Behavioral/Cognitive

Hippocampal Threat Reactivity Interacts with Physiological Arousal to Predict PTSD Symptoms


1Department of Psychology and Neuroscience, Temple University, Philadelphia, Pennsylvania 19121, 2Department of Psychiatry and Behavioral Sciences, Emory University School of Medicine, Atlanta, Georgia 30329, 3Department of Depression and Anxiety, McLean Hospital, Belmont, Massachusetts 02478, 4Department of Psychiatry, Harvard Medical School, Boston, Massachusetts 02115, 5Department of Psychiatry and Behavioral Neurosciences, Wayne State University, Detroit, Michigan 48202, 6Department of Emergency Medicine, Washington University School of Medicine, St. Louis, Missouri 63110, 7Department of Emergency Medicine & Department of Health Services, Policy, and Practice, Alpert Medical School of Brown University, Rhode Island Hospital, and Miriam Hospital, Providence, Rhode Island 02930, 8Institute for Trauma Recovery, Department of Anesthesiology, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27559, 9Departments of Psychiatry and Neurology, University of California San Francisco, San Francisco, California 94143, 10Department of Biomedical Informatics, Emory University School of Medicine, Atlanta, Georgia 30332, 11Department for Technology in Psychiatry, McLean Hospital, Belmont, Massachusetts 02478, 12Institute for Technology and Neuroscience & Department of Sociology, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27559, 13Institute for Technology in Psychiatry/Department of Emergency Medicine, University of Massachusetts Medical School, Worcester, Massachusetts 01655, 14Department of Emergency Medicine, Vanderbilt University Medical Center, Nashville, Tennessee 37232, 15Department of Emergency Medicine, Henry Ford Health System, Detroit, Michigan 48202, 16Department of Emergency Medicine, Indiana University School of Medicine, Indianapolis, Indiana 46202, 17Department of Emergency Medicine, University of

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Correspondence should be addressed to Vishnu P. Murty at Vishnu.murty@temple.edu.

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Hippocampal impairments are reliably associated with post-traumatic stress disorder (PTSD); however, little research has characterized how increased threat sensitivity may interact with arousal responses to alter hippocampal reactivity, and further how these interactions relate to the sequelae of trauma-related symptoms. In a sample of individuals recently exposed to traumatic stress disorder (PTSD), decreased hippocampal function linked to threat-related arousal is reliably associated with post-traumatic stress disorder (PTSD); however, how these alterations relate to the sequelae of trauma-related symptoms is unknown. Prior models based on nontrauma samples suggest that arousal may impact hippocampal neurophysiology leading to maladaptive behavior. Here we show that decreased hippocampal threat sensitivity interacts with fear-potentiated startle to predict PTSD symptoms. Specifically, individuals with high fear-potentiated startle and low, transient hippocampal threat sensitivity showed the greatest PTSD symptomology. These findings bridge literatures of threat-related arousal and hippocampal function to better understand PTSD risk.

Key words: arousal; fear; fMRI; hippocampus; trauma

Introduction
Threat is known to alter hippocampal function, a region critically implicated in supporting memory (Eichenbaum, 2001). Whereas moderate threat increases hippocampal sensitivity (Joëls et al., 2006), excessive threat impairs hippocampal function (Kim and Diamond, 2002; McEwen, 2007; Henckens et al., 2009; Schwabe and Wolf, 2012; Bisby and Burgess, 2013, 2017). In post-traumatic stress disorder (PTSD), decreased hippocampal

Significance Statement
Alterations in hippocampal function linked to threat-related arousal are reliably associated with post-traumatic stress disorder (PTSD); however, how these alterations relate to the sequelae of trauma-related symptoms is unknown. Prior models based on nontrauma samples suggest that arousal may impact hippocampal neurophysiology leading to maladaptive behavior. Here we show that decreased hippocampal threat sensitivity interacts with fear-potentiated startle to predict PTSD symptoms. Specifically, individuals with high fear-potentiated startle and low, transient hippocampal threat sensitivity showed the greatest PTSD symptomology. These findings bridge literatures of threat-related arousal and hippocampal function to better understand PTSD risk.
engagement propagates traumatic memories (Hayes et al., 2011) and impairs discrimination between danger and safety signals, leading to the overgeneralization of fear (Besnard and Sahay, 2016; Asok et al., 2019), which underlies PTSD (e.g., Hayes et al., 2011). Further, lower hippocampal engagement during inhibitory tasks has been associated with PTSD (van Rooij et al., 2016; van Rooij, 2018). However, contradictory evidence shows increased hippocampal engagement during trauma-related memory and imagery in individuals with PTSD (Bremner et al., 2003; Tural et al., 2018). These inconsistencies may result from the functional demands placed on the hippocampus (HPC; threat vs safety detection) and the neuromodulatory profile in which these demands occur (high vs low arousal). Here, we characterize the relationship among hippocampal function, threat-related arousal, and PTSD symptomatology in a large sample of trauma-exposed individuals.

We previously developed a model of how threat-related arousal alters hippocampal function, biasing information processing away from HPC to other learning structures because of arousal-mediated norepinephrine (NE) engagement (Murty and Adcock, 2017; Clewett and Murty, 2019). Specifically, we predict that threat-related arousal disrupts behavioral and neural indices of hippocampal function. Thus, this model posits that an individual’s threat sensitivity, including heightened defensive arousal, can determine downstream impairments in hippocampal function and associated symptoms (Murty and Adcock, 2017).

Many aspects of PTSD fall within this theoretical framework. Threat-predictive behaviors, such as fear-potentiated startle (FPS) responses to danger and safety cues, are heightened in PTSD (Grillon and Morgan, 1999; Grillon and Baas, 2003; Glover et al., 2011; Norrholm and Jovanovic, 2018), and are associated with increased NE engagement (Yehuda et al., 1996). Patients with PTSD (1) show greater arousal in response to cues of both danger and safety (Jovanovic et al., 2010, 2012; Shin and Liberzon, 2010; Pitman et al., 2012; Briscione et al., 2014); (2) fail to inhibit fear responses during fear extinction (Milad et al., 2009; Jovanovic et al., 2010, 2012; Maren and Holmes, 2016; Cacciaiglia et al., 2017; Maeng and Milad, 2017); and (3) overgeneralize fear responses (Hoffmann et al., 2014). Yet these profiles of threat sensitivity have yet to be directly related to hippocampal function. However, our model predicts that these increases in arousal may divert information processing resources away from the HPC, leading to PTSD risk.

In the current study, we extend our model to trauma-related behavioral impairment by characterizing hippocampal dysfunction in relation to heightened arousal and PTSD symptom severity in trauma-exposed participants. We operationalize hippocampal threat sensitivity as responses to fearful versus neutral face stimuli with functional imaging, and arousal as FPS responses to learned danger and safety cues. We also make a distinction between the anterior HPC (aHPC) and posterior HPC (pHPC) portions of the HPC, given aHPC is reportedly more responsive during fear learning and trauma-related arousal (Bannerman et al., 2004; Dolcos et al., 2004; Murty et al., 2010; Hayes et al., 2011; Strange et al., 2014; Abdallah et al., 2017). Our main analyses characterize transient HPC responses reflecting initial threat sensitivity in this region, but we also conduct exploratory analyses reflecting more sustained activity indicating contextual processing. We hypothesized that (1) reductions in HPC threat sensitivity, specifically the aHPC, will predict PTSD symptom severity in trauma-exposed individuals and (2) associations between HPC-threat sensitivity and PTSD symptoms will be mediated by FPS responses.

Materials and Methods

Participants. Participants were recruited from United States emergency departments (EDs) as part of a multisite longitudinal study: Advancing Understanding of Recovery after Trauma (AURORA; U01MH110925) (McLean et al., 2020). Twenty-two EDs within the Northeast, Southern, mid-Atlantic, or Midwest regions of the United States enrolled patients in the ED within 72 h of trauma exposure. All participants were ages 18-75, able to speak and read English, oriented in time and place, physically able to use a smartphone, and possessed a smart phone for >1 year. Potential participants were excluded if they had a solid organ injury >Grade 1, significant hemorrhage, required a chest tube or general anesthesia, or were likely to be admitted for >72 h. MRI scans were collected between 2 and 3 weeks later (M20 = 18, SD20 = 6, referred to as 2 week assessment from here on) at a laboratory visit which included MRI and psychophysiology at four hub sites: McLean Hospital, Emory University, Temple University, or Wayne State University. All participants gave written informed consent as approved by each study site’s Institutional Review Board.

Data collection for the parent study is ongoing and released in specific data freezes. For the second large deep-phenotyping freeze of 202 participants, we focused analyses on using fMRI data during an emotional face processing task and startle data in a fear conditioning paradigm to predict concurrent and future PTSD symptoms (for the timeline of assessments, see Table 1). A total of 116 participants (age: mean = 35.19, SD = 12.51 years, 76 female) were included after excluding for missing PTSD data, and fMRI preprocessing (see fMRI preprocessing) in the release. Participant demographics and psychometric averages are reported in Table 1.

Psychometric assessments. PTSD symptoms were assessed using the PTSD Symptom Checklist for DSM-5 (PCL-5). The PCL-5 is a 20 item self-report questionnaire assessing the presence and severity of various post-traumatic stress symptoms (Weathers et al., 2013). Participants rated symptoms on a scale of 0 (not at all) to 4 (extremely) for the severity of each symptom. A raw total score was computed from summing the individual items and converted to a T-score, reflecting a more general score. Our main analyses focused on the symptom severity at 2 weeks. In an exploratory analysis, we also tested how PTSD symptoms changed from 2 weeks to 8 weeks, and to 3 months after trauma exposure (Fig. 1).

Acquisition and analysis of FPS. Fear conditioning was assessed with a fear-potentiated startle experimental paradigm used successfully in adult trauma populations (Glover et al., 2011; Norrholm et al., 2011). Participants completed this task during the laboratory visit for the MRI scans within the 2 weeks of their trauma exposure (Fig. 1). Participants were seated ~3 feet from a computer screen and asked to remain still and watch the monitor. The protocol consisted of a habituation, acquisition, and extinction phase, all on the same day, lasting a total of 45-60 min. The habituation phase included four trials of each type: startle noise alone (NA); a conditioned stimulus (CS), which would be paired with the unconditioned stimulus (US) during acquisition (CS+); and a CS, which would not be reinforced during acquisition (CS-). The acquisition phase followed habituation and contained three blocks with 12 trials each (36 total acquisition trials). The US was an aversive 250 ms air blast with an intensity of 140 psi directed at the larynx. Both CSs were colored shapes presented on the monitor in front of the participant using a smart phone for 1 s, followed by a 100 ms gap, and CS. The CS+ coterminated with the US 0.5 s after the presentation of the startle stimulus. The shape and color of the CS+ and CS- were counterbalanced across subjects. The CS+ was reinforced with the air blast on 100% of the acquisition trials. The air blast was emitted by a compressed air tank attached to polyethylene tubing and controlled by a solenoid switch. This US has been used in several of our previous studies and consistently produces robust FPS (Jovanovic et al., 2005; Norrholm et al., 2011). The extinction phase occurred 10 min after acquisition and consisted of four blocks of four trials each (NA, CS+, CS-) for a total of 16
Trials of each type. During extinction, the CS− was no longer paired with the air blast. In all phases, the intertrial intervals were randomized to be 9-22 s in duration.

The acoustic startle response data were acquired using the EMG Biomnadix module of the Biopac MP160 for Windows (Biopac Systems). Participants were screened for hearing impairment with an audiometer (Grason-Stadler, Model GS1710), and were required to hear tones ranging from 250 Hz to 4000 Hz above 30 dB. The eyelink component of the acoustic startle response was measured by EMG recordings of the right orbicularis oculi muscle with two 5 mm Ag/AgCl electrodes. One electrode was positioned 1 cm below the pupil of the right eye, and the other was placed 1 cm below the lateral canthus. Impedance levels were <6 kilo-ohms for each participant. The startle probe was a 108-db [A] SPL, 40 ms burst of broadband noise, delivered binaurally through headphones.

EMG data were sampled at 1000 Hz, and the acquired data were filtered with low- and high-frequency cutoffs at 28 and 500 Hz in MindWare software (MindWare Technologies) and exported for statistical analyses. The maximum amplitude of the eyelink muscle contraction 20-200 ms after presentation of the startle probe was used as a measure of the acoustic startle response. FPS was calculated as a percent potentiation: First, a difference score is calculated by subtracting average startle magnitude to the NA trials from average startle magnitude to the CS+. The difference score was then divided by the startle magnitude to NA trials, and finally multiplied by 100. Percent potentiation scores were used because they have been highly replicated and are in line with the National Institutes of Health Research Domain Criteria constructs (Insel et al., 2010).

Figure 1. Experimental timeline. Participants were recruited from EDs after exposure to trauma. Trauma symptoms were assessed 2 weeks, 8 weeks, and 3 months after trauma using PCL-5. As part of the 2 week assessments, participants also completed a fear conditioning task, and a face viewing task in the MRI scanner. During fear conditioning, colored shapes were either reinforced (CS+) or not reinforced (CS−) with air blast, and FPS responses to the CS+ and CS− stimuli were measured. In the fMRI study, participants passively viewed fearful and neutral faces in the scanner.

MRI data acquisition. Before scanning, participants were screened for MR contraindications or other exclusion criteria. Female participants and participants who were potentially childbearing completed a pregnancy test before entering the MR environment. MRI scans were completed on 3T Siemens scanners at each site. Scan sequences were largely harmonized between imaging sites with some variability in sequence parameters because of hardware differences (for overview of all imaging parameters, see Table 2). Following familiarization with the MR environment, participants completed first the T1-weighted anatomic imaging, and then the fMRI. T1-weighted images were used for coregistration (see Preprocessing). Below we report on the passive viewing of fearful faces during fMRI scan (for the details of all MRI scans not reported here, see McLean et al., 2020).

fMRI task design. Integral to the assessment of neural circuitry related to PTSD in the peritraumatic and post-traumatic periods is the inclusion of stimuli and tasks to probe various cognitive and affective processes. Three separate tasks were chosen for the AURORA study; the neural substrates activated within each task have been highly replicated and are in line with the Task Domain Criteria constructs (Insel et al., 2010). Participants completed passive viewing of fearful faces (Stevens et al., 2013), a go/no-go task (Jovanovic et al., 2013), and a card-guessing (reward) task (Delgado et al., 2000).

We report on the fearful face processing task (Stevens et al., 2013). This task has been used in several PTSD studies and has consistently demonstrated greater activation of the amygdala to fearful, compared with neutral, faces (Shin et al., 2005; Stevens et al., 2013; Kim et al., 2019). Participants viewed alternating blocks of either neutral or fearful faces of white race from the Ekman and Friesen faces library (Ekman and Friesen, 1976). Before the task participants were told that they will be shown a series of faces and instructed to “be alert and pay attention to the faces.” Blocks of fearful and neutral stimuli were sequentially presented with the order of fearful and neutral blocks counterbalanced across participants (15 blocks each). In each block, a total of eight faces (four male, four female) were presented for 500 ms each with a 500 ms fixation cross presented after each face. Every 10th block, participants received a 10,000 ms fixation cross as a “rest period” and instructed to “relax and look at the screen” (Kim et al., 2019). No behavioral responses were collected from participants during this task to minimize artifacts because of other neural processes not related to processing the visual stimulus.

MRI data conversion and quality control. DICOM images were converted to NIfTI format with Brain Imaging Data Structure nomenclature using dcm2nii (Li et al., 2016) and were visually inspected for conversion errors and data exclusion criteria (e.g., signal drop-out from Faux calibration, anatomic abnormalities). Further quality control was achieved by running the MRIQC pipeline (version 0.10.4 in a Docker container) (Esteban et al., 2017) on the structural and functional images.

fMRI preprocessing. fMRI preprocessing was performed with FSL 6.0.1 (Jenkinson et al., 2012). First, the T1-weighted anatomic image was skull-stripped using the Brain Extraction Tool. This image was used to assist in spatial normalization processes detailed below. Brain tissue

<table>
<thead>
<tr>
<th>Table 1. Demographic and clinical characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Characteristic</td>
</tr>
<tr>
<td>Age, yr</td>
</tr>
<tr>
<td>Gender, female/male</td>
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<tr>
<td>Race</td>
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<tr>
<td>Black</td>
</tr>
<tr>
<td>White</td>
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<tr>
<td>Hispanic/Latino</td>
</tr>
<tr>
<td>Other</td>
</tr>
<tr>
<td>Family income</td>
</tr>
<tr>
<td>≤$19,000</td>
</tr>
<tr>
<td>$19,001-$35,000</td>
</tr>
<tr>
<td>$35,001-$50,000</td>
</tr>
<tr>
<td>$50,001-$75,000</td>
</tr>
<tr>
<td>$75,001-$100,000</td>
</tr>
<tr>
<td>&gt;$100,000</td>
</tr>
<tr>
<td>Highest education completed</td>
</tr>
<tr>
<td>Some high school</td>
</tr>
<tr>
<td>High school</td>
</tr>
<tr>
<td>Associate’s degree</td>
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<tr>
<td>Bachelor’s degree</td>
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<tr>
<td>Master’s degree</td>
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<tr>
<td>Professional school degree</td>
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<tr>
<td>Doctoral degree</td>
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<tr>
<td>Clinical characteristics</td>
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<tr>
<td>PTSD symptom severity</td>
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<tr>
<td>PCL-5 total scores at 2 wk (n = 116)</td>
</tr>
<tr>
<td>PCL-5 total scores at 3 mo (n = 116)</td>
</tr>
<tr>
<td>Trauma type</td>
</tr>
<tr>
<td>Motor vehicle collision</td>
</tr>
<tr>
<td>Physical assault</td>
</tr>
<tr>
<td>Sexual assault</td>
</tr>
<tr>
<td>Fall</td>
</tr>
<tr>
<td>Nonmotorized collision</td>
</tr>
<tr>
<td>Burns</td>
</tr>
<tr>
<td>Other</td>
</tr>
</tbody>
</table>
segmentation of white matter, gray matter, and CSF was performed on the brain extracted T1-weighted images using FAST. These segmentations were used to extract time series from the white matter and CSF for reduction of noise in our preprocessing stream. fMRI preprocessing was completed using the fmRI Expert Analysis Tool version as implemented in FSL 6.0.1. Using a pipeline designed to minimize the effects of head motion (Murty et al., 2018). This included simultaneous head motion correction, and nonlinear warping to the MNI space, but no temporal or spatial filtering.

Following preprocessing, we ran a GLM, where the onset of fearful and neutral blocks of faces were modeled as separate regressors, and were convolved with a double-gamma HRF as an event-related response capturing the block onset. Six head-motion parameters, and their first derivatives, and time series extracted from CSF and white matter were added as covariates to the model to reduce noise. For our exploratory analysis of sustained responses, a second GLM was run with the additional regressors to model the entire duration (8 s) for the fearful and neutral blocks in addition to the transient on-set block (i.e., to model the sustained activity). The GLMs were run using fmRI Expert Analysis Tool version 6.0 as implemented in FSL 6.0.3. First level contrast of fearful versus neutral contrasts were estimated using the middle tertile in this analysis as signals from this region have been shown to be a mixture of anterior versus posterior hippocampal activity. The significance was set at p < 0.00125. Importantly, we tested two additional models, which included activity from both left and right hemispheres as covariates (separately for aHPC and pHPC). Then for each subregion, we tested whether the coefficients differed between left and right to test any effects of laterality.

Next, we tested whether threat-related activity in the HPC relates to arousal responses. Twenty-two subjects were removed from these models because of missing startle data (N = 95, 62 female). We first tested whether the fear acquisition elicited the intended effects, comparing participants’ FFS responses to CS+ (danger signal) and CS− stimuli (safety signal). Next, we tested whether FFS is predicted by the threat-related activity in the HPC. Finally, we tested whether startle responses interacted with fear-related hippocampal reactivity in predicting the PTSD symptoms at 2 weeks after trauma. Importantly, we tested this assumption only in the regions whose activity yielded significant effects on the PTSD symptoms at 2 weeks (for more details, see Results). Therefore, we tested a total of two models here, with significance set at p < 0.05 (uncorrected) and Bonferroni correction set at p < 0.025.

We next tested a time-based hypothesis that hippocampal threat sensitivity, together with physiological threat sensitivity, would predict PTSD symptom change across the follow-up assessments (8 weeks and 3 months after trauma). To that end, we first tested a mixed-effects model with a two-way interaction between threat-related activity and time (2 weeks, 8 weeks, and 3 months), separately in aHPC and pHPC. We then tested a second mixed-effects model with a three-way interaction model between threat-related hippocampal activity, FFS responses, and time, separately in anterior and posterior subregions. Across all four models, significance was set at p < 0.05 (uncorrected), while Bonferroni corrections for multiple comparisons were set at p < 0.0125.

We next conducted an exploratory analysis. Specifically, we tested whether the sustained hippocampal activity related to PTSD symptomatology differently than transient activity. To that end, we repeated the analyses above using the activity extracted from the fearful > neutral contrast from the GLM where sustained activity was modeled. Therefore, we tested four initial models where PTSD symptoms at 2 weeks were predicted by the sustained HPC activity. The significance was set at p < 0.05 (uncorrected) and Bonferroni correction set at p < 0.0125 for these models. For the regions with significant effects on PTSD outcome that survived the Bonferroni correction, we then proceeded with the additional tests.
with the interaction models (FPS difference by hippocampal activity). This resulted in two additional tests, for which the significance set at \( \rho = 0.05 \) (uncorrected) and Bonferroni correction set at \( \rho < 0.025 \).

The unstandardized \( \beta \) coefficients are reported for all our significant results. All analyses were performed using R software (R package version 3.4.1) using the anova (the stats library), glm (the stats library), glmer (the lme4 library), linearHyphothesis (the car library), and simple_slopes (the reghelper library) functions depending on the test. Finally, regression models predicting PTSD symptoms were tested using a Poisson distribution (family = Poisson (link= ”log”)) since the symptom distribution was positively skewed. Age, gender, and scanner type (to control potential effects of different scanners on the hippocampal signal) were added in all of the models as covariates. Finally, all continuous variables were standardized before testing the regression models. Analysis scripts are available on request.

**Results**

**HPC does not differentiate between fearful and neutral faces**

Four separate one-way ANOVAs testing the effect of emotion (fearful, neutral) on the neural activity were run in the left aHPC, right aHPC, left pHPC, and right pHPC. The models did not reveal any significant main effect of emotion (left anterior: \( F_{(2,230)} = 0.01, \rho = 0.8 \); right anterior: \( F_{(2,230)} = 0.001, \rho = 0.9 \); left anterior: \( F_{(2,230)} = 1.2, \rho = 0.3 \); right anterior: \( F_{(2,230)} = 0.06, \rho = 0.8 \)), suggesting that HPC does not differentiate between fearful and neutral faces.

**Decreased transient left hippocampal fear-related activity predicts PTSD symptoms**

Threat-related transient activity in left aHPC (left: \( \beta = -0.08, \rho < 0.0001 \)) and left pHPC (\( \beta = -0.09, \rho < 0.0001 \)) was associated with PTSD symptom severity at 2 weeks (Fig. 2; Table 3), such that relatively less threat-related reactivity in the HPC the greater their 2 week PTSD symptom. All of the reported models with a significant effect survived Bonferroni correction (\( \rho_{\text{adjusted}} = 0.0125 \)). However, right HPC was not a significant predictor of PTSD symptoms at 2 weeks (anterior: \( \rho = 0.22 \); posterior: \( \rho = 0.05 \)); thus, we did not test the following FPS-related models in right aHPC and pHPC.

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**Table 3. Predicting PTSD Symptoms at 2-Weeks from Transient Hippocampal Threat (F>N) Reactivity and Fear-Potentiated Startle (FPS) Difference between Danger (CS⁺) and Safety (CS⁻)**

<table>
<thead>
<tr>
<th></th>
<th>Model 1 (PTSD ~ Left aHPC)</th>
<th>Model 2 (PTSD ~ Right aHPC)</th>
<th>Model 3 (PTSD ~ Left pHPC)</th>
<th>Model 4 (PTSD ~ Right pHPC)</th>
<th>Model 5 (PTSD ~ Left aHPC + FPS Diff)</th>
<th>Model 6 (PTSD ~ Left pHPC + FPS Diff)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Left aHPC (std)</strong></td>
<td>-0.081*** (0.018)</td>
<td>-0.222 (0.018)</td>
<td>-0.085*** (0.018)</td>
<td>-0.031 (0.021)</td>
<td>-0.058*** (0.021)</td>
<td></td>
</tr>
<tr>
<td><strong>Right aHPC (std)</strong></td>
<td>-0.0022 (0.018)</td>
<td>-0.0085*** (0.018)</td>
<td>-0.035 (0.018)</td>
<td>-0.031 (0.021)</td>
<td>-0.058*** (0.021)</td>
<td></td>
</tr>
<tr>
<td><strong>Left pHPC (std)</strong></td>
<td>-0.046** (0.018)</td>
<td>-0.044 (0.018)</td>
<td>-0.040 (0.018)</td>
<td>-0.042 (0.018)</td>
<td>-0.035 (0.018)</td>
<td>-0.058*** (0.021)</td>
</tr>
<tr>
<td><strong>Right pHPC (std)</strong></td>
<td>-0.092 (0.028)</td>
<td>-0.041 (0.017)</td>
<td>-0.092 (0.028)</td>
<td>-0.092 (0.028)</td>
<td>-0.092 (0.028)</td>
<td>-0.092 (0.028)</td>
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<tr>
<td><strong>FPS Diff. (std)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (std)</td>
<td>0.142** (0.041)</td>
<td>0.188** (0.039)</td>
<td>0.187** (0.039)</td>
<td>0.193** (0.039)</td>
<td>0.287** (0.048)</td>
<td>0.347** (0.047)</td>
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<tr>
<td>Female</td>
<td>0.006 (0.043)</td>
<td>-0.053 (0.042)</td>
<td>-0.063 (0.042)</td>
<td>-0.063 (0.042)</td>
<td>0.075 (0.051)</td>
<td>0.102** (0.050)</td>
</tr>
<tr>
<td>Scanner: TrioTim (&gt; Prisma)</td>
<td>-0.095 (0.043)</td>
<td>-0.053 (0.042)</td>
<td>-0.063 (0.042)</td>
<td>-0.063 (0.042)</td>
<td>0.075 (0.051)</td>
<td>0.102** (0.050)</td>
</tr>
<tr>
<td>Scanner: Verio (&gt; Prisma)</td>
<td>0.015 (0.048)</td>
<td>0.047 (0.048)</td>
<td>0.037 (0.048)</td>
<td>0.036 (0.046)</td>
<td>0.183** (0.053)</td>
<td>0.174** (0.052)</td>
</tr>
<tr>
<td>Left aHPC (std): FPS Diff. (std)</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Left pHPC (std): FPS Diff. (std)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Constant</td>
<td>3.269** (0.045)</td>
<td>3.217** (0.044)</td>
<td>3.220** (0.044)</td>
<td>3.219** (0.044)</td>
<td>3.010** (0.054)</td>
<td>2.957** (0.055)</td>
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<td>Observations</td>
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<td>116</td>
<td>116</td>
<td>116</td>
<td>116</td>
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<tr>
<td>Log Likelihood</td>
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<td>-845.250</td>
<td>-834.567</td>
<td>-844.101</td>
<td>-619.638</td>
<td>-615.919</td>
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<tr>
<td>Akaike Inf. Crit.</td>
<td>1,684.674</td>
<td>1,702.500</td>
<td>1,681.133</td>
<td>1,700.202</td>
<td>1,255.276</td>
<td>1,247.838</td>
</tr>
</tbody>
</table>

*The models did not reveal any significant main effect of emotion (left anterior: \( F_{(2,230)} = 0.01, \rho = 0.8 \); right anterior: \( F_{(2,230)} = 0.001, \rho = 0.9 \); left anterior: \( F_{(2,230)} = 1.2, \rho = 0.3 \); right anterior: \( F_{(2,230)} = 0.06, \rho = 0.8 \)), suggesting that HPC does not differentiate between fearful and neutral faces.**

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**Figure 2.** Reduced threat-related transient activity in hippocampus predicts PTSD severity. Increased threat-related transient activity in left aHPC and left pHPC, as measured by the fearful > neutral face image contrasts, predicted lower PTSD symptom severity at 2 weeks, concurrent with the timing of the fMRI scan. The effects are shown as follows: (A) left aHPC; (B) left pHPC.
It is important to note that left aHPC and left pHPC activity was correlated \((r_{114} = 0.21, p = 0.03)\); however, the low correlation between the two subregions emphasizes the relative orthogonality of the aHPC and pHPC activity in predicting PTSD symptom severity. Finally, comparing coefficients from left and right hemisphere for both hippocampal subregions revealed that the association between hippocampal activity and PTSD symptom severity was stronger in the left than right hemisphere (anterior: \(\chi^2_{(109)} = 10.69, p = 0.001\); posterior: \(\chi^2_{(109)} = 13.4, p = 0.0003\)).

### Increased FPS responses during fear acquisition predict PTSD symptoms

Participants had greater FPS response to the CS\(^+\) (danger) compared with the CS\(^-\) (safety) during fear acquisition \((t_{93} = 3.4, p = 0.001)\), suggesting that they learned to discriminate between the danger and safety cues. Therefore, we focused on the FPS difference between danger and safety cues as our main predictor in the startle models. To that end, we first tested whether FPS difference was associated with the PTSD symptoms at 2 weeks. The results revealed that increased FPS difference was associated with higher PTSD symptoms \((\beta = 0.07, SE = 0.02, p = 0.0002)\).

#### Fear-related transient activity in the HPC and startle responses during fear acquisition interactions predict PTSD symptoms

The models testing whether threat-related activity in the HPC was associated with FPS responses did not reveal any significant relationship (left anterior: \(F_{(3,90)} = 0.7, p = 0.6\); left posterior: \(F_{(3,90)} = 0.5, p = 0.7\)). Critically, we found that significant interactions between transient threat-related hippocampal activity and FPS difference predicted 2 week PTSD symptoms (left anterior: \(\beta = -0.04, SE = 0.02, p = 0.017\); left posterior: \(\beta = -0.09, SE = 0.03, p = 0.001\)). Results from both left aHPC and left pHPC survived Bonferroni corrections \((p_{adjusted} = 0.025)\). To determine whether these findings generalized to alternative approaches to estimating FPS, we separately calculated FPS by using a residualization approach (i.e., using the residual FPS to CS\(^+\) and CS\(^-\) after regressing out the average startle magnitude to the NA trials). This approach yielded results similar to HPC \(\times\) FPS interactions in the posterior, but not anterior, HPC (anterior: \(\beta = 0.007, SE = 0.063\); posterior: \(\beta = 0.08, SE = 0.004\)), which suggests that the reported FPS-related PTSD outcomes in the pHPC are specific to threat-related arousal instead of individual differences in baseline startle responses.

Simple slopes analyses revealed that the inverse relationship between transient left anterior hippocampal threat reactivity and PTSD symptoms at 2 weeks was stronger for high (+1 SD) FPS differentiation \((\beta = -0.07, SE = 0.03, t = -2.8, p = 0.005)\). Moreover, the relationship between transient left posterior hippocampal threat reactivity and PTSD symptoms was stronger for both mean and high (+1 SD) FPS differentiation (mean: \(\beta = -0.06, SE = 0.02, t = -2.82, p = 0.005\); high: \(\beta = 0.15, SE = 0.04, t = -3.99, p < 0.0001\) (Fig. 3). These effects suggest that individuals with higher FPS differentiation and lower transient hippocampal reactivity to threat report higher PTSD symptoms.

#### Independent contributions of fearful and neutral hippocampal reactivity to PTSD symptoms

To better decompose the component effects guiding the relationships above, we next tested whether our hippocampal effects were driven by changes in the HPC activity specific to the fearful (fearful > baseline) or neutral (neutral > baseline) faces. The fearful-only analyses revealed that decreased transient activity in left aHPC and pHPC was associated with greater PTSD symptoms at 2 weeks (anterior: \(\beta = -0.06, SE = 0.02, p < 0.0004\); posterior: \(\beta = -0.04, SE = 0.02, p = 0.015\), both effects survive Bonferroni adjustments at \(p_{adjusted} = 0.025\)). However, there were no significant interactions between the transient fearful-only hippocampal activity and FPS difference in predicting PTSD symptoms at 2 weeks.

On the other hand, increased transient neutral-only activity in left pHPC was associated with increased PTSD symptoms at 2 weeks \((\beta = 0.04, SE = 0.02, p = 0.038\), albeit it did not survive Bonferroni corrections at \(p = 0.025\)). Importantly, the neutral-only activity in left pHPC significantly interacted with FPS difference score in predicting PTSD symptoms at 2 weeks \((\beta = 0.06, SE = 0.03, p = 0.02)\). Simple slopes analysis revealed that this association was significant at the lower end of the FPS difference \((-1 SD, p = 0.045\) and at the moderate (mean: \(p = 0.003\) and higher \((+1 SD, p < 0.0001)\) left posterior hippocampal activity to neutral faces. These results suggest that decreased transient activity to fearful stimuli and increased transient activity to neutral stimuli in HPC both contribute to increased PTSD symptomatology.

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**Figure 3.** FPS interacts with transient hippocampal threat reactivity in predicting PTSD at 2 weeks. Increased FPS differentiation between danger (CS\(^+\)) and safety (CS\(^-\)) cues had a significant effect on the inverse relationship between the increased hippocampal threat reactivity and lower PTSD symptoms at 2 weeks in (A) left aHPC and (B) left pHPC.
PTSD symptom change across time

We took a growth modeling approach to analyze whether the symptom change from 2 weeks to 8 weeks and 3 months follow-ups is predicted by hippocampal threat reactivity and/or FPS differentiation. For these analyses, we focused on the left aHPC and left pHPC given their significant role in 2 week PTSD outcomes. Analyses revealed a main effect of time (Table 4), such that PTSD symptoms decreased from 2 weeks to 8 weeks and 2 weeks to 3 months follow-up assessments. However, there were no significant interactions between time, hippocampal threat reactivity, and FPS differentiation (Table 4).

Age, gender, and scanner effects on PTSD

Age, gender, and scanner type were included as covariates in all models. In all the 2 weeks PTSD models reported above, gender was a significant predictor of PTSD symptoms (Table 3) such that female subjects reported higher PTSD symptom scores compared with male participants. Age was also a significant predictor of PTSD symptoms in the simple 2 weeks models, but this effect was no longer evident when the FPS difference was added to the models as an interaction term (Table 3). Finally, including the scanner type as a covariate ensured that the reported significant hippocampal effects were not influenced by the scanner related differences across the study sites.

Sustained fear-related activity in the HPC predicts increased PTSD symptoms

In a set of exploratory analyses, we next tested whether sustained fear-related hippocampal activity relates to PTSD symptoms differently than the transient activity. Notably, these analyses included both sustained and transient activity within the same fMRI model when estimating single-subject parameters, highlighting independent contributions of sustained activity. The results revealed that increased sustained fear-related activity in left and right posterior (left: $\beta = 0.05$, $SE = 0.02$, $t = 2.69$, $p = 0.007$; right: $\beta = 0.06$, $SE = 0.02$, $t = 3.17$, $p = 0.002$) HPC was associated with increased PTSD symptoms at 2 weeks (Fig. 4A, B). These results suggest that sustained posterior hippocampal reactivity to fear-related information relates to higher PTSD symptomatology (Table 5). Importantly, interactions between the sustained pHPC and FPS difference significantly predicted PTSD symptoms at 2 weeks (left: $\beta = 0.04$, $SE = 0.02$, $t = 2.27$, $p = 0.024$; right: $\beta = 0.04$, $SE = 0.02$, $t = 2.45$, $p = 0.015$, both effects survive Bonferroni corrections at $p_{adjusted} = 0.025$) (Fig. 4C,D). Simple slopes analyses revealed that this interaction effect was stronger at the higher levels of FPS difference (+1 SD: $p = 0.0007$ in left posterior; $p < 0.0001$ in right posterior). Moreover, the interaction effects were also stronger for the moderate (mean: $p < 0.0001$ in left posterior; $p < 0.0001$ in right posterior) and higher levels of sustained pHPC activity (+1 SD: $p < 0.0001$ in left posterior; $p < 0.0001$ in right posterior). Accordingly, individuals with higher sustained fear-related activity in pHPC and higher FPS difference report higher PTSD symptoms at 2 weeks.

Discussion

Heightened arousal because of threatening events alter hippocampal activity (Kim and Diamond, 2002; Henckens et al., 2009; Schwabe and Wolf, 2012; Bisby and Burgess, 2013, 2017), which has been suggested to strengthen traumatic memories and exacerbate symptoms (Hayes et al., 2011). Here, we assessed the relationship between threat sensitivity, hippocampal function, and PTSD symptomatology in a group of individuals recently exposed to trauma (McLean et al., 2020).

We first showed that decreased transient hippocampal threat sensitivity was related to PTSD symptom severity at 2 weeks after trauma exposure. Specifically, we found that participants who showed reduced transient threat reactivity in left aHPC and left pHPC reported more severe PTSD symptoms. This is consistent with previous research that showed reduced left HPC activity in
Table 5. Predicting PTSD symptoms at 2 weeks from sustained hippocampal threat reactivity and FPS differentiation between danger ($\text{CS}^-$) and safety ($\text{CS}^+$)

<table>
<thead>
<tr>
<th></th>
<th>Model 1 (PTSD $\rightarrow$ Left pHPC)</th>
<th>Model 2 (PTSD $\rightarrow$ Right pHPC)</th>
<th>Model 3 (PTSD $\rightarrow$ Left pHPC + FPS Diff)</th>
<th>Model 4 (PTSD $\rightarrow$ Right pHPC + FPS Diff)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Left pHPC (std)</td>
<td>0.047** (0.018)</td>
<td>0.055*** (0.017)</td>
<td>0.040 (0.020)</td>
<td>0.054** (0.021)</td>
</tr>
<tr>
<td>Right pHPC (std)</td>
<td></td>
<td>0.041* (0.018)</td>
<td>0.041* (0.018)</td>
<td>0.019 (0.020)</td>
</tr>
<tr>
<td>FPS Diff. (std)</td>
<td></td>
<td>0.019 (0.017)</td>
<td>0.019 (0.017)</td>
<td>0.012 (0.020)</td>
</tr>
<tr>
<td>Age (std)</td>
<td>0.198*** (0.039)</td>
<td>0.198*** (0.039)</td>
<td>0.343*** (0.046)</td>
<td>0.331*** (0.046)</td>
</tr>
<tr>
<td>Female</td>
<td></td>
<td></td>
<td>0.089 (0.050)</td>
<td>0.110* (0.051)</td>
</tr>
<tr>
<td>Scanner: TrioTim (&gt;) Prisma</td>
<td>-0.046 (0.042)</td>
<td>-0.046 (0.042)</td>
<td>0.166*** (0.051)</td>
<td>0.193*** (0.055)</td>
</tr>
<tr>
<td>Scanner: Verio (&gt;) Prisma</td>
<td>0.034 (0.047)</td>
<td>0.019 (0.047)</td>
<td>0.042* (0.018)</td>
<td>0.036* (0.015)</td>
</tr>
<tr>
<td>Left pHPC (std): FPS Diff. (std)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right pHPC (std): FPS Diff. (std)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Constant</td>
<td>3.210*** (0.044)</td>
<td>3.211*** (0.044)</td>
<td>2.975*** (0.054)</td>
<td>2.964*** (0.054)</td>
</tr>
</tbody>
</table>

|                         |                                         |                                         |                                                  |                                                  |
|                         | Pseudo R²: 0.04                        | Pseudo R²: 0.04                        | Pseudo R²: 0.10                                   | Pseudo R²: 0.11                                   |
|                         | Akaik Inf. Crit.: 1,693.687            | Akaik Inf. Crit.: 1,690.896            | Akaik Inf. Crit.: 1,251.667                       | Akaik Inf. Crit.: 1,244.650                       |

*p < 0.05, **p < 0.01, ***p < 0.001; aHPC: anterior hippocampus; pHPC: posterior hippocampus; std: Standardized; F>N: Fearful > Neutral contrast; FPS Diff: Fear-Potentiated Startle Difference

Figure 4. Effects of sustained hippocampal activity. Increased sustained threat-related activity in (A) left pHPC and (B) right pHPC predicted higher PTSD symptoms at 2 weeks. Increased FPS differentiation between danger ($\text{CS}^-$) and safety ($\text{CS}^+$) cues had a significant effect on the relationship between the increased hippocampal threat reactivity and increased PTSD symptoms at 2 weeks in (C) left pHPC and (D) right pHPC.
PTSD patients when remembering trauma-related memories (Bremner, 2001; Bremner et al., 2003; Hayes et al., 2011) or recently learned negative information (Bisby and Burgess, 2017). Relatedly, reduced hippocampal activation during a response inhibition task has also been associated with increased PTSD symptoms in chronically traumatized individuals (van Rooij et al., 2016; van Rooij and Jovanovic, 2019), and predicted future PTSD symptoms in recently traumatized civilians (van Rooij et al., 2018). Together with these earlier findings, our study supports an account of intact hippocampal function playing a role in trauma resilience (van Rooij et al., 2021).

An important distinction between our findings and the previous research, however, is that previous research has shown that the association between the hippocampal dysfunction and PTSD was driven by the anterior portion of the HPC (Dickie et al., 2011; Hayes et al., 2011; Abdallah et al., 2017), a region that is often implicated in fear learning (Kjelstrup et al., 2002; Bannerman et al., 2004; Murty et al., 2010; Strange et al., 2014). However, we did not find a functional distinction between anterior and posterior portions of the HPC in predicting PTSD symptom severity, and our pHPC results were more robust to characterizing interactions with FPS in predicting PTSD symptoms. Moreover, albeit low, the activity in aHPC and pHPC was correlated in the current sample. Therefore, our results are more in line with the results of Lazarov et al. (2017), who recently showed that the functional distinction between aHPC and pHPC in their connectivity to regions in the default mode network (e.g., ventromedial PFC, precuneus, and posterior cingulate cortex), which are often implicated in PTSD patients, is eliminated in individuals with PTSD but not in trauma-exposed controls.

Our findings suggest a complex role of the HPC in threat sensitivity since it is highly sensitive to threatening stimuli after traumatic experiences. This heightened hippocampal sensitivity protects the individual from developing severe symptoms of PTSD, but only to the extent that it can process the negative information. We found that the relationship between hippocampal threat reactivity and PTSD symptom severity is modulated impaired ability to differentiate threat from safety (CS'). Specifically, our data demonstrated greater threat anticipation, as evidenced by the greater differentiation between FPS responses to CS' and to CS, was associated with lower reactivity in the left HPC. Moreover, this interaction between the reduced hippocampal activity and greater threat anticipation was linked with PTSD symptom severity at 2 weeks after trauma. Although previous research has established an association between reduced hippocampal activity and arousal symptoms of PTSD (Hayes et al., 2011), and between an impairment in delineating danger and safety cues and the development of PTSD (Jovanovic et al., 2010, 2012; Shin and Liberzon, 2010; Pitman et al., 2012; Briscione et al., 2014; Maeng and Milad, 2017), our results are unique in demonstrating that the same individuals who are highly reactive to threat cues also show impaired hippocampal engagement in the processing of threat cues, which is associated with PTSD symptom severity.

These findings may be surprising in the context of the prior PTSD literature, but our results are consistent with our recent model detailing arousal-related impairments in hippocampal function. Our model suggested that threat-related arousal impairs hippocampal function, biasing information processing away from the HPC to other learning structures, particularly when arousal-mediated systems, such as the NE system, are engaged (Clewett and Murty, 2019). Critically, PTSD studies have shown increased NE release in response to stress (for review, see Bremner, 2006), which may bias hippocampal threat reactivity. Given this evidence, we conclude that physiological arousal, a putative marker of the NE system, represents an important individual difference measure predicting whether the HPC will propagate or mitigate PTSD symptoms.

In a set of exploratory analyses, we also explored the relationship of more sustained hippocampal responses to threat and how they relate to PTSD symptoms. Specifically, we found unlike transient threat processing in the HPC, increased sustained engagement of the HPC in response to threatening stimuli positively predicted PTSD symptoms. These effects were even more pronounced in individuals who showed greater differentiation between threat and safety cues as measured by FPS. The opposing directions of these sustained responses compared with transient responses suggest that differential mechanisms may be at play when considering fast, event-evoked responses and more prolonged, sustained responses. Critically, the HPC has been shown to subserve multiple roles, including subserving the formation and retrieval of episodic memories (Eichenbaum, 2001), but also regulating stress responses that underlie hyper-salience and defensive behaviors (Herman et al., 2016; Jimenez et al., 2018; Goldfarb et al., 2020). While highly speculative, we suggest that the more transient responses in the HPC reflects more adaptive forms of memory encoding that can protect individuals from developing PTSD symptoms, whereas the more sustained responses may reflect sustained signals that propagate HPA-axis engagement, leading to greater susceptibility to the damaging effects of trauma. However, more empirical work that includes explicit, dynamic measures of episodic memory formation and hyper-salience are needed to confirm these hypotheses.

The current study had a few features that limited our ability to fully interpret our findings, that should be addressed in future work. First, our fearful face processing task did not include dynamic assays of behavior, such as eye-tracking, subsequent memory, or physiological arousal, to help us integrate our neural findings with behavioral outcomes. Including more behavioral variables related to real-time assessments of hippocampal threat sensitivity could provide clear relationships to PTSD symptoms. Second, all participants in our study were exposed to trauma in recent history. Thus, our study lacks the baseline of a normative, non–trauma-exposed cohort, which could help us determine whether individuals with low PTSD reflect signals of resilience and/or compensation. Third, our current sample of trauma participants consisted mainly of individuals in recent automobile accidents, with relatively low sampling of other forms of trauma. Thus, the current dataset was unable to disambiguate how different forms of trauma relate to PTSD symptoms, which has important implications for the development of tailored therapeutics.

Together, our findings are consistent with a novel model of the involvement of the HPC in mediating PTSD symptomology. Specifically, we propose that decreased threat sensitivity in the HPC, a structure known to support safety learning, contributes to both concurrent PTSD symptoms as well as the propagation of these symptoms into the future. However, our model further specifies that an important mediator of this relationship is state-dependent physiological arousal. Thus, physiological arousal may divert information processing away from the HPC during threat learning, yielding vulnerability and risk. Future studies are warranted linking engagement of the hippocampal system to memory fragmentation and threat-related memory, as prior work has specified this relationship in normative populations.
References


