature shows promising results for the effect of prolonged exposure (PE) treatment on brain region volume and its effect on decreasing PTSD symptoms, especially for women who already show a generally positive trend between left rACC volume and PSS scores at 1 month post-trauma (Helpman et al., 2016). PE treatment may decrease and/or eliminate maladaptive trauma associations, and may promote structural change and synaptic plasticity in the rACC (Helpman et al., 2016). Early therapy modalities also impact different PTSD trajectories. Early cognitive behavioral therapy (CBT) was found to be effective for the recovery class of symptomatic trauma survivors, and accelerated their recovery (Galatzer-Levy et al., 2013). Thus, future research analyzing the effects of different therapy modalities on certain brain volume regions and class distinctions may help specify the best treatments based on the specific presentation of symptoms of the individual across different timepoints post-trauma.

Another future avenue that would be useful to study is how PTSD onset and symptom severity may manifest differently in children vs. adults who experience trauma, in relation to brain volume, and how childhood trauma impacts brain volume. One study found reduced bilateral hippocampal volume in adults with childhood maltreatment-related PTSD compared with healthy controls, but no significant change in hippocampal volume in children with maltreatment-related PTSD (Woon et al., 2008). This suggests that hippocampal volume

deficits from childhood maltreatment may not be apparent until adulthood (Woon et al., 2008). Dorsal ACC grey matter volume abnormalities were found in adolescents with childhood sexual abuse (Rinne-Albers et al., 2017). Dorsal ACC volume was also found to be associated with altered evaluative emotion processing of fear and anxiety, although not directly with PTSD severity in adolescents (Rinne-Albers et al., 2017). Furthermore, a history of childhood maltreatment and abuse has been associated with reduced hippocampal volumes in adult males, but not in adult females (Samplin et al., 2013). Additional studies may help explain differences in age group and how that manifests differently when considering PTSD symptom severity and having brain region volume as a structural biomarker. Furthermore, future studies may help increase our understanding of how childhood trauma affects adults later.

Conclusion

Overall, this thesis provided greater insight into how early brain volume can serve as a predictor of later PTSD onset and symptom severity, and how these correlations and consequent symptoms may present themselves differently in women versus men. Coupled with future work such as those outlined above would strengthen the current field and further our understanding of brain volume as a biomarker of PTSD and the impact of gender. This will hopefully help establish better early intervention and treatment modalities focused on certain symptoms of PTSD based on brain volume and other biological and psychosocial data of the individual. It may also help predict risk for developing other mental health disorders post-trauma, particularly those with higher prevalence in women than in men, such as depression.

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