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Longitudinal Changes in Resting-State Functional Connectivity of the Salience Network Among Individuals At-Risk for PTSD Development

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LONGITUDINAL CHANGES IN RESTING-STATE FUNCTIONAL CONNECTIVITY OF THE SALIENCE NETWORK AMONG INDIVIDUALS AT-RISK FOR PTSD DEVELOPMENT

by

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ABSTRACT

LONGITUDINAL CHANGES IN RESTING-STATE FUNCTIONAL CONNECTIVITY OF THE SALIENCE NETWORK AMONG INDIVIDUALS AT-RISK FOR PTSD DEVELOPMENT

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Predicting post-traumatic stress disorder (PTSD) following a traumatic event has been a focus of recent neuroimaging research in the hopes of identifying key biomarkers that contribute to the disorder's development. One possibility relies on understanding the connectivity between intrinsic connectivity networks (ICNs), including the salience network (SN). Prior research has consistently identified hyperconnectivity among SN regions among those with chronic PTSD, and this study aimed to examine the role of SN connectivity over time on PTSD symptom development. To do so, this study recruited individuals presenting to the Emergency Department with traumatic injuries to complete two resting-state fMRI scans: one at two-weeks post-trauma (T1) and one at six-months post-trauma (T2). The current analyses used an intrinsic connectivity contrast (ICC) within a SN mask of salience-related regions to assess the connectivity of particular SN regions with the entirety of the network. There were no significant relationships between T2 connectivity and total PTSD symptom severity at T2, nor was there any significant findings for the relationship between T1 connectivity and total PTSD symptom severity at T2. While the change in total PTSD symptom severity scores did not significantly relate to changes in SN connectivity over time, a significant cluster within the dACC was found to be hyperconnected with the rest of the SN for the interaction between Time and Reexperiencing symptom severity score. This result remained significant when additional covariates were added to the model. Overall, this study highlights the importance of tracking changes in neurocircuitry from the acute trauma response to chronic PTSD, suggesting that chronic exposure to reexperiencing symptoms of PTSD leads to small changes in SN connectivity that slowly rewire ICN circuitry over time.

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LIST OF ABBREVIATIONS

AFNI	Analysis of Functional NeuroImages
AUDIT	Alcohol Use Disorder Initial Testing
CEN	Central Executive Network
DAST	Drug Abuse Screening Test
DASS	Depression, Anxiety, Stress Scale
dACC	Dorsal Anterior Cingulate Cortex
DMN	Default Mode Network
dlPFC	Dorsolateral Prefrontal Cortex
GLM	General Linear Model
ICC	Intrinsic Connectivity Contrast
ICN	Intrinsic Connectivity Network
LME	Linear Mixed Effects (Model)
MDD	Major Depressive Disorder
mPFC	Medial Prefrontal Cortex
PCC	Posterior Cingulate Cortex
PCL-5	PTSD Checklist for DSM-5
PTSD	Post-Traumatic Stress Disorder
ROI	Region of Interest
SN	Salience Network
SUD	Substance Use Disorder
T1	Timepoint collected at 2-weeks post-trauma
T2	Timepoint collected at 6-months post-trauma

Longitudinal Changes in Resting-State Functional Connectivity of the Salience Network among Individuals At-Risk for PTSD Development

Post-Traumatic Stress Disorder

Trauma is an unfortunately common experience; approximately 70% of individuals worldwide and nearly 90% of Americans will experience a traumatic event in their lifetime (Kessler et al., 2017; Kilpatrick et al., 2013). While exposure to trauma is high, the majority of individuals are resilient, with only four percent of the world population and eight percent of the US population diagnosed with posttraumatic stress disorder (PTSD; Kessler et al., 2017; Kilpatrick et al., 2013; Liu et al., 2017). PTSD is characterized by symptoms of reexperiencing intrusions, hypervigilance, hyperarousal, and negative cognitions following a single or prolonged perceivable threat to one's safety (Bisson et al., 2015). These symptoms may develop immediately after the traumatic experience; however, PTSD cannot be diagnosed until one month after the trauma has passed (American Psychiatric Association, 2013) . With the discrepancy in rates of trauma exposure and PTSD prevalence, research has turned towards identifying underlying factors that make some individuals more likely to developing PTSD after traumatic exposures.

A wide variety of socioenvironmental factors can contribute to the development of symptoms including trauma type, history of trauma exposures, comorbid diagnoses, gender, and age (Kessler et al., 2017). Negative affect (e.g., unpleasant emotions such as sadness, anger, shame, guilt, fear, etc.) is another factor that may influence the development of PTSD as well as the biological circuitry involved. Importantly, negative affect extends beyond diagnostic boundaries, leading to comorbidities that complicate the interpretation of mechanisms for psychopathology. Both major depression (MDD) and alcohol/substance use disorders (AUD/SUDs) often coincide with PTSD, and all three are strongly linked to negative affect (Dorison et al., 2020; Erwin et al., 2019; Gandelman et al., 2018; Post et al., 2011; Tull et al., 2018). Beyond socioenvironmental factors and negative affect, there is a growing movement dedicated to identifying biological risk factors uniquely contributing to PTSD susceptibility, and altered neurocircuitry has been presented as one potential biomarker (Admon et al., 2013).

Intrinsic Connectivity Networks

Development of PTSD is theorized to result in part from to changes in cognitive function and brain activity (Liu et al., 2017; Martynova et al., 2020; Zhang et al., 2015). Specifically, dysregulated intrinsic connectivity networks have been proposed to be contributors to PTSD symptom development. Intrinsic connectivity networks (ICNs) refer to large-scale networks of brain regions (nodes) that are functionally related, stable across time and tasks, and correspond to structural connectivity pathways (Menon, 2011; Sripada et al., 2012). Functional relationships across a network describes regions that are co-activated in the same conditions, and these relationships can be reliably identified over time and different task paradigms (Sripada et al., 2012). Hyperactivity within a network leads to increased functional connectivity between regions of the network, whereas hypoactivity leads to decreased functional connectivity. Further, these functional networks correspond to structural connectivity pathways that substantiate their interactions with one another (Huang & Ding, 2016).

One such ICN implicated in the neurobiology of PTSD is the salience network (SN). The major nodes of the SN include the insula, amygdala, and dorsal anterior cingulate cortex (dACC), which together are responsible for directing attention toward internal and external stimuli, either from interoception sensory signals or from scanning the environment, respectively. The SN acts as a dynamic switch between the default mode network (DMN), which supports self-referential processing, and the central executive network (CEN), which is engaged during cognitively demanding tasks (V. Menon, 2015; V. Menon, 2011). These networks represent some of the most basic functions of human cognition, and dysregulation of these three core networks is implicated across diagnostic boundaries of psychopathology. For example, while elevated DMN activity has been implicated in depression and CEN dysconnectivity has been found in schizophrenia, alterations in all three core networks have also been identified in PTSD (Fornito et al., 2011; Jiang et al., 2017; B. Menon, 2019; Qin et al., 2012). For proper functioning, these networks must be in sync with one another and communicate between each other effectively (Menon, 2011).

Network Connectivity in PTSD

In PTSD, alterations within and between these networks have been reported, which may contribute to development of symptoms (Zhang et al., 2015). Investigations of network-level connectivity can provide additional information on how the engagement of regions of interest (ROIs) is influenced by interactions with other regions and how network connectivity may relate to psychopathology. Traditionally, PTSD has been conceptualized as a dysregulation of nodes within the DMN, CEN, and SN. For instance, prior ROI-based investigations have suggested hypoactive dorsolateral and medial prefrontal cortices (dlPFC; mPFC), nodes within the CEN and DMN, respectively, lead to a decrease in the top-down regulation of the amygdala, a node in the SN resulting in it becoming hyperactive among individuals with PTSD (Martynova et al., 2020). In addition, other SN nodes such as the dACC have exhibited increased activity at rest among individuals with PTSD while activity in prefrontal regions of the DMN or CEN is often decreased (Akiki et al., 2017). As such, the hypoactive CEN and decreased regulation of the SN leads to weakened connectivity in the CEN and DMN and strengthened connectivity in the SN, specifically elevations in amygdala—insula and amygdala—dACC connectivity (Akiki et al., 2017; Y. Liu et al., 2017). The heightened coupling between SN nodes at rest in PTSD is suggested to represent a state of "primed" saliency, and been associated with symptoms of arousal, hypervigilance, and intrusion. In contrast, changes in the CEN and DMN are more likely associated with negative cognitions, dissociation, and avoidance (Akiki et al., 2017; Koch et al., 2016). As such, the SN appears to be working over-time with greater sensitivity to environmental triggers and flashbacks.

In contrast, negative affect tends to differentially present in different disorders across these three ICNs. For example, depression is often explained in terms of DMN hyperconnectivity, while AUD is linked with decreased SN integrity, suggesting alcohol use may have the opposite effect on the SN compared to PTSD (Galandra et al., 2018). Therefore, comorbidity of PTSD with other disorders, such as major depression (MDD), has distinct implications for salience related connectivity. Patients with comorbid PTSD and MDD exhibit reduced connectivity between the insula and hippocampus and increased connectivity within components of the ventral ACC, a node of the DMN (Kennis et al., 2014).

However, very few neuroimaging studies have investigated the neurocircuitry of comorbid PTSD and substance use disorders (SUDs). One study among women with SUDs found that those with comorbid PTSD exhibited reduced task-related functional connectivity of an orbitofrontal network with the bilateral insula (Poppa et al., 2019). This emphasizes the importance of PTSD and SUD comorbidity changes in salience-related connectivity as insular connectivity outside of the SN is typically increased in PTSD alone (Cisler et al., 2014). For these reasons, it is important to consider the potential influences of negative affect (i.e., depression and substance use) on the neurocircuitry of PTSD.

Longitudinal Changes in Neurocircuitry

Prior studies have focused on cross-sectional comparisons that take place a substantial amount of time after the trauma occurred (Cisler et al., 2014; Dunkley et al., 2018; Zhang et al., 2015). Most cross-sectional neuroimaging studies compare individuals who have already been diagnosed with PTSD with individuals who have experienced trauma, but not developed PTSD; however, these studies are limited in their ability to explain the process leading up to symptom onset. In fact, there are currently only a few studies that have taken a longitudinal approach, although they typically have not included the acute phase of trauma exposure (Dopfel et al., 2019; Dunkley et al., 2018; Heyn et al., 2019); and existing prospective studies have yet to identify SN changes that predict subsequent PTSD (Qin et al., 2012; Quidé et al., 2021; Zhou et al., 2012). Given the vast amount of data in chronic PTSD compared to the dearth in acute data, it is still important to elucidate the role of SN connectivity in relation to PTSD development.

The acute phase of trauma exposure, ranging from immediately following the trauma up to one-month post-trauma, is often overlooked. However, this acute phase provides a critical window for researchers to investigate how the brain responds in the immediate aftermath of trauma, how network connectivity changes over the duration of the acute period, and if these changes can predict later brain function or PTSD symptomatology. While there are few studies that prospectively test the role of functional network connectivity alterations on PTSD symptom development (Ben-Zion et al., 2019; Lanius et al., 2010; McLean et al., 2020; Qin et al., 2012), there are some findings that indicate resting-state connectivity patterns may play a role in predicting future PTSD symptom severity.

The posterior parietal cortex (PCC), a node of the DMN, has shown promise as a potential indicator of future PTSD symptom severity. In a prospective study, Qin and colleagues (2012) investigated the relationship between functional connectivity of the PCC two days following a traumatic exposure and PTSD symptoms at one- and six-months post trauma. Compared to trauma-exposed controls, individuals who developed PTSD exhibited different resting-state connectivity patterns, notably showing increased anterior cingulate cortex and insula connectivity with the PCC (Qin et al., 2012). Further, the strength of PCC—mPFC connectivity was negatively correlated with PTSD symptom severity. This finding strengthens the idea of increased DMN—SN connectivity leading to worsening symptoms, whereas the loss in DMN—CEN connectivity with increasing symptom severity may reflect the loss of mPFC regulation on the SN. In a subsequent study among individuals with PTSD, researchers reported that the strength of connectivity between the PCC and left superior temporal gyrus and right hippocampal gyrus/right amygdala was negatively correlated with PTSD symptom severity (Zhou et al., 2012). These results have been consistently supported, with increased connectivity within the SN and between the SN and DMN among individuals with PTSD routinely reported (Koch et al., 2016; Sripada et al., 2012). Taken together, these results suggest that there may be an imbalanced shift away from CEN—DMN connectivity and towards stronger SN—DMN connectivity in the aftermath of trauma exposure, which leads to a lack of inhibitory control over the SN and allows it to influence self-referential thought (Qin et al., 2012). The intrusion of the SN into DMN function might influence symptom development, particularly of hypervigilance, reexperiencing intrusions, and physiological arousal, when no threat is present (Sripada et al., 2012).

Current Study

The overarching goal of this study was to investigate the relationship between PTSD symptom severity and resting-state functional connectivity over time. By including two scanning timepoints—two weeks (T1) and six months (T2) post-trauma —the study aimed to elucidate substantial information on the critical period between acute trauma exposure and chronic PTSD symptoms. The current study addresses questions that remain unanswered in the literature regarding the impact of longitudinal changes

in functional connectivity on PTSD symptomology, and is among the first to investigate how changes in SN connectivity influence risk and resilience for post-traumatic symptomatology among an adult population. By examining the role of SN connectivity during the acute and chronic phases following trauma exposure, this study determined symptom development in particular symptom domains may be related to changes in functional connectivity.

This study aimed to 1) replicate findings of increased SN connectivity in chronic PTSD (T2), 2) assess whether SN connectivity during the acute phase (T1) can predict chronic PTSD symptom severity at T2, and 3) investigate how changes in SN connectivity over time may influence PTSD symptom severity. In Aim 1, I conducted a regression analysis to investigate the relationship between the T2 connectivity data and T2 symptom severity score. Based on previous findings, I hypothesized that greater intraconnectivity of the SN (i.e., connectivity within the SN) would be associated with greater symptom severity. For Aim 2, I investigated the relationship between T1 SN connectivity and T2 PTSD symptom severity using a regression analysis. I hypothesized that elevated T1 SN connectivity would be associated with greater T2 PTSD symptom severity. Aim 3 used a linear mixed effects model to assess the predictive ability of the longitudinal changes in SN connectivity on PTSD symptom severity. For this aim, I hypothesized there to be a significant interaction between Time (T1; T2) and PTSD symptom severity (T1; T2) on the intrinsic connectivity of the SN such that the greater difference in connectivity between T1 and T2 would be associated with greater symptom change between T1 and T2. Finally, to investigate possible distinctions between network connectivity and specific symptoms, post-hoc analyses were conducted to assess the relationship of SN connectivity with individual symptom domains of PTSD, symptoms of depression, and substance use.

Methods

Procedure

Individuals who were treated for traumatic injuries at the Froedtert Hospital Emergency Department (ED) were recruited either directly in the ED or over the phone as a part of a larger study conducted through the University of Wisconsin-Milwaukee and the Medical College of Wisconsin. The

initial visit occurred two weeks following ED discharge, and participants completed consent, self-report questionnaires, and an MRI scan. Participants returned at a date six months after the traumatic event and repeated the same assessments and scanning protocol as the two-week visits with the addition of structural clinical interviews to assess current psychopathology.

Participants

Two hundred and six individuals who had experienced a traumatic event and received acute care from the Froedtert Hospital ED were included in the study. Participants were included if they were between the ages of 18-60, presented to the ED with a Glascow Coma Score greater than 13 (i.e. no moderate to severe brain damage), and experienced a traumatic event that meets the DSM-5 criterion A for a PTSD diagnosis. There was not specific inclusion criteria regarding trauma type, as only sustaining a traumatic injury was required for inclusion. Thus, the sample consists of survivors of motor vehicle accidents, assault (physical and sexual), violence, and accidental injuries. Exclusion criteria included self-inflicted traumatic injury, severe hearing or vision impairments, history of psychotic or manic symptoms, current use of antipsychotic medications, a diagnosis of an SUD in their medical chart, initial screening scores on the Alcohol Use Disorder Identification Test above 7, on police hold to be released to jail, and any MRI scanning contraindications such as claustrophobia, pregnancy, or metal objects or fragments in the body. Participants were also excluded if they do not have usable data from both timepoints, and an anticipated 15-20% loss of subjects is expected due to motion.

Measures

PTSD Symptoms. PTSD symptoms were assessed at both timepoints with the PTSD Checklist for DSM-5 (PCL-5; Blanchard et al., 1996) The PCL is a 20-item self-report measure on which respondents indicate severity of symptoms experienced in the past month on a five-point Likert scale (0= “Not at all” to 4 = “Extremely”; Blanchard et al., 1996) The measure provides a total severity score (PCL-Total) ranging from 0-80 with a proposed cut-off score between 31-33 suggesting probable PTSD diagnosis. Individual symptom cluster scores (PCL-sxs) can be calculated by summing the scores from the items within the scale related to a specific symptom cluster. For example, the reexperiencing scale

(PCL-Reexp; Cluster B) has 5 items and a range of severity scores from 0-20. The avoidance scale (PCL-Avoid; Cluster C) has 2 items and a range of severity scores from 0-8, the negative cognitions scale (PCL-Neg; Cluster D) has 8 items with a range of severity scores from 0-32, and the hyperarousal scale (PCL-Hyper; Cluster E) has 6 items with a range of severity scores from 0-24 (Weathers et al., 2014). While change scores for the PCL-5 are currently being determined, the PCL-IV offers the opportunity to measure symptomatic change over time, with a 5-10 point change representing reliable change not due to chance and a 10-20 point change representing clinically significant change (Blanchard et al., 1996). It is expected that the PCL-5 exhibits similar ranges in clinically reliable and relevant change scores. Two-sample t-tests were used to determine significant changes between T1 and T2 PCL-Total and PCL-sxs scores.

Comorbid Psychopathology. In addition to PTSD, depression and anxiety symptom severity were assessed at T2 with the Depression, Anxiety, and Stress Scale (DASS; Brown et al., 1997) at both the two week and six month timepoints. The DASS consists of 21 items on a 4-point Likert scale (0 = "Did not apply to me at all" to 3 = "Applied to me very much, most of the time"), and the scale assesses the presence of symptoms over the past week. Each of the individual scales (depression, anxiety, stress) includes 7 distinct items, and scale scores were each multiplied by 2 to obtain the severity scores, resulting in a severity score range of 0-42 for each scale. For depression, normal = 0-9, mild = 10-12, moderate = 13-20, severe = 21-27, and extremely severe = 28-42; for anxiety, normal = 0-6, mild = 7-9, moderate = 10-14, severe = 15-19, and extremely severe = 20-42; and for stress, normal = 0-10, mild = 11-18, moderate = 19-26, severe = 27-34, and extremely severe = 35-42. The DASS has high internal consistency across the three scales ($\alpha_{depression} = 0.96$, $\alpha_{anxiety} = 0.89$, $\alpha_{stress} = 0.93$; Brown et al., 1997). Because of a high comorbidity with PTSD and a specific interest in other measures of negative affect, the depression scale (DASS-Dep) was the main factor used from the DASS.

Substance Use. Substance use has been shown to have significant effects on brain structure and activation (Luijten et al., 2017; Silveri et al., 2016; Squeglia & Gray, 2016). While participants with a clinical history of SUDs as defined in their medical chart were excluded at intake, they were not excluded

if they began drinking or using after enrollment. As such, the abbreviated Alcohol Use Disorder Identification Test (AUDIT; Saunders et al., 1993) and the Drug Abuse Screening Test (DAST; Skinner, 1982), were administered at T2 to assess alcohol or drug abuse, respectively. The abbreviated AUDIT consists of three questions assessing for the alcohol consumption frequency and quantity and binge drinking frequency (Bush et al., 1998). Scores range from 0-12, with each question consisting of a 5-point Likert scale (a= 0 points to e = 4 points). In men, a score of 4 or more is considered positive, and a score of 3 is considered positive for women. The AUDIT has good sensitivity and specificity for active alcohol abuse or dependence in both men and women with a score of 4 or more (Sensitivity = 0.79, Specificity = 0.56) and women with a score of 3 or more (Sensitivity= 0.80, Specificity = 0.87; (Bush et al., 1998)). The DAST consists of 28 categorical items to assess for drug abuse in the past year. Participants are asked to report if they had on any occasion used prescribed or over-the-counter mediation in excess of the recommended amounts or used any non-medical drugs. A cut-off score of 6 or more provides excellent sensitivity and satisfactory specificity for identifying individuals with a substance use disorder, and a cut-off score of 12 or more is a definite indication of a substance use problem (Skinner, 1982).

MRI Acquisition

An 8-minute resting MRI scan was conducted using a 3.0T short bore GE Signa Excite system with the following parameters: repetition time (TR)/echo time (TE) = 2000 ms/25 ms; number of TRs = 240; number of slices = 41; sagittal orientation; field of view = 22.4 mm; matrix = 64 x 64; slice thickness = 3.5 mm; flip angle 77°; voxel size = 3.5 x 3.5 x 3.5 mm^3. Functional data was registered to high-resolution T1 spoiled gradient recalled (SPGR) images (TR/TE = 8.2 ms/3.2 ms; number of slices = 150; sagittal orientation; field of view = 240 mm; matrix = 150 x 256 × 256; slice thickness = 1 mm; flip angle = 12°; voxel size = 1 x 0.9375 × 0.9375 mm^3).

Image Preprocessing

The acquired images were preprocessed using a pipeline in the CONN MATLAB toolbox. The first three TRs were removed for magnetic field stabilization. Following this, the preprocessing steps included: 1) realignment and unwarping, 2) outlier detection and scrubbing using a conservative

framewise displacement of 0.3mm such that participants were excluded from analysis if more than 20% of the volumes were scrubbed 3) segmentation of grey matter, white matter, and cerebrospinal fluid; 4) normalization to MNI space using both linear and nonlinear steps; and 5) spatial smoothing with a Gaussian kernel of 6mm. To reduce the signal-to-noise ratio, a temporal bandpass filter of 0.09-.9 Hz was applied.

Network Isolation

A mask of the SN was created using a Neurosynth generated template. Neurosynth is an online, open access platform that synthesizes fMRI data across numerous studies and generates meta-analysis images related to a given term of interest. A Neurosynth search using the term "salience" yields a meta-analysis of 396 studies and a uniformity test map displaying regions that are consistently active across studies that are highly associated with salience processing (Yarkoni et al., 2011). A mask was created from the test map with a cluster threshold size of 250 voxels in order to ensure substantial regional engagement in salience-related activity (Figure 1). Subject resting-state scans were restricted to this mask in the first-level analysis step in CONN in order to isolate salience-specific regions for second-level analyses.

Analytic Approach

Intrinsic Connectivity Analysis The first-level analysis used an intrinsic connectivity contrast (ICC) approach within the SN mask, where ICC is defined as the root mean square of correlation coefficients between a given voxel and all the voxels in the brain, or mask in this case (Martuzzi et al., 2011). While the ICC analysis is capable of assessing whole-brain connectivity, this study aimed to understand the overall connectivity within the SN alone. As such, all subject scans were masked to restrict the ICC analysis to only SN regions. The ICC analysis and SN mask combination was chosen over other methods due to its ability to assess a voxel-to-voxel connectivity within the entire network (sometimes referred to as node centrality) without the need to select *a priori* regions of interest that may exhibit increased ICC values within the network. An intrinsic connectivity map was produced representing the

ICC across all voxels to characterize the strength of connectivity between each voxel and the entirety of the SN mask.

Covariates of Interest There are several potential factors that may contribute to the relationship between PCL-Total / PCL-sxs scores and SN connectivity. Of note, these include gender, age, negative affect, and substance use. Gender and age were included as covariates as brain function varies over the lifespan and can differ across genders (Heyn et al., 2019; Shvil et al., 2014). Further, several additional measures assessing aspects of negative affect, including the six-month DASS-Dep score, the six-month AUDIT score, and the six-month DAST score, were added in an additional model to isolate any effects that may be due to the overall contribution of negative affect on connectivity.

Linear Models Regarding Aims 1 and 2, general linear models (GLMs) were run in CONN to assess the connectivity between salience clusters and the symptom severity across subjects. The GLM examined the independent relationships of the T1 (Aim 2) and T2 (Aim 1) scans with symptom severity at T2. For Aim 3, between-subject differences in SN restricted ICC maps and changes in PTSD symptoms over time were assessed with the linear mixed effects models (LMEs) using the 3dLME command in AFNI. PCL-Total scores from both T1 and T2 were entered as a repeated measure in the model. Time was included as a fixed effect to parse out differences associated with each timepoint. For PCL-sxs scores (PCL-Reexp, PCL-Avoid, PCL-Neg, PCL-Hyper), the individual PCL-sxs score was entered in place of the PCL-Total score. The models used in 3dALME are as listed below:

*connectivity ~ Time*PCL-Total + age + gender + (1|subject)*

*connectivity ~ Time*PCL-Total + DASS-Dep + AUDIT + DAST + age + gender + (1|subject)*

Results

Sample Characteristics

Following exclusion for motion, 120 subjects were included in the current analyses. General demographic information is included in Table 1. Overall, the average age of participants was 33, and the sample was 54% women, 52.5% Black, 31.8% White, 7.5% multiracial, 1.7% Asian, and 7.5% unknown

or unreported. By far, the main type of index trauma experienced was a motor vehicle accident (67.5%) with the second most experienced being an assault, either physical or sexual (14.2%). Other types of index trauma experienced were less than 3% and are listed in Table 1 (with the exception that the "Other" category was 7.5%).

A summary of the results from the self-report symptom scales are provided in Table 2 and 3. On average, subjects reported subthreshold levels of PTSD symptoms under the cut-off score of 31 (Figure 2). The PCL-Total and PCL-sxs scores all significantly decreased between the two timepoints (PCL-Total: $t = 3.11(237.91)$, $p = .002$; PCL-Reexp: $t = 3.68(235.33)$, $p < .001$; PCL-Avoid: $t = 2.81(236.82)$, $p = .005$; PCL-Neg: $t = 2.14(236.82)$, $p = .034$; PCL-Hyper: $t = 2.73(235.52)$, $p = .007$). As for the other clinical scales, the sample exhibited low levels of depression, moderate amounts of alcohol abuse (average score just above the cut-off score of 4), and low amounts of substance use. For more information on these scales, please see Table 2.

Aim 1: Relationship between T2 SN Connectivity and PCL Scores

Aim 1 hypothesized that greater T2 PCL-Total score would be related to increased T2 connectivity within the SN which would be represented by clusters within the SN exhibiting significantly higher ICC values. No significant clusters were found that exceeded threshold limits of $| T(111) | > 3.38$ and $p < .001$. The relationships between six-month ICC values and the six-month PCL-sxs scores were also insignificant.

Aim 2: Relationship between T1 SN Connectivity and T2 PCL Scores

Aim 2 hypothesized that greater PCL-Total scores at T2 would be related to increased T1 connectivity within the SN, which would be represented by clusters within the SN exhibiting significantly higher ICC values. No significant clusters were found that exceeded threshold limits of $| T(111) | > 3.38$ and $p < .001$. The relationships between two-week ICC values and the two-week PCL-sxs scores were also insignificant.

Aim 3: Effect of Time on PCL Scores and SN Connectivity

Aim 3 hypothesized that Time would influence the relationship between SN connectivity and PCL-Total score, such that greater change between the two timepoints in PCL-Total score would interact with greater differences in SN connectivity of the two timepoints. This was to be represented by clusters within the SN exhibiting significantly higher ICC values in the PCL x Time interaction term of the LME results. Cluster threshold size was found to be best at 13 voxels in order to account for a p-value > .001. This provided a threshold F value to be set at 11.395. At this level, for both the simple model (age and gender only) and the larger model (age, gender, DASS-Dep, DAST, AUDIT), no significant clusters were identified for the PCL-Total score. However, post-hoc analyses of the PCL-sxs revealed a significant cluster in the dACC associated with the PCL-Reexp domain (Figure 3). While the ICC value of the dACC cluster decreased with increasing PCL-Reexp scores at T1, dACC ICC values increased with increasing PCL-Reexp scores at T2 meaning that the intrinsic connectivity of the dACC with the entire SN increases over time as symptoms grow more severe. This relationship remained significant when DASS-Dep, DAST, and AUDIT were added to the model ($F(1,117) = 23.1167$, $p < .001$, cluster size = 14, x = -6, y = -20, z = 26). Notably, no other symptom clusters had any significant interactions with time.

Discussion

This study is among the first investigations examining the effects of longitudinal changes in functional connectivity on PTSD symptom development. Using a SN specific mask, ICC analyses comparing T2 ICC values with T2 PCL-Total score (Aim 1), T1 ICC values with T2 PCL-Total score (Aim 2), and changes in ICC and PCL-Total score over time (Aim 3), were run to assess alterations in SN connectivity associated with increased PTSD symptoms. There were no significant results between the T2 ICC and T2 PCL-Total or PCL-sxs scores, or between the T1 ICC and T2 PCL-Total or PCL-sxs scores, nor was there an interaction between the Time and PCL-Total score in changes in ICC. However, the interaction between Time and PCL-Reexp score identified a single cluster in the dACC that remained significant even after the addition of additional negative affect covariates.

SN Connectivity at T2 and PTSD Symptoms at T2

The current findings contradict established theories of PTSD being associated with increased SN connectivity (Akiki et al., 2017; Martynova et al., 2020). With this in mind, SN intraconnectivity alone may not be a significant contributor to PTSD symptoms, and the interaction of the SN with other regions and networks is important in PTSD symptom severity. Meta-analyses have shown that SN activity may not be as reliable as an indicator of PTSD compared to DMN and CEN regions; while the dACC is often found to be *hyper*active in PTSD, there are some findings of *hypo*activation in a smaller, more ventral area of the dACC (Patel et al., 2012). In contrast, alterations in CEN and DMN nodes are more consistent in differentiating PTSD from trauma-exposed controls (Akiki et al., 2017; Patel et al., 2012). However, new research may support the current findings. In a whole-brain ICC analysis, Quidé et al. (2021) also found no significant differences in intrinsic connectivity between healthy controls, trauma-exposed controls, and PTSD participants. The Quidé et al. (2021) study differs from others in that the subjects all completed the study at six-months post-trauma, which, when combined with the current findings, suggests six-months may be too early to be considered "chronic" PTSD compared to other studies of PTSD that have identified SN changes conducted years after the trauma occurred. Alterations in ICN connectivity may be a result of chronic PTSD symptoms that over time rewire the core neurocircuitry as other studies have found (Cisler et al., 2014; Dunkley et al., 2018; Zhang et al., 2015). Overall, these findings emphasize the importance of considering the time since the trauma has occurred in studies on the pathophysiology of PTSD.

Another possible interpretation of the current results could be that this sample has relatively low severity of PTSD symptoms with the T2 average being subthreshold for PTSD diagnosis. This is in contrast to many extant findings that examine group differences between controls and PTSD patients with an average PTSD severity score much higher than the diagnostic cutoff (Harricharan et al., 2020; Rabinak et al., 2011; van Rooij et al., 2016; Zhang et al., 2015). This could indicate that the range for detecting the effects of SN changes was too low and that only in more severe cases of PTSD would there be significant increases in SN connectivity. Interestingly, in an investigation of combat veterans with subthreshold PTSD scores, significant alterations in activity of regions in all three core ICNs were found during an

affective Stroop task (White et al., 2015). Taken with the current findings, this suggests that specifically SN resting-state intraconnectivity may not be detectable at subthreshold levels of PTSD, at least at rest. Given the nuances of all of these findings, it is important to continue investigating the contribution of SN connectivity on PTSD symptom severity.

SN Connectivity at T1 and PTSD Symptoms at T2

The current finding that acute post-trauma SN connectivity does not relate to PTSD symptoms at six-months suggests SN intraconnectivity alone is not a significant predictor of future PTSD symptom severity. Interconnectivity of the SN with outside regions or networks may be more influential on PTSD symptom development than SN intraconnectivity alone. Further, SN connectivity changes may not occur in the acute phase, as other prospective investigations support the idea of greater predictive qualities in non-salience regions. While SN centrality (ICC strength) may not be able to distinguish effects of PTSD among trauma-exposed individuals, Quidé et al. (2021) found that trauma exposed controls did not differ from PTSD participants in the centrality of the PCC at three-weeks post-trauma; however, the trauma-exposed controls did differ from healthy controls in the PCC. This is corroborated by other prospective studies that identified the PCC, a node of the DMN, as a key predictor of later PTSD symptom severity (Qin et al., 2012; Zhou et al., 2012). PTSD participants did exhibit greater centrality in the middle/superior occipital gyrus at three-weeks post-trauma compared to either control group, suggesting that acute occipital, not salience, connectivity may predict future PTSD diagnosis (Quidé et al., 2021). This is logical as occipital and salience-related regions have been found to be activated in concert with one another among traumatized samples (Sartory et al., 2013). In addition to DMN and occipital regions, acute connectivity of a CEN node, the dlPFC, with limbic regions including the amygdala, hippocampus, and other subcortical arousal regions, is inversely related to PCL-Total scores at three-months (Harnett et al., 2021). These early investigations into the role of neurocircuitry in PTSD development provide a frame of reference to conceptualize prior research and the current findings. For instance, alterations in the CEN and DMN may be early indicators of the effect of trauma on the brain, and these changes, along with repeated experience of PTSD symptoms, may lead to chronic changes in SN connectivity. This fits within

the framework of a loss of top-down regulation from the CEN on the SN leading to heightened PTSD symptoms (Martynova et al., 2020). Taken together, these results suggest the importance of studying internetwork connectivity between all three of the core ICNs both acutely and chronically.

While other investigations have not identified differences in acute SN interconnectivity, salience intraconnectivity in the acute phase may still differ between trauma-exposed controls and PTSD groups. Because the current sample exclusively included trauma-exposed individuals who did not necessarily meet threshold for PTSD, it may be that the sample did not exhibit high enough severity scores at T2 to be predicted by T1 SN connectivity, meaning that any significant increases in SN connectivity at T1 may have been identified if the sample had a higher average in PCL-Total scores at six months. Prior prospective studies that have found altered connectivity in PTSD have included samples with higher PTSD severity scores (Qin et al., 2012; Quidé et al., 2021; Zhou et al., 2012), supporting the possibility of significant acute connectivity changes only being detectable among more severe PTSD samples. That said, however, the AURORA study, which has a similar study design and sample size as the current study, had more moderate mean PCL scores and identified altered acute connectivity in CEN and DMN nodes, but not the SN (Harnett et al., 2021). Regardless, the question of SN intra- and inter-network connectivity warrants further investigation to elucidate the true nature of its predictive capacity for PTSD symptom severity.

SN Connectivity and PTSD Symptoms over Time

The hypothesis that changes over time would influence the relationship between SN connectivity and PCL-Total score was not supported. While the differences in PCL-Total and PCL-sxs scores between the two timepoints were significant, the trend for changes was in decreasing scores rather than increasing scores which would be more likely to relate to SN changes (Qin et al., 2012; Zhou et al., 2012). This trend was not ideal for identifying connectivity changes associated with increasing symptom severity; however, it is in line with prior literature showing that the majority of people who experience a trauma exhibit some acute symptoms that tend to decrease over time, particularly among those who experience a non-intentional trauma like motor vehicle accidents – the main trauma type of this sample (Santiago et al.,

2013; Thompson et al., 2018). Given that the base rate for PTSD remains relatively low, this pattern highlights the aspect of resilience most individuals express following a trauma (Kilpatrick et al., 2013; H. Liu et al., 2017). Further, the significant reduction in PCL-Total score still may not have been substantial to exhibit changes in connectivity in either direction as the overall change was relatively minor. This is supported by treatment studies that have shown task-related activation changes in salience ROIs in remitting patients whose scores, on average, decreased by 42 points, while persistent patients who did not show activation differences had relatively stable or less pronounced change scores (van Rooij et al., 2016). Therefore, this sample may not have exhibited the higher severity in PCL scores necessary to observe significant changes in SN connectivity. Further, while SN hyperconnectivity has been associated with PTSD, these studies often include participants who have had PTSD for a year or longer (Cisler et al., 2014; Dunkley et al., 2018; Zhang et al., 2015). The six-month T2 timepoint of the present study might be too early to observe particular effects. Indeed, other longitudinal prospective studies of PTSD have also failed to find significant changes over time in samples with greater trauma and symptom severity (Quidé et al., 2021). Because there was no significant relationship with PCL-Total or PCL-sxs scores at either T1 or T2, the sample may have been too acutely traumatized to exhibit the expected changes in SN connectivity.

While the PCL-Total score may not be indicative of salience related connectivity changes, individual symptom clusters, specifically the PCL-Reexp score, may be more representative of SN changes over time. The PCL-Reexp x Time interaction was associated with a small cluster in the dACC while all other symptom domains failed to identify any significant relationships with the ICC values over time. This stands to reason as increased SN activity and connectivity can be linked specifically to elevated reexperiencing symptoms (Akiki et al., 2017). The dACC is responsible for the appraisal of negative emotions and evaluations of threatful stimuli as evidenced by its consistent activation in fear conditioning paradigms (Etkin et al., 2011). Certainly, the dACC has been implicated in the pathophysiology of PTSD, with meta-analyses finding a majority of studies identifying hyperactivation of the dACC in PTSD compared to trauma-exposed controls (Patel et al., 2012). Further, the dACC is also involved in emotional

conflict, and increased dACC activity during an emotion processing task has been shown to predict both short-term and long-term follow-up CAPS scores (Etkin et al., 2011; Kennis et al., 2017; van Rooij et al., 2016). These findings implicate the dACC as a vital center for communication within the SN and may suggest that there is a break down in the system's threat evaluation, leading to misinterpretation of particular safe cues as threats and resulting in the presence of reexperiencing symptoms. The dACC might also act as the catalyst for change in the chronic development of altered SN connectivity. Small changes over time, such as the increased dACC centrality within the first six months post-trauma, may accumulate into the altered neurocircuitry observed in chronic PTSD, implying that the chronic stress of PTSD symptoms lead to SN hyperconnectivity rather than the contrary. This may also contribute to the persistence of PTSD even after treatment, implicating the dACC as an important factor in reducing both reexperiencing and overall PTSD symptoms (Kennis et al., 2017; van Rooij et al., 2016).

Methodological Limitations

This study aimed to provide a novel investigation of the longitudinal changes in resting-state functional connectivity, however, it is not without limitations. First, the ICC is a measure of degree centrality for a given voxel with the rest of the brain (Martuzzi et al., 2011). This is ideal for investigations with no *a priori* hypotheses or assumptions; however, this investigation chose to isolate the SN as the sole network of interest. In isolating only the SN, the ICC analysis had very narrow parameters within which changes in connectivity were expected to be found, limiting the results to only include *intra*-connectivity of regions included in the SN mask. It is possible, therefore, that the changes in SN connectivity related to PTSD may not be represented by *intra*-connectivity changes but rather *inter*-connectivity changes which were then excluded from this analysis. Further investigations should consider alternative approaches for network-based analyses, such as an Independent Component Analysis if the study allows for it.

Further limitations include the choice to focus on the PCL and other measures of negative affect, such as the DASS-Dep, AUDIT, and DAST, but future studies should also focus on other factors that may influence PTSD such as past trauma exposure. Additionally, the first scan was conducted two weeks post-

exposure rather than immediately after the trauma when the patient is in the ED. This may result in altered connectivity patterns at the two-week timepoint that may have developed within the two weeks or may have been present prior to the trauma. Natural observations of behavioral and neurobiological factors following trauma are difficult to conduct as it is impossible to predict who may be traumatized and when it might occur, and longitudinal studies that follow individuals throughout their lifetime in order to collect pre-trauma data are costly. However, one remedy to this problem could be to administer fMRI resting-state scans at the time of ED admittance to account for immediate effects of trauma exposure.

Conclusions

Psychopathology neuroimaging research has long asked the "which came first" question in regard to symptoms and alterations in the brain. Without longitudinal investigations, these questions cannot be answered. This study is among the first to include both acute and chronic scanning sessions, allowing for comparison between the two stages of post-trauma exposure. Taken in context with the current literature, these findings suggest that the acute and chronic phases of PTSD symptoms may not be the same as acute and chronic phases of trauma-related neurocircuitry. Further, while the study did not identify significant relationships between PTSD symptoms and SN connectivity at two-weeks or six-months individually, the identification of a significant dACC cluster in the interaction between Time and PTSD symptom severity indicates the importance of small changes that may build over time to contribute to chronic changes in neurocircuitry and exacerbated symptoms. With the methodological limitations in mind, it remains vital to chart the longitudinal behavioral and neurocircuitry changes over time in order to track who may be more susceptible to PTSD and to provide earlier interventions that can target the dysregulated connections in the SN.

Tables

Overall Demographics	N = 120 *n (%) / M (SD)*
Age	33.2 (10.43)
Gender	
Female	65 (54.2%)
Male	55 (45.8%)
Race	
Black	63 (52.5%)
White	37 (30.8%)
Asian	2 (1.7%)
Multiracial	9 (7.5%)
Unknown	9 (7.5%)
Years of Education	14.9 (2.17)
Employed	93 (77.5%)
Trauma Type	
Motor Vehicle Accident	81 (67.5%)
Motorcycle Accident	3 (2.5%)
Struck Pedestrian	3 (2.5%)
Assault (physical or sexual)	17 (14.2%)
Domestic Violence	3 (2.5%)
Stabbing	1 (0.8%)
Fall	3 (2.5%)
Other	9 (7.5%)

Table 1. Participant demographic information. Total sample included 120 subjects.

Scale *Range*	**2 Weeks (T1)** *M (SD)*	**6 Months (T2)** *M (SD)*
PCL Total	26.792 (17.541)	19.808 (17.212)
0 - 80	*0 -73*	*0 -76*
PCL Reexperiencing	7.467 (5.291)	5.075 (4.755)
0 - 20	*0 -20*	*0 -20*
PCL Avoidance	3.300 (2.431)	2.450 (2.256)
0 - 8	*0 - 8*	*0 - 8*
PCL Negative Mood	7.558 (7.097)	5.667 (6.613)
0 - 32	*0 - 28*	*0 - 26*
PCL Hyperarousal	8.467 (4.970)	6.617 (5.510)
0 - 24	*0 - 23*	*0 - 24*
DASS - Dep		7.117 (9.133)
0 - 42		*0 - 40*
AUDIT Total		4.692 (2.263)
0 -12		*0 - 11*
DAST Total		2.783 (4.059)
0 - 28		*0 - 24*

Table 2. Mean scores and standard deviations of the PCL total and individual symptom scales, DASS-Dep scale, AUDIT scale, and DAST scale.

PCL Scale	***t(df)***	***p-value***
Total	3.11(237.91)	.002
Reexperiencing	3.68(235.33)	< .001
Avoidance	2.81(236.69)	.005
Negative Cognitions	2.14(236.82)	.034
Hyperarousal	2.73(235.52)	.007

Table 3. Results of two-sample paired t-tests of PCL scale scores between T1 and T2.

Figures

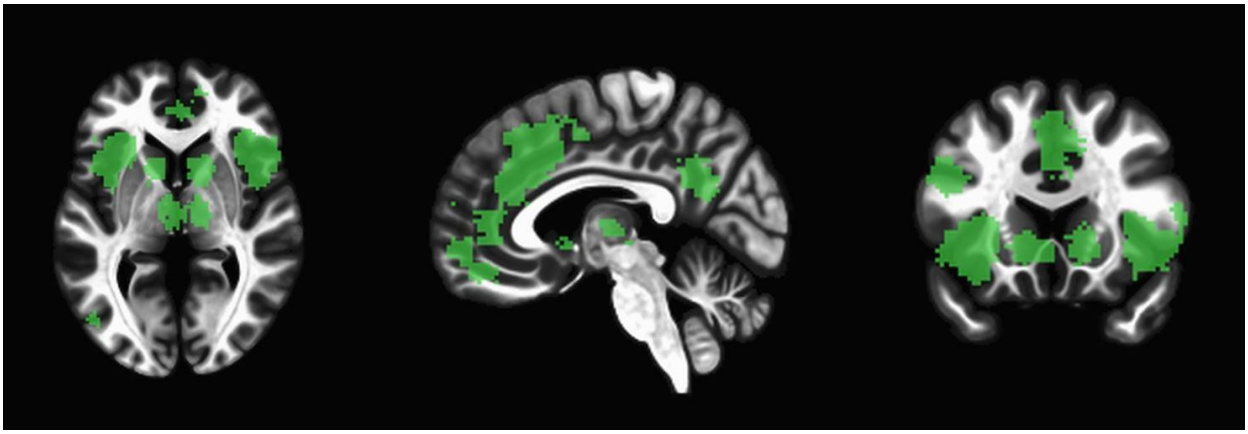


Figure 1. Salience mask derived from Neurosynth.org meta-analysis. Green indicates voxels included in the ICC analysis. Coordinates of slices shown: x = -5, y = -16, z = 2.

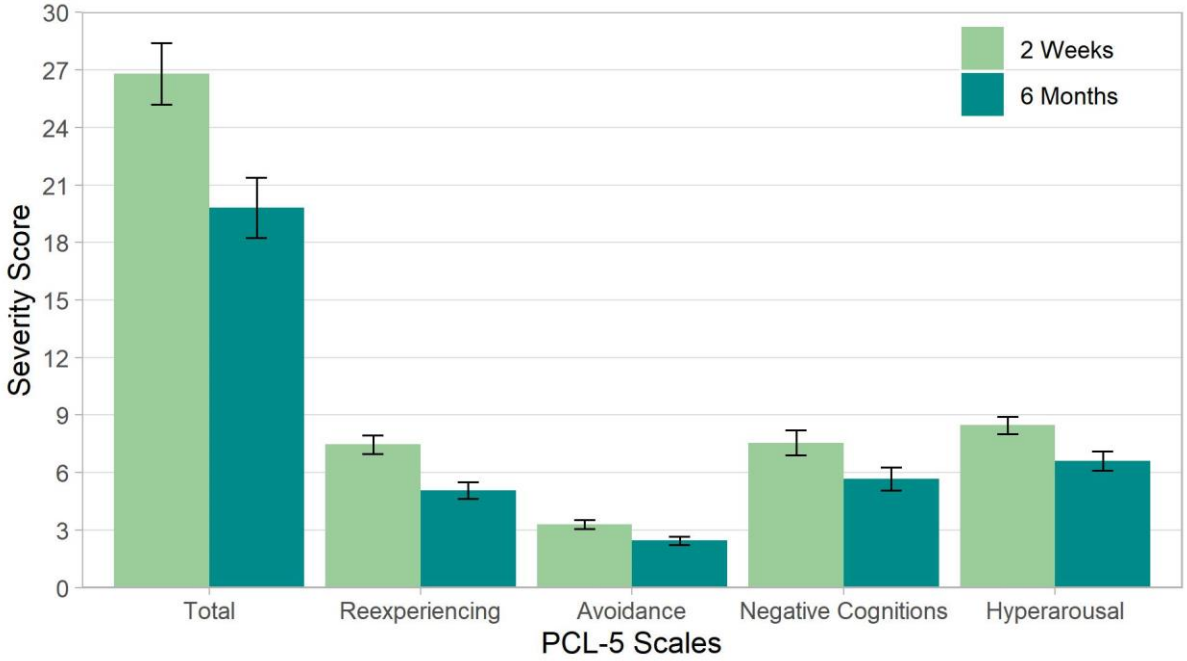
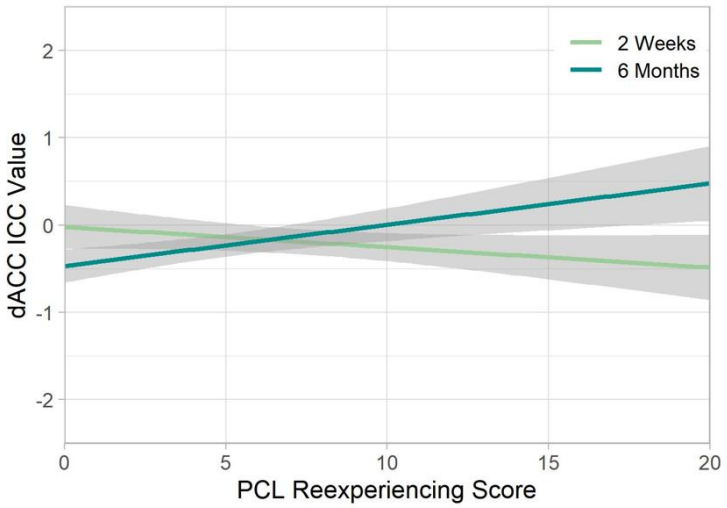
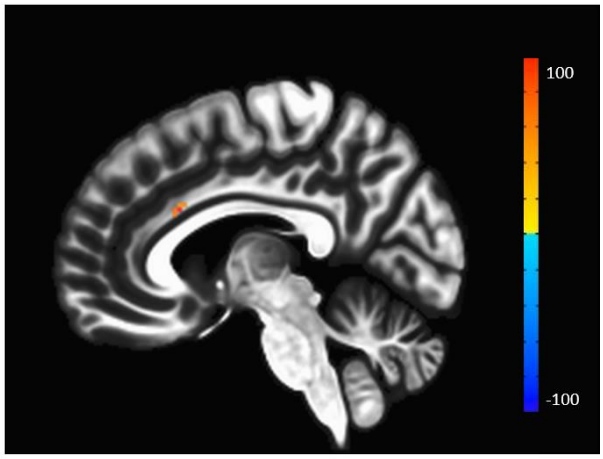


Figure 2. Mean severity scores and standard error of the Total and individual symptom cluster scales on the PCL-5. Light green indicates scores at two-weeks post-trauma (T1), and dark green indicates scores at six-months post-trauma (T2).





Cluster Location	Threshold	p-value	F-stat	Cluster Size	x	y	z
dACC	11.395	.001	23.1167	14	-6	-20	26

Figure 3. Significant result of the interaction between time and PCL Reexperiencing scores. *Left)* Plot of interaction between PCL-Reexp score and Time. The y axis represents the average ICC value in the identified dACC cluster for a given subject while the x axis represents their severity score on the PCL-Reexp score. The light green line represents the relationship between T1 ICC and T1 PCL-Reexp score and the dark green line represents the relationship between T2 ICC and T2 PCL-Reexp score. Grey shaded regions represent confidence intervals for each given timepoint. *Right)* Sagittal view of the ACC cluster that survived correction, $F(1,117)=23.1167$, $p < .001$. *Bottom)* Table displaying details of the dACC cluster identified in the 3dLME model testing the interaction of PCL-Reexp and Time. Model included the DASS-Dep, DAST, AUDIT, age, and gender as covariates.

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