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Trauma exposure acutely alters neural function during Pavlovian fear conditioning

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Abstract

Posttraumatic stress disorder (PTSD) is associated with dysfunction of the neural circuitry that supports fear learning and memory processes. However, much of what is known about neural dysfunction in PTSD is based on research in chronic PTSD populations. Less is known about neural function that supports fear learning acutely following trauma exposure. Determining the acute effects of trauma exposure on brain function would provide new insight into the neural processes that mediate the cognitive-affective dysfunction associated with PTSD. Therefore, the present study investigated neural activity that supports fear learning and memory processes in recently Trauma-Exposed (TE) and Non-Trauma-Exposed (NTE) participants. Participants completed a Pavlovian fear conditioning procedure during functional magnetic resonance imaging (fMRI). During fMRI, participants' threat expectancy was continuously monitored. NTE participants showed greater threat expectancy during warning than safety cues, while no difference was observed in the TE group. This finding suggests TE participants overgeneralized the fear association to the safety cue. Further, only the TE group showed a negative relationship between fMRI signal responses within dorsomedial prefrontal cortex (PFC) and threat expectancy during safety cues. These results suggest the dorsomedial PFC mediates overgeneralization of learned fear as an acute result of trauma exposure. Finally, neural activity within the PFC and inferior parietal lobule showed a negative relationship with PTSD symptom severity assessed three months posttrauma. Thus, neural activity measured acutely following trauma exposure predicted future PTSD symptom severity. The present findings elucidate the acute effects of trauma exposure on cognitive-affective function and provide new insight into the neural mechanisms of PTSD.

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Conflict of Interests

The authors declare no conflicts of interest.

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Keywords

fMRI; PTSD; Fear; Trauma; Emotion

1. Introduction

Understanding the neural dysfunction that mediates the deleterious effects of posttraumatic stress is critical for improving diagnostic tools, early intervention efforts, and treatment approaches for posttraumatic stress disorder (PTSD). Prior PTSD research has identified aberrant activity within brain circuitry that supports important emotional learning and memory processes (Liberzon & Sripada, 2007; Milad et al., 2009; Rauch et al., 2000; Shin et al., 2011). However, much of this prior work has largely focused on neural function in chronic PTSD and provides limited information about neural dysfunction that may manifest acutely following trauma exposure (Stevens et al., 2017; van Rooij et al., 2017). Thus, there is a significant gap in our understanding of the acute effects of trauma exposure on brain function in humans. Determining the acute effect of trauma exposure on brain function will enhance our understanding of the etiology of PTSD and open new avenues to investigate trauma and stress-related disorders. Therefore, the current study investigated brain activity during Pavlovian fear conditioning in recently (i.e., < 1 month) Trauma-Exposed (TE) individuals. The present study offers unique insight into the effects of trauma exposure on neural activity that supports fear learning and memory processes.

Posttraumatic stress is associated with the disruption of important fear learning and memory processes. For example, prior research has demonstrated that fear acquisition, expression, extinction, and extinction recall are disrupted in chronic PTSD (Blecher, Michael, Vriends, Margraf, & Wilhelm, 2007; Fani et al., 2012; Garfinkel et al., 2014; Grillon & Morgan, 1999; Milad et al., 2009; Norrholm et al., 2011). Further, previous research has demonstrated that acute stress exposure modulates associative fear learning (Goodman, Harnett, Wheelock, et al., 2018; Raio & Phelps, 2015), which is disrupted in chronic PTSD (Blecher et al., 2007; Fani et al., 2012; Garfinkel et al., 2014; Grillon & Morgan, 1999; Milad et al., 2009; Norrholm et al., 2011). For example, physical and psychosocial stress exposure appears to sensitize fear learning processes such that fear-related associations are formed more rapidly, and expressed more strongly and indiscriminately (Dunsmoor, Otto, & Phelps, 2017; Goodman, Harnett, Wheelock, et al., 2018; Rau, DeCola, & Fanselow, 2005; Shors, Weiss, & Thompson, 1992). Thus, the prior literature suggests stress exposure can disrupt healthy fear learning and memory processes.

The dysfunctional fear learning and memory observed in chronic PTSD appears to be mediated by disruption of prefrontal cortex (PFC) and amygdala activity. Prior research indicates the amygdala is critical for fear conditioning and expression of the conditioned fear response (Cheng, Knight, Smith, & Helmstetter, 2006; Cheng, Knight, Smith, Stein, & Helmstetter, 2003; Knight, Nguyen, & Bandettini, 2005; LaBar, Gatenby, Gore, LeDoux, & Phelps, 1998). Further, the dorsolateral, dorsomedial, and ventromedial PFC appear to regulate the fear learning processes that are mediated by the amygdala (Delgado, Nearing, LeDoux, & Phelps, 2008; Fullana et al., 2016; Knight, Smith, Stein, & Helmstetter, 1999;

Milad et al., 2007; Motzkin, Philippi, Wolf, Baskaya, & Koenigs, 2015; Wheelock et al., 2014). Importantly, chronic PTSD patients often show enhanced activity within the dorsomedial PFC and amygdala, and reduced activity within the dorsolateral and ventromedial PFC (Aupperle et al., 2012; Bremner et al., 2005; Bryant et al., 2008; Milad et al., 2009; Patel, Spreng, Shin, & Girard, 2012; Pitman et al., 2012; Rauch et al., 2000). Further, PTSD patients show altered functional connectivity between these brain regions (Morey et al., 2015; Sripada et al., 2012; Stevens et al., 2013). Taken together, the disrupted fear learning and memory processes observed in chronic PTSD patients appear to be mediated by dysfunction of PFC and amygdala circuitry.

Although prior work has clearly demonstrated dysfunction of the neural circuitry that supports fear learning in chronic PTSD, these processes have not been assessed acutely following trauma exposure. Importantly, the acute effects of trauma exposure may differ from the chronic effects of PTSD. For example, prior animal model research has demonstrated that acute stress enhances glutamatergic activity within the PFC, which improves memory performance (Yuen et al., 2009; Yuen et al., 2011). In contrast, chronic, repeated stress disrupts glutamatergic systems within the PFC and impairs memory performance (Yuen et al., 2012). This prior work suggests the impact acute and chronic stress have on neural processes can be dramatically different. Therefore, the acute effects of trauma exposure may differ from the effects of chronic posttraumatic stress on the brain function that supports associative fear learning in humans.

The present study investigated brain activity during Pavlovian fear conditioning using functional magnetic resonance imaging (fMRI) in recently TE and Non-Trauma-Exposed (NTE) participants. Specifically, the acute impact of trauma exposure on brain and behavioral responses was assessed via comparisons of the TE and NTE groups. Further the relationship between posttraumatic stress severity and neurobehavioral function was assessed by comparing inter-individual variability in Posttraumatic Diagnostic Scale (PDS) scores to brain/behavioral responses. We hypothesized that threat expectancy, skin conductance responses (SCR), and fMRI signal responses would be greater during warning than safety cues. Further, we expected TE and NTE participants would show differences in brain activity within regions that support associative fear learning. In addition, we expected TE participants would show greater behavioral responses (i.e., greater threat expectancy and greater SCRs) than NTE participants. Finally, we hypothesized that brain and behavioral responses would vary with acute and future posttraumatic stress severity within the TE group. The results of the present study offer new insight into the neural processes that underlie PTSD.

2. Methods

Participants:

Volunteers in the present study participated in prior published work that investigated the relationship between trauma exposure and brain glutamate/glutamine levels (Harnett et al., 2017). In total, forty TE (n = 21) and NTE (n = 19) volunteers participated in the present study. One TE participant terminated participation prior to completing fMRI. Thus, 20 TE participants were included in the present analysis (Table 1). TE participants were recruited

from the University of Alabama at Birmingham's Acute Trauma Care Unit, Trauma Burn Nursing Unit, Trauma Burn Intensive Care Unit, and Emergency Department, and were scanned within 30 days of trauma exposure ($M = 22.55$ days, $SD = 5.68$ days). TE participants had experienced 1) a physical injury requiring a visit to a trauma unit/ Emergency Department and 2) exposure to a traumatic event identified on the PDS within the last 30 days (Table S1). Nineteen NTE control participants were recruited from the Birmingham-Metropolitan Area. NTE participants did not endorse any of the traumatic events listed in the PDS. All participants were screened for exclusion criteria prior to the study. Exclusion criteria included: history of blood or circulation disorders (e.g., anemia or sickle-cell), diabetes, brain or spinal abnormalities, pregnancy, previous or current head injury (e.g., traumatic brain injury, concussion, or loss of consciousness), or previous/current diagnosis of mental illness that began prior to the traumatic event. All participants provided written informed consent as approved by the University of Alabama at Birmingham Institutional Review Board.

Stimuli:

Conditioned stimulus (CS) presentations consisted of two pure tones (10 s duration; 700 Hz and 1300 Hz). One CS (CS+) co-terminated (24 trials at a 100% pairing rate) with the unconditioned stimulus (UCS; 100-dB white noise, 0.5 s duration), while the other CS (CS-) was presented (24 trials) without the UCS. In addition, the UCS was also presented alone (UCS alone) on 24 trials. A total of 72 trials (18 s inter-trial interval) were presented across two fMRI scans (36 trials per scan; 12 CS+, 12 CS-, 12 UCS alone trials). Stimuli were presented in a pseudorandom order such that no more than two trials of any stimulus (CS+, CS-, and UCS alone) were presented consecutively. The present study focused on brain and behavioral responses to CS presentations (i.e., CS+ and CS-). Responses to UCS presentations were not analyzed for the current study as they were included to address questions related to predictable and unpredictable threat that are not the focus of the present report (Goodman, Harnett, & Knight, 2018; Knight, Waters, King, & Bandettini, 2010; Wood, Ver Hoef, & Knight, 2012).

UCS Expectancy:

A UCS (i.e., threat) expectancy rating scale was presented on an IFIS-SA LCD (Invivo Corp.; Gainesville, FL) video screen using Presentation software (Neurobehavioral Systems, Inc.; Albany, CA). The screen was located above the participant's head and viewed through a mirror attached to the head coil. An MRI compatible joystick (Current Designs; Philadelphia, PA) was used to monitor participants' expectancy of UCS presentation on a moment-by-moment basis throughout the scan (i.e., continuously during both ITI and CS presentations). The joystick controlled a rating bar that was presented throughout the session on the video screen. Each participant was instructed to rate their UCS expectancy on a continuous scale from 0 to 100 (0 = certain the UCS would not be presented, 50 = uncertain whether the UCS would be presented, 100 = certain the UCS would be presented). UCS expectancy was calculated as the average response during the second (40 Hz sample rate) prior to UCS onset. Additional details on this methodology have been published previously (Knight & Wood, 2011).

Skin Conductance Response:

An MRI compatible physiological monitoring system (Biopac Systems; Goleta, CA) was used to collect SCR data. SCR was sampled (10 kHz) with a pair of disposable radio-translucent electrodes (1 cm diameter, Biopac Systems; Goleta, CA) from the thenar and hypothenar eminences of the nondominant hand. SCR data were processed using Biopac AcqKnowledge 4.1 software. A 1 Hz low pass digital filter was applied and SCR data were resampled at 250 Hz. Conditioned SCRs were assessed from the phasic increase in conductance during the 10 s CS presentation. Responses smaller than 0.05 μ S were assigned a value of zero. SCR data were square-root transformed prior to statistical analyses. SCR data for two TE participants were not collected due to injuries (e.g., burn) that prevented the use of psychophysiological monitoring sensors. Thus, 18 TE participants were used in group-level SCR data analyses.

Posttraumatic Diagnostic Scale:

Participants completed the PDS, a self-report assessment of stress disorder symptoms that assessed re-experiencing, avoidance, and arousal symptoms (Foa, 1995; Foa, Cashman, Jaycox, & Perry, 1997). NTE control participants were screened for any exposure to a traumatic event as defined by the PDS. The traumatic events listed on the PDS include serious accident, natural disaster, nonsexual or sexual assault, military combat, life threatening illness, torture, or other traumatic event. No NTE participants endorsed experiencing a traumatic event on the PDS within their lifetime and all subsequent items were scored as 0 (i.e., participants had no posttraumatic stress symptoms as there was no trauma). Total scores on the PDS (reflecting total PTSD symptom severity) have a range from 0 to 51. Participants also completed the PDS a second time 3 months after trauma exposure. Scores from the PDS were compared between the TE and NTE groups to confirm differences in posttraumatic stress symptoms (Table 2).

Psychosocial Risk Factor Survey:

Participants completed the Psychosocial Risk Factor Survey (PRFS) to assess levels of depression, anxiety, anger/hostility, social isolation, and emotional guardedness (Eichenauer, Feltz, Wilson, & Brookings, 2010). Total and subscale (i.e., depression, anxiety, anger/hostility, social isolation, and emotional guardedness) PRFS scores were compared between TE and NTE groups (Table 3).

Wechsler Test of Adult Reading:

Participants completed the Wechsler Test of Adult Reading (WTAR) to estimate a Full Scale Intelligence Quotient (FSIQ) (Wechsler, 2001). One TE participant did not complete the WTAR. FSIQs were compared between the TE and NTE groups.

Magnetic Resonance Imaging:

Structural and functional imaging data were acquired on a 3 Tesla Siemens Allegra scanner. High resolution anatomical magnetization prepared rapid acquisition gradient echo (MPRAGE) images were obtained in the sagittal plane using a T1 weighted series (TR=2300 ms, TE=3.9 ms, flip angle=12°, FOV=25.6 cm, matrix=256×256, slice thickness=1 mm, 0.5

mm gap) to serve as an anatomical reference. Whole-brain blood-oxygen-level-dependent (BOLD) fMRI was completed using a gradient-echo echoplanar pulse sequence in an oblique-axial orientation (TR=2000 ms, TE=30 ms, flip angle=70°, FOV=24 cm, matrix=64×64, slice thickness=4 mm, no gap) during each block of stimulus presentations. Functional image processing was performed with the Analysis of Functional NeuroImages (AFNI) software package (Cox, 1996). The first 2 volumes of fMRI data were discarded due to transient magnetization effects. Echo-planar time series data were corrected for slice timing offset, motion corrected, concatenated, reregistered to the fifth volume of the first imaging block, and spatially smoothed using a 4 mm full-width-at-half-maximum Gaussian kernel.

Functional MRI data were analyzed at the individual subject level using multiple linear regression (i.e., 3dDeconvolve). Brain activity was modeled by convolving a Gamma variate hemodynamic response function with reference waveforms reflecting the onset of CS+ and CS− presentations. Regressors of no interest were modeled with a Gamma variate hemodynamic response function to account for brain activity not related to the CS+ or CS−, and included reference waveforms for UCS presentations and joystick movement. Six motion parameters (i.e., three displacement and three rotation parameters) were included as additional regressors of no interest to account for participant head motion. Time-series volumes with high motion (i.e., more than 3% of voxels were outliers defined by 3dToutcount) were censored from the first-level regression analyses. Percent signal change was used as an index of the amplitude of the fMRI signal response to stimulus presentation. Spatial maps of the percent signal change were normalized to the Talairach and Tournoux stereotaxic coordinate system (Talairach & Tournoux, 1988) and resampled to 1 mm³ resolution. Functional MRI data for one TE participant was corrupted by irreconcilable artifact and was excluded from fMRI data analyses. Therefore, 19 TE participants were included in group level fMRI data analyses.

Group analyses of the fMRI data were completed using a linear mixed-effects model (i.e., 3dLME) (Chen, Saad, Britton, Pine, & Cox, 2013). General linear tests (z-tests) within the model were used to test *a priori* contrasts of interest that included CS+ > CS−, CS− > CS+, TE versus NTE, and the interaction between stimulus type and group. The analysis was restricted using an anatomical mask that included the amygdala, hippocampus, and cortical gray matter. Cluster correction methods were used to maintain $\alpha = 0.05$ (i.e., multiple comparisons corrected). Specifically, Monte Carlo simulations were implemented in 3dClustSim to determine the corresponding cluster extent volume (determined as $k = 529$ and $k = 434$ for one and two-tailed tests respectively) at a cluster forming threshold of $p = 0.005$ to maintain $\alpha = 0.05$. Estimates of random noise smoothness were generated from the spatial autocorrelation function derived from the residuals in the first-level model (i.e., the -acf option was used in 3dFWHMx and the resulting group-level parameters were used in 3dClustSim to generate random noise fields). However, the amygdala often does not meet whole-brain cluster correction thresholds due to its relatively small size in comparison to corrected cluster extent volumes. Therefore, given our *a priori* hypothesis about the amygdala, the amygdala was also included in an anatomical region of interest (ROI) analysis whereby the percent fMRI signal change to the CS+ and CS− was extracted and used in a separate repeated-measures analysis of variance (ANOVA).

Statistical Analyses:

Statistical analyses were completed using SPSS Statistics Version 24 (IBM Corp., Armonk, NY). Demographic and self-report data were compared between the groups using independent-samples t-tests and Chi-square tests as appropriate. We completed ANOVAs to tests effects of stimulus type, group, and the interaction on UCS expectancy and SCR. Follow-up analyses on fMRI data were also completed using SPSS by extracting the average fMRI signal (% change) over significant clusters of activation. We completed ANOVAs, analyses of covariance (ANCOVAs), and multivariate analyses of covariance (MANCOVAs) to assess relationships between fMRI signal responses, behavioral, and self-report data. A nominal significance threshold of $p < 0.05$ was used.

3. Results

3.1 Participant demographics

We first compared the TE and NTE groups on demographic variables (Table 1). The demographics of both the TE and NTE groups were similar and FSIQ scores were not significantly different (all $p > 0.05$). However, TE participants were older than NTE participants ($p < 0.05$). TE participants also reported greater posttraumatic stress symptoms than the NTE participants (Table 2). Specifically, total, re-experiencing, avoidance, and arousal scores on the PDS were greater for the TE than NTE group. The criteria for PTSD on the PDS requires participants to endorse each of the main symptom dimensions (re-experiencing, avoidance, and arousal symptoms), significant distress/impairment, and a duration of symptoms greater than 1 month. Of the 20 TE participants in the present study, 4 TE participants met criteria for preclinical PTSD (i.e., acute stress disorder; endorsement of all PTSD symptom dimensions and significant distress that lasted less than one month) at the < 1 month assessment (assessed via the PDS). At the 3 month assessment, these 4 TE participants still met criteria for PTSD as did 1 other TE participant (i.e., 5 TE participants met criteria for PTSD at the 3 month assessment).

The present study assessed PDS scores at < 1 month and 3 months post-trauma. Six TE participants did not complete the 3 month assessment and were therefore not included in analyses assessing the relationship between future posttraumatic stress severity and brain or behavioral measures. There were no demographic or behavioral differences between participants that completed follow-up ($n = 14$) compared to those that did not ($n = 6$; Table S2). The total PDS scores for the 14 participants that completed follow-up did not significantly differ ($p > 0.05$), as a group, between the < 1 month ($M = 15.29$, $SD = 10.46$) and 3 month follow-up ($M = 13.29$, $SD = 12.91$) assessments. In addition, total PDS scores at the < 1 month assessment were positively correlated with PDS scores at the 3 month follow-up [$r = 0.70$, $p < 0.01$]. These findings suggest that PDS scores did not change for the TE group. However, some participants' PDS scores increased, while other participants' scores decreased such that, as a group, scores remained unchanged despite significant individual variability (i.e., within-subject) in PDS scores over time. Therefore, the absolute value of the difference in PDS scores from < 1 to 3 months was included in a one-sample t-test against zero (i.e., no change in PDS score). The analysis revealed significant within-subject variability in PDS scores over time [$t(13) = 4.21$, $p < 0.005$]. Specifically, some

participants showed improvement while others showed worsening of their symptom expression on the PDS between the < 1 and 3 month assessments (five participants worsened, seven participants improved, and two participants did not change over time). On average, PDS scores varied by 7.00 (SD = 6.23) points from < 1 to 3 months. These findings indicate there was significant individual variability in PDS scores between the < 1 and 3 month assessments.

3.2 UCS Expectancy

A repeated-measures ANOVA containing a within-subjects factor of stimulus type (CS+ versus CS-) and a between-subjects factor of group (TE versus NTE) was completed. The analysis did not demonstrate a main effect of stimulus type [$F(1,37) = 3.01, p > 0.05$] or group [$F(1,37) = 1.10, p > 0.05$]. However, a significant stimulus type by group interaction was observed [$F(1,37) = 5.66, p < 0.05$] (Figure 1). Post-hoc comparisons revealed NTE participants demonstrated greater expectancy [$t(18) = 2.20, p < 0.05$] during the CS+ (M = 69.31, SEM = 4.85) than the CS- (M = 55.51, SEM = 5.09). In contrast, there was no difference in expectancy [$t(19) = -0.80, p > 0.05$] during the CS+ (M = 67.06, SEM = 4.57) and CS- (M = 69.23, SEM = 3.59) within the TE group. Further, UCS expectancy was greater in the TE group than the NTE group during CS- trials [$t(37) = 2.22, p < 0.05$], but not CS+ trials [$t(37) = -0.34, p > 0.05$]. Taken together, these data suggest the TE group expected the UCS during both the CS+ and CS- trials, while the NTE group only expected the UCS on CS+ trials.

The prior results suggested UCS expectancy differed between the TE and NTE groups. We next investigated whether UCS expectancy varied with either acute (i.e., < 1 month) or future (i.e., 3 months) posttraumatic stress symptom severity within the TE group. UCS expectancy was included as the dependent variable in a repeated-measures ANCOVA that included a within-subjects factor of stimulus type (CS+ versus CS-) and a continuous covariate of either acute or future PDS scores (two separate models). No significant main effects or interactions were observed (all $p > 0.05$).

3.3 Skin Conductance Response

A repeated-measures ANOVA containing a within-subjects factor of stimulus type (CS+ versus CS-) and a between-subjects factor of group (TE versus NTE) was completed. The analysis did not reveal significant main effects for stimulus type [$F(1,35) = 0.06, p > 0.05$] or group [$F(1,35) = 0.31, p > 0.05$], or a significant stimulus type by group interaction [$F(1,35) = 0.40, p > 0.05$].

3.4 Functional Imaging

Voxel-wise analysis of the fMRI data was completed using 3dLME and general linear tests were completed to test *a priori* contrasts of interests (Table 4) (Figure 2). Significant differential activation (i.e., CS+ > CS-) was observed within the dorsolateral PFC (Figure 3), dorsomedial PFC, superior frontal gyrus (SFG), ventrolateral PFC, and inferior parietal lobule (IPL). There were no regions that showed greater activity during CS- presentations than CS+ presentations. The group contrast (i.e., TE versus the NTE) revealed significant differences in brain activity within the dorsolateral PFC, dorsomedial PFC, SFG, precentral

gyrus, precuneus, superior temporal gyrus (STG), cuneus, insula, superior parietal lobule, and IPL. Across these brain regions, the TE group demonstrated significantly greater fMRI signal responses to CS presentations than the NTE group. Finally, the stimulus by group interaction contrast revealed significant effects within the ventrolateral PFC, STG, and temporal pole. The TE group demonstrated greater fMRI signal responses to the CS+ than the CS- within the ventrolateral PFC [$t(18) = 2.62, p < 0.05$], STG [$t(18) = 2.91, p < 0.01$], and the temporal pole [$t(18) = 2.74, p < 0.05$]. However, the NTE group demonstrated greater fMRI signal responses to the CS- than the CS+ within the ventrolateral PFC [$t(18) = -3.16, p < 0.01$], STG [$t(18) = -2.71, p < 0.05$], and temporal pole [$t(18) = -4.10, p < 0.005$]. Taken together, these data suggest the TE group had greater neural reactivity to conditioned stimuli than the NTE group. Further, the TE group showed altered conditioned responses (CRs) to CS+ and CS- presentations compared to the NTE group.

Given our *a priori* hypotheses about the role of the amygdala in Pavlovian fear conditioning, an anatomical ROI-based analysis was completed to investigate the relationship between amygdala activity, trauma exposure, and fear learning. Bilateral amygdala activity was extracted and used as the dependent variable in an ANOVA with a within-subjects measure of stimulus type (CS+ versus CS-), a within-subjects measure of brain hemisphere (left and right), and a between-subjects measure for group (TE versus NTE). No main effects or interactions for hemisphere were observed (all $p > 0.05$). Further, no main effect of group on amygdala activity was observed [$F(1,36) = 0.12, p > 0.05$]. However, we did observe a significant main effect of stimulus type [$F(1,36) = 5.43, p < 0.05$] such that amygdala activation was greater during CS+ than CS- trials. We also observed a significant stimulus type by group interaction such that amygdala activity to the CS+ and CS- varied with trauma exposure [$F(1,36) = 9.34, p < 0.005$]. Post-hoc paired t-test comparisons revealed greater amygdala activity during the CS+ compared to the CS- [$t(18) = 3.49, p < 0.005$] within the TE group, but no difference was observed within the NTE group [$t(18) = -0.57, p > 0.05$]. Interestingly, while the amygdala response to CS+ was greater in the TE than NTE group [$t(36) = 2.31, p < 0.05$], the amygdala response to CS- did not differ between groups [$t(36) = -1.63, p > 0.05$]. These data suggest trauma exposure facilitates differential fear conditioning within the amygdala.

The prior analyses demonstrated differences in neural activity between stimulus type (i.e., CS+ versus CS-) and group (i.e., TE versus NTE). Further, our behavioral data demonstrated the TE and NTE groups differed in UCS expectancy during CS- trials. Therefore, we investigated whether the TE and NTE groups differed in the relationship between neural activity and UCS expectancy during CS- trials. The dependent variables (i.e., fMRI signal responses from regions that showed a CS+ > CS- effect) reflected the same underlying process (i.e., neural response to safety cues). Therefore, we tested a multivariate hypothesis that neural responses to safety would vary by group and UCS expectancy. A MANCOVA was completed that included a between-subjects factor of group and a continuous covariate of UCS expectancy during CS- presentations. The fMRI signal responses to the CS- from brain regions (i.e., the bilateral dorsolateral PFC, dorsomedial PFC, bilateral ventrolateral PFC, left SFG, and left IPL) that showed a stimulus effect (i.e., CS+ > CS-) in the voxel-wise analysis were included as multiple dependent variables. Although the MANCOVA did not reveal a main effect of UCS expectancy [$F(7,28) = 1.65, p$

> 0.05, Wilk's $\lambda = 0.71$], a significant main effect of group [$F(7,28) = 3.45$, $p < 0.01$, Wilk's $\lambda = 0.54$] was observed. Follow-up univariate ANCOVAs revealed the group effects were present within dorsomedial PFC [$F(1,34) = 9.90$, $p < 0.005$] and left ventrolateral PFC [$F(1,34) = 4.45$, $p < .05$]. The MANCOVA also revealed a significant group by UCS expectancy interaction [$F(7,28) = 3.00$, $p < 0.05$, Wilk's $\lambda = 0.57$]. Follow-up univariate ANCOVAs revealed the interaction was only present within the dorsomedial PFC [$F(1,34) = 6.78$, $p < 0.05$] (Figure 4). Within the dorsomedial PFC, TE participants demonstrated a significant negative ($r = -0.47$, $p < 0.05$) relationship between the fMRI signal response and UCS expectancy during CS- trials, while no relationship was observed for NTE participants ($r = 0.32$, $p > 0.05$). Taken together, these data suggest the dorsomedial PFC may underlie group differences in UCS expectancy during CS- presentations.

Findings from the prior analyses suggested there were differences in neural processing between the TE and NTE groups. We next sought to determine if brain activity within the TE group was related to acute (i.e., < 1 month post-trauma) or future (i.e., 3 months post-trauma) posttraumatic stress severity. We completed repeated-measures ANCOVAs to test the relationship between brain activity and acute/future posttraumatic stress and whether the relationship was moderated by brain region or stimulus type. Specifically, the data from the seven regions of activation (i.e., the bilateral dorsolateral PFC, dorsomedial PFC, bilateral ventrolateral PFC, left SFG, and left IPL) that showed greater fMRI signal responses to the CS+ than CS- were used as dependent variables in the repeated-measures ANCOVA. The ANCOVA included within-subjects factors for stimulus type (CS+ versus CS-) and brain region (seven levels) in addition to a continuous covariate for either acute or future PDS scores (two separate models). In the first model (i.e., < 1 month PDS scores included as a covariate), we observed no significant effects (all $p > 0.05$) indicating neural activity did not vary with acute posttraumatic stress severity. In the second model (i.e., 3 month PDS score included as a covariate), we observed a significant main effect of 3 month posttraumatic stress severity on brain activity [$F(1,11) = 5.11$, $p < 0.05$]. Specifically, the average fMRI signal responses across these brain regions varied inversely with 3 month PDS scores ($r = -0.56$, $p < 0.05$). Thus, TE participants with greater fMRI signal responses within the bilateral dorsolateral PFC, dorsomedial PFC, bilateral ventrolateral PFC, left SFG, and left IPL showed diminished 3 month posttraumatic stress severity. Given the repeated-measures ANCOVA revealed a main effect of 3 month PDS scores, the average fMRI signal response (i.e., the average response to both CS across regions) was included in follow-up analyses with 3 month PDS subscale scores. Followup exploratory correlation analyses demonstrated the fMRI signal response varied with 3 month arousal severity ($r = -0.76$, $p < 0.005$), but not re-experiencing severity ($r = -0.32$, $p > 0.05$) or avoidance severity ($r = -0.39$, $p > 0.05$). Exploratory follow-up correlation analyses were completed to determine the brain regions in which fMRI signal responses varied with the 3 month PDS scores. The analyses revealed the 3 month PDS scores significantly varied only with left IPL activity ($r = -0.64$, $p < 0.05$) (Table S3). In addition, the exploratory analyses showed PDS arousal scores were correlated with the fMRI signal response within the dorsomedial PFC, bilateral ventrolateral PFC, left SFG, and left IPL (Table S3). Taken together, these data suggest neural activity during the acute phase following trauma exposure varies with future posttraumatic stress severity.

The prior analyses suggested the fMRI signal response varied with 3 month PDS scores, but not < 1 month PDS scores. Therefore, we next sought to determine if the fMRI signal response was related to the change (from < 1 to 3 month assessments) in PDS scores. A repeated-measures ANCOVA that contained within-subjects factors for stimulus type (CS+ versus CS-) and brain region (seven levels), and a continuous covariate for the change in PDS scores (i.e., the difference in PDS scores between the < 1 month and 3 month assessments) was completed. No interactions between the change in PDS scores and either stimulus type or brain region were observed (all $p > 0.05$). However, we did observe a significant main effect of change in PDS scores [$F(1,11) = 4.98$, $p < 0.05$]. Specifically, the fMRI signal response across these brain regions (i.e., the bilateral dorsolateral PFC, dorsomedial PFC, bilateral ventrolateral PFC, left SFG, and left IPL) varied inversely with the change in PDS scores ($r = -0.56$, $p < 0.05$). Follow-up correlation analyses revealed that the fMRI signal response across these brain regions varied inversely with the change in avoidance ($r = -0.57$, $p < 0.05$) and arousal ($r = -0.63$, $p < 0.05$) scores, but not the change in re-experiencing ($r = -0.21$, $p > 0.05$) scores. Exploratory follow-up correlation analyses were completed to determine which of the brain regions showed fMRI signal responses that varied with the change in PDS scores. The analyses revealed the change in total PDS scores varied with both left ($r = -0.67$, $p < 0.05$) and right ($r = -0.60$, $p < 0.05$) ventrolateral PFC activity (Table S4). The exploratory analyses also revealed significant correlations between bilateral ventrolateral PFC activity and the change in PDS avoidance and arousal scores (Table S4).

As described above, the fMRI signal response varied with both 3 month and the change in posttraumatic stress severity. However, it was unclear if neural activity varied with affective dysfunction more broadly. Thus, we investigated the relationship between the fMRI signal response and total and subscale PRFS scores. Given that the prior analysis revealed a main effect of 3 month PDS scores (i.e., a relationship between the PDS scores and the fMRI signal response), the fMRI signal response was included in a correlation analysis with PRFS scores. No significant correlation was observed between total PRFS score and the fMRI signal response within the TE and NTE groups separately (all $p > 0.05$). Across all participants (i.e., TE and NTE combined), a positive correlation was observed between the fMRI signal response and PRFS anxiety ($r = 0.34$, $p < 0.05$) and PRFS anger/hostility ($r = 0.35$, $p < 0.05$) scores.

4. Discussion

Determining the acute effects of trauma exposure on neural function is crucial for improving our understanding of the etiology of PTSD. New insights into the etiology of PTSD would advance efforts to improve diagnostic techniques and develop early intervention tools. Therefore, the present study investigated the acute effects of trauma exposure on brain function by recruiting recently TE (< 1 month post-trauma) and NTE participants to complete a Pavlovian fear conditioning task during fMRI. The TE group showed greater threat (i.e., UCS) expectancy during safety cues (i.e., CS-) than the NTE group. These differences in threat expectancy varied with dorsomedial PFC activity. Further, neural activity within the dorsolateral PFC, dorsomedial PFC, ventrolateral PFC, SFG and IPL varied inversely with posttraumatic stress symptom severity at follow-up (i.e., 3 months

post-trauma). The current results suggest that trauma exposure acutely impacts the neural circuitry that supports important fear learning and memory processes.

The current findings demonstrate trauma exposure acutely disrupts associative fear learning. In the present study, the TE group expected threat during both warning (i.e., CS+) and safety (i.e., CS-) cues, while threat (i.e., UCS) expectancy in the NTE group showed a more typical pattern with greater expectancy during the warning than safety cue. Further, the TE group had greater threat expectancy during safety cues than the NTE group. These findings demonstrate both the TE and NTE group formed a fear association between the warning cue and threat (i.e., the CS-UCS association). However, the TE group overgeneralized that fear association, resulting in greater threat expectations during the safety cue. Previous research has demonstrated that prior stress exposure facilitates the generalization of learned fear in healthy individuals (Dunsmoor et al., 2017). Thus, the present findings suggest trauma exposure facilitates fear generalization processes. Interestingly, chronic PTSD patients also show fear overgeneralization as evidenced by heightened emotional responses and threat expectations during safety cues (Grillon & Morgan, 1999; Jovanovic et al., 2010; Rabinak, Mori, Lyons, Milad, & Phan, 2017). Taken together trauma exposure appears to acutely disrupt fear learning processes and lead to the fear overgeneralization observed in chronic PTSD.

The dorsomedial PFC appears to mediate fear overgeneralization following trauma exposure. In the present study, the fMRI signal response to the safety cue within the dorsomedial PFC varied negatively with threat expectancy in the TE group. The dorsomedial PFC supports fear learning and memory processes (Buchel, Morris, Dolan, & Friston, 1998; Etkin, Egner, & Kalisch, 2011; Knight, Cheng, Smith, Stein, & Helmstetter, 2004; Milad et al., 2007), and plays an important role in the generalization of fear associations in both healthy individuals and PTSD patients (Dunsmoor, Prince, Murty, Kragel, & LaBar, 2011; Kaczurkin et al., 2017; Lange et al., 2017; Lissek et al., 2014). Notably, dorsomedial PFC responses to the warning cue emerge once the warning cue-threat association has been learned (Maier et al., 2012). Further, prior work suggests the dorsomedial PFC promotes, rather than inhibits, conditioned fear responses (Milad et al., 2007). Thus, following acquisition of the warning cue-threat association, greater dorsomedial PFC activity should reflect facilitation of fear learning. Therefore, if TE participants generalize the fear associated with the warning cue to the safety cue, then one might predict a positive relationship should be observed between threat expectancy and dorsomedial PFC responses to safety cues. However, the TE group in the present study showed a negative relationship between dorsomedial PFC activity and threat expectancy during safety cues. Interestingly, the NTE group showed a significantly different pattern, which more closely approximates what may be hypothesized from prior work (i.e., a positive brain-behavior relationship). Thus, our results suggest that there is an acute effect of trauma exposure that modulates the function of the dorsomedial PFC in regards to threat expectancy. Recent research demonstrates acute stress impacts neural activity within the dorsomedial PFC (Wheelock et al., 2016), which has subsequent effects on fear learning-related activity within this region of the brain (Goodman, Harnett, Wheelock, et al., 2018). Thus, acute posttraumatic stress may alter cognitive functions supported by the dorsomedial PFC that lead to changes in fear learning processes. Importantly, the dorsomedial PFC supports error detection, conflict

monitoring, and selective attention processes (Carter et al., 1998; Carter & van Veen, 2007; Goodman, Harnett, & Knight, 2018; MacDonald, Cohen, Stenger, & Carter, 2000; Weissman, Gopalakrishnan, Hazlett, & Woldorff, 2004). Therefore, we speculate that greater dorsomedial PFC activity within the TE group may reflect the engagement of cognitive processes that reduce fear generalization to safety cues. Specifically, TE individuals may be primed to overgeneralize learned fear associations to non-threatening stimuli (i.e., the CS-). Greater engagement of dorsomedial PFC functions (e.g., error detection, conflicting monitoring, and attentional processes) may underlie better discrimination of safety from warning cues to avoid fear overgeneralization. In contrast, the failure to engage the dorsomedial PFC may lead to the overgeneralization of fear to non-threatening stimuli and play an important role in the future development of chronic PTSD.

The amygdala is another region that plays a critical role in fear learning and memory. The amygdala is important for the formation of CS-UCS associations and expression of the peripheral emotional response (Buchel et al., 1998; Cheng et al., 2006; Cheng et al., 2003; Knight et al., 2005; LaBar et al., 1998). Prior research demonstrates stress exposure facilitates amygdala-mediated fear learning processes (Shors et al., 1992). Interestingly, we observed a significant stimulus type (CS+ versus CS-) by group (TE versus NTE) interaction within the amygdala in the present study. Specifically, amygdala fMRI signal responses to the warning versus the safety cue were greater in the TE than NTE group. Thus, trauma exposure led to greater differential amygdala activity during the fear conditioning procedure. Importantly, while the amygdala response to the warning cue was greater in the TE than NTE group, amygdala reactivity to the safety cue did not differ between groups. Thus, our findings suggest trauma exposure enhances amygdala reactivity to signals that warn of impending threat, but does not lead to indiscriminate amygdala reactivity. These findings suggest that the amygdala does not underlie the fear overgeneralization observed within the TE group in the present study. Given that the amygdala is essential for the acquisition and expression of fear memories (Campeau & Davis, 1995; Cheng et al., 2006; Helmstetter, 1992; Hitchcock & Davis, 1986; LaBar et al., 1998), heightened amygdala reactivity to the safety cue would be expected if this region mediated the fear generalization observed in the TE group. In fact, prior research has found the amygdala underlies expression of generalized fear in healthy adults (Dunsmoor et al., 2011). However, amygdala reactivity to the safety cue did not differ between TE and NTE groups as would be expected if it mediated fear overgeneralization. Thus, amygdala reactivity to the safety cue cannot explain the generalization of threat expectancy observed in the present study. Instead, these findings suggest that the fear overgeneralization observed in the TE group must be mediated by a different neural mechanism. The dorsomedial PFC findings discussed above suggest that dysfunctional cognitive processes supported by the dorsomedial PFC underlie the fear overgeneralization observed in the present study.

The current findings suggest neural activity measured acutely following trauma exposure predicts future PTSD symptom severity (i.e., both symptom severity at 3 months and the change in symptom severity from < 1 to 3 months). Specifically, we observed an inverse relationship between fMRI signal responses (i.e., across the dorsolateral PFC, dorsomedial PFC, ventrolateral PFC, SFG, and IPL) and total PDS scores during the 3 month assessment as well as the change from < 1 to 3 months. Given fMRI was completed months prior to the

3 month PTSD symptom assessment, the results suggest greater engagement of these brain regions acutely following trauma attenuates future PTSD development. A growing body of work has begun to characterize how neural activity following trauma relates to future PTSD in civilians (Stevens et al., 2017; van Rooij et al., 2017). This prior research has demonstrated activity within the amygdala, hippocampus, and PFC is tied to later development of PTSD symptoms. For example, human amygdala reactivity varies positively with future PTSD symptoms, while the habituation of ventromedial PFC activity varies negatively with future PTSD symptoms (Stevens et al., 2017). Further, inhibition-related activity within the PFC and hippocampus varies negatively with future PTSD symptoms (van Rooij et al., 2017). In the present study, we found that CS-elicited fMRI signal responses across the dorsolateral PFC, ventrolateral PFC, dorsomedial PFC, SFG, and IPL were negatively associated with future PTSD symptoms. Interestingly, the relationship between the fMRI signal response and PDS scores did not vary by stimulus type (i.e., CS+ versus CS-) in the present study. Although one might expect that posttraumatic stress symptoms should have a different impact on the brain's response to warning and safety cues, the current findings suggest that is not the case. Instead, our findings suggest posttraumatic stress symptoms modulate the neural response to important events (e.g., warning and safety cues) regardless of their intended emotional valence. This finding is consistent with the threat expectancy results from the present study, which suggest that individuals exposed to trauma overgeneralize fear associations from warning to safety cues. In combination with prior research (Stevens et al., 2017; van Rooij et al., 2017), our findings suggest that key brain regions, that support emotional learning and memory processes, partially underlie the future development of PTSD.

Interpretations from the present study should be tempered by several limitations. First, a relatively small sample was available for the initial and follow-up analyses. The limited sample size may affect the generalizability of the current findings. Second, only one imaging session was performed, which limits our ability to determine how functional brain activity may change over time. Specifically, brain activity during fear conditioning may be facilitated acutely following stress but may become attenuated over time. Assessing changes in brain activity following trauma that develop over time may further elucidate the neural processes that mediate the development of PTSD. Future work employing a longitudinal study design may be able to better disentangle interactions between time and neural activity that underlie the development of PTSD.

In conclusion, the present study used fMRI to investigate the neural substrates of Pavlovian fear conditioning in individuals recently exposed to trauma. The TE group demonstrated fear overgeneralization to safety cues. Fear overgeneralization appeared to be mediated by activity within the dorsomedial PFC. Further, engagement of the dorsolateral PFC, dorsomedial PFC, ventrolateral PFC, SFG, and IPL varied with PTSD symptom severity (i.e., PTSD symptoms 3 months post-trauma and the change in PTSD symptoms over time). Together, the current findings suggest recently traumatized individuals may be susceptible to disruptions in fear learning and memory processes that are mitigated by greater engagement of the dorsolateral PFC, dorsomedial PFC, ventrolateral PFC, SFG, and IPL. Failure to engage these regions following trauma exposure may facilitate future development of PTSD symptoms. These findings elucidate the acute effects of trauma exposure on the neural

substrates of fear learning and provide new insight into the neural mechanisms underlying PTSD susceptibility.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Trauma exposure and UCS expectancy. Trauma-Exposed (TE) participants showed safety learning deficits. Specifically, participants in the TE group expected the UCS during CS+ (i.e., warning signals) and CS- (i.e., safety signals) trials. In comparison, the Non-Trauma-Exposed (NTE) group showed greater UCS expectancy during CS+ than CS- trials. The TE group showed greater UCS expectancy during CS- trials than the NTE group. The graph depicts UCS expectancy during the CS+ (black bars) and CS- (gray bars) for the TE (left) and NTE (right) groups. Error bars reflect standard error of the mean. Asterisk indicates a significant difference at a $p < 0.05$ level.

Figure 2. Neural activation during Pavlovian fear conditioning. Significant activation was identified in several brain regions reflecting an effect of stimulus-type (CS+ > CS-) (red), an effect of group (Trauma-Exposed versus Non-Trauma-Exposed) (blue), and a stimulus type by group interaction (green).

Figure 3.

Neural reactivity to CS+ and CS- presentations. Both the Trauma-Exposed (TE) and Non-Trauma-Exposed (NTE) groups demonstrated greater fMRI signal responses to the CS+ and CS- in several brain regions. The TE group showed greater fMRI signal responses to both the CS+ and CS- (i.e., the average response) than the NTE group. The graphs depict the percent fMRI signal response during the CS+ (black bars) and CS- (gray bars) for the TE and NTE groups. Error bars reflect standard error of the mean. Asterisk indicates a significant difference at a $p < 0.05$ level.

Figure 4.

Brain-behavior relationship and trauma exposure. Trauma-Exposed (TE) and Non-Trauma-Exposed (NTE) groups differed in the relationship between UCS expectancy and the fMRI signal response to the CS– (i.e., safety cue) within the dorsomedial prefrontal cortex. While the TE group showed a significant negative relationship between brain activity and UCS expectancy, the NTE group showed no significant relationship. The graph depicts the percent fMRI signal response to the CS– and a median split (for illustrative purposes) of UCS expectancy to the CS– for the TE (black triangles) and NTE (gray circles) groups. Error bars reflect standard error of the mean.

Table 1.

Participant demographics.

	TE	NTE	p-value
Age	29.65 (9.11)	24.26 (3.43)	< 0.05
Race	9 AA/11 EA	9 AA/10 EA	n.s.
Sex	6 F/14 M	5 F/14 M	n.s.
Handedness	18 R/2 L	17 R/2 L	n.s.
FSIQ	91.74 (13.02)	98.74 (14.87)	n.s.

Note: FSIQ = Full Scale Intelligence Quotient; EA = European-American; AA =African-American; M = Male; F = Female; R = Right; L = Left. Values for Trauma-Exposed (TE) and Non-Trauma-Exposed (NTE) groups are presented as: Mean (Standard Deviation).

Table 2.

Posttraumatic stress symptoms.

	TE	NTE	p-value
PDS Total Severity (< 1 month)	13.90 (10.09)	0 (0)	< 0.001
<u>Re-experiencing Severity</u>	4.35 (3.79)	0 (0)	< 0.001
<u>Avoidance Severity</u>	4.25 (3.77)	0 (0)	< 0.001
<u>Arousal Severity</u>	5.30 (4.18)	0 (0)	< 0.001
PDS Total Severity (3 month)	13.29 (12.91)	.	.
<u>Re-experiencing Severity</u>	4.00 (4.08)	.	.
<u>Avoidance Severity</u>	4.07 (4.87)	.	.
<u>Arousal Severity</u>	5.21 (5.25)	.	.

Note: PDS = Posttraumatic Diagnostic Scale. Values for Trauma-Exposed (TE) and Non-Trauma-Exposed (NTE) groups are presented as: Mean (Standard Deviation).

Table 3.

Psychosocial risk factor survey.

	TE	NTE	p-value
PRFS Total Score	108.35 (30.41)	97.89 (27.73)	n.s.
<u>Depression</u>	16.75 (8.96)	16.26 (11.04)	n.s.
<u>Anxiety</u>	19.75 (12.13)	14.21 (7.47)	n.s.
<u>Anger/Hostility</u>	26.70 (8.31)	21.21 (8.69)	n.s.
<u>Social Isolation</u>	18.90 (8.52)	19.89 (8.82)	n.s.
<u>Emotional Guardedness</u>	26.25 (3.73)	26.32 (4.80)	n.s.

Note: PRFS = Psychosocial Risk Factor Survey. Values for Trauma-Exposed (TE) and Non-Trauma-Exposed (NTE) groups are presented as: Mean (Standard Deviation).

Table 4.

Regions showing significant activation.

Structure	Hemisphere	Z-Statistic	Cluster (mm ³)	Talairach Coordinates (X,Y,Z)		
<u>CS+ > CS−</u>						
Dorsolateral PFC	Left	3.94	1488	−35	37	31
	Right	3.61	1035	40	41	15
Dorsomedial PFC	Bilateral	2.95	531	−4	20	30
SFG	Left	4.11	874	−11	−7	63
Ventrolateral PFC	Left	3.89	596	−47	5	8
	Right	3.90	1393	50	7	13
IPL	Left	4.03	1810	−55	−38	34
<u>TE versus NTE</u>						
Dorsolateral PFC	Right	3.78	445	43	33	20
Dorsomedial PFC	Bilateral	3.84	3174	4	−3	51
SFG	Right	4.40	2035	15	−15	64
Precentral Gyrus	Left	4.14	641	−30	−15	54
Precuneus	Left	3.43	639	−11	−80	44
SPL	Left	5.40	7000	−23	−55	54
IPL	Left	4.63	1218	−44	−35	23
	Right	4.10	2582	44	−40	49
	Right	3.98	845	49	−26	21
Insula	Left	4.11	1286	−33	−3	11
	Left	3.91	584	−35	−19	9
STG	Left	4.35	967	−59	−13	−1
	Right	5.13	2806	61	−7	1
Cuneus	Left	4.04	574	−11	−98	2
<u>Stimulus x Group</u>						
Ventrolateral PFC	Left	4.03	708	−45	29	2
STG	Right	4.47	502	48	−5	−10
Temporal Pole	Left	5.13	439	−49	4	−31

Location, Z-Statistic, volume, and Talairach and Tournoux (1988) coordinates of the peak voxel for significant ($\alpha = 0.05$; corrected) areas of activation. NTE = Non-Trauma-Exposed, TE = Trauma-Exposed, CS = Conditioned Stimulus, PFC = Prefrontal Cortex, SFG = Superior Frontal Gyrus, IPL = Inferior Parietal Lobule, SPL = Superior Parietal Lobule, STG = Superior Temporal Gyrus.