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Fear-potentiated Startle During Extinction is Associated with White Matter Microstructure and Functional Connectivity

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Abstract

Background—Extinction of conditioned fear is an associative learning process that involves communication among the hippocampus, medial prefrontal cortex, and amygdala. Strength of connectivity between the hippocampus and the anterior cingulate cortex (ACC), and between the amygdala and ventromedial prefrontal cortex (vmPFC), may influence fear-potentiated startle (FPS) responses during extinction. Specific white matter tracts, the cingulum and uncinate fasciculus (UF), serve as primary routes of communication for these areas. Our objective was to investigate associations between FPS during extinction and cingulum and UF connectivity.

Method—Diffusion tensor imaging (DTI) and probabilistic tractography analyses were used to examine cingulum and UF structural connectivity in 40 female African-Americans with psychological trauma exposure. FPS responses during fear conditioning and extinction were assessed via EMG of the right orbicularis oculi muscle. Secondarily, functional connectivity analyses were performed with the seed ROIs used for tractography.

Results—A significant negative association between cingulum microstructure and FPS during early extinction (r=−.42, p=.01) and late extinction (r=−.36, p=.03) was observed after accounting for the effects of age, trauma exposure, and psychopathology (posttraumatic stress disorder symptoms); this pattern was similar for early extinction and functional connectivity between these

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regions ($p<.05_{corrected}$). No significant correlations were observed between FPS and UF microstructure.

Conclusions—These data indicate that structural integrity of the cingulum is directly associated with extinction learning and appears to influence functional connectivity between these regions. Decrements in cingulum microstructure may interfere with extinction learning, thereby increasing risk for the development of pathological anxiety.

Keywords

fear-potentiated startle; extinction; cingulum; hippocampus; anterior cingulate cortex; connectivity

1. Introduction

Pathological anxiety has been linked to deficits in extinction of learned fear responses, as indicated by data from clinical and animal studies using fear conditioning methods (Delgado, Olsson, & Phelps, 2006; Graham & Milad, 2011). Fear conditioning involves repeated pairings of a neutral (conditioned) stimulus (CS) with an innately aversive (unconditioned) stimulus (US); extinction represents a process by which the CS is repeatedly presented in the absence of the US, with the expectation that defensive, fear-related physiological responses to the cue will attenuate as learning occurs (Myers & Davis, 2007). In high- or pathologically-anxious individuals, this attenuation in fear response may be delayed or diminished. Extinction involves learning to inhibit the conditioned fear response; impairments in this inhibition learning process are thought to be an underlying mechanism of pathological anxiety (Graham & Milad, 2011; Milad et al., 2009; Norrholm et al., 2011). To address this problem, evidence-based approaches to the treatment of pathological anxiety, such as exposure therapy, incorporate techniques that facilitate extinction of cues that trigger anxiety.

Although a number of studies have compared extinction processes between anxious and non-anxious groups of individuals (e.g., Jovanovic et al., 2010; Norrholm et al., 2011) and identified relative deficiencies in anxious groups, it is also valuable to examine extinction learning as a dimensional construct. Examining individual patterns of variation in extinction in association with other neurobiological information, such as neural connectivity (both structural and functional), may give insights into vulnerability for a disorder and likewise inform treatment selection.

A network of limbic and prefrontal brain regions work in concert during extinction learning, including the amygdala, hippocampus, and medial prefrontal cortical regions, particularly, aspects of the anterior cingulate cortex (ACC) and orbitofrontal cortex (OFC). The amygdala, which includes the central nucleus and basolateral complex, has been consistently associated with the physiological expression of fear during conditioning and extinction (Davis, Myers, Chhatwal, & Ressler, 2006). The amygdala complex receives inhibitory communication from both the hippocampus and ventromedial aspects of the prefrontal cortex (vmPFC; a term that references rostral aspects of the ACC or OFC) during extinction (Hartley & Phelps, 2010; Phelps, Delgado, Nearing, & LeDoux, 2004). The hippocampus is involved with contextual aspects of fear encoding, extinction, and recall of extinction

(Maren, Phan, & Liberzon, 2013; Myers & Davis, 2007). This region also has reciprocal connections to the ACC, which regulates inhibition of conditioned fear responses during extinction (Lang et al., 2009).

In general, limbic-prefrontal connections appear to be essential for efficient extinction of conditioned fear-related responses; amygdala, hippocampal and prefrontal regions participate in an integrated manner during this process. The hippocampus engages in the presence of contextual stimuli and signals the need to increase or diminish inhibitory responses by the ACC. Earlier studies have demonstrated involvement of the hippocampus and ACC during extinction learning and recall (Kalisch et al., 2006; Milad et al., 2007), and a recent study highlights the importance of connections between these areas for extinction processes. Lang and colleagues (2009) applied electrical stimulation as the US in a fear conditioning paradigm that involved colored shapes; they found that dorsal anterior cingulate (ACC) activation positively correlated with activation in the hippocampus, as well as the amygdala and OFC, during extinction (Lang et al., 2009). Animal studies further support the notion that ACC-hippocamapal connections directly affect extinction. Griffin and Berry (2004) observed that, compared to rabbits without ACC lesions, ACC-lesioned rabbits continued to demonstrate conditioned responses during extinction trials and did not demonstrate the suppressed hippocampal responses that were characteristic of extinction learning (Griffin & Berry, 2004). A question that remains unexplored is whether these associations were influenced by the integrity of structural/white matter connections. The cingulum is a primary white matter connection between hippocampal and frontal regions; this region has repeatedly shown relevance to learning and memory processes (Charlton, Barrick, Lawes, Markus, & Morris, 2010; Sepulcre et al., 2008; Sexton et al., 2010; van der Holst et al., 2013).

Connectivity between the amygdala and vmPFC also appears to influence extinction, as well as general emotion regulation, as suggested by studies using functional connectivity methods. Delgado and colleagues (2008) employed a fear conditioning paradigm that incorporated an emotion regulation task; participants were instructed to use positive reappraisal strategies when presented with particular conditioned stimuli. The authors found that amygdala activation was positively coupled with activation in the vmPFC during attempts to regulate emotion (Delgado, Nearing, Ledoux, & Phelps, 2008). Compared to anxious individuals, low trait-anxious and psychopathology-free controls have demonstrated heightened amygdala/vmPFC connectivity, whereas highly anxious individuals have shown an opposite pattern (Kim, Gee, Loucks, Davis, & Whalen, 2011; Roy et al., 2013; Stevens et al., 2013). This pattern of (decreased) functional connectivity has characterized individuals with bipolar disorder (Ladouceur et al., 2011), generalized anxiety disorder (Roy et al., 2013), social anxiety disorder (Prater, Hosanagar, Klumpp, Angstadt, & Phan, 2013), and post-traumatic stress disorder (Stevens et al., 2013).

The structural integrity of amygdala/vmPFC connections may influence these functional outcomes. The uncinate fasciculus (UF) provides a connection between the amygdala (as well as the hippocampus) to the vmPFC. Kim and colleagues (2009) observed a negative correlation between trait anxiety and integrity of white matter connections between the amygdala and vmPFC (Kim & Whalen, 2009). In addition, a recent study found that

individuals with generalized anxiety disorder demonstrated reduced microstructural integrity (measured through fractional anisotropy; FA) in the UF, compared to psychopathology-free controls (Tromp do et al., 2012). Thus, the UF is another tract of potential relevance to extinction; degraded tract integrity may lead to increased susceptibility to the development of pathological anxiety, possibly via impairment of extinction learning.

Notably, no studies to date have examined associations between extinction and structural connectivity. Examining the associations between extinction learning and cingulum and UF microstructure connectivity could inform current models of anxious psychopathology; it is possible that structural alterations in these paths may increase susceptibility to extinction deficits, thus heightening vulnerability for the development of anxiety disorders. We used a fear-potentiated startle (FPS) paradigm, diffusion tensor imaging (DTI) and probabilistic tractography methods to examine associations between extinction and structural connectivity of the UF and cingulum; seed regions for these analyses were, respectively, the amygdala and vmPFC and the hippocampus and ACC. Fear-potentiated startle was defined as the increase in the acoustic startle reflex in the presence of the CS compared to baseline levels of startle; FPS was assessed during fear conditioning and extinction. Given that individuals in our sample had experienced varying degrees of trauma exposure and PTSD symptoms, we controlled for these factors in our primary analyses. We hypothesized that greater connectivity in these pathways would be associated with lower FPS during extinction.

Our secondary objective was to examine whether similar patterns would emerge with extinction and functional connectivity of our selected gray matter regions of interest (ROI) during performance of a response inhibition (go/nogo) task; our previous data indicated associations between ACC function and extinction during the “nogo” condition, which involved response inhibition (Jovanovic et al., 2013). Although the two paradigms are dissimilar, both the extinction paradigm and the go-nogo task involve inhibition of prepotent response to neutral cues. Functional connectivity analyses were conducted with ROIs that were significantly associated with FPS in white matter connectivity analyses.

2. Methods

2.1 Participants

The Institutional Review Board of Emory University approved all study procedures. A total of 48 African-American women aged 21–62 years were recruited through an ongoing study of risk factors for PTSD. Individuals were approached in general medical clinics of a publicly-funded hospital that serves low income individuals in inner-city Atlanta. Eligibility criteria for participation included ability to understand English (assessed by a study researcher) and willingness to provide informed consent. Participants were initially screened for the following exclusion criteria: current psychotropic medication use, current alcohol or substance dependence, medical or physical conditions that preclude MRI scanning (e.g., metal implants), a history of bipolar disorder, schizophrenia or other psychotic disorder, medical conditions that contribute significantly to psychiatric symptoms (e.g., dementia), history of head injury, loss of consciousness for longer than 5 minutes, or a history of neurological illness. Participants were additionally excluded if they were unable to detect tones at 30 dB ranging in frequency between 250 and 4000 Hz, as assessed by an

audiometer. Clinical assessments were administered during a separate appointment. Participants were given a pregnancy test to confirm that they were not pregnant and a urine drug screen to rule out substance use on the day prior to scanning. Sample demographics and clinical characteristics are provided in Table 1. Participants reported experiencing 0–12 types of traumatic events throughout their lifetime, with a mode of 2, and an average of 4.8.

2.2 Clinical measures

The Traumatic Events Inventory (TEI) was administered to detail frequency and type of trauma(s) experienced; total level of trauma exposure was measured by a sum score reflecting the total number of different types of traumas to which a participant had been exposed over the course of their life (TEI total) or adulthood (TEI adult trauma). The PTSD Symptom Scale (PSS; Falsetti, Resnick, Resick, & Kilpatrick, 1993) is a self-report questionnaire that provides a measure of PTSD symptoms that have occurred in the 2 weeks prior to test administration. The PSS was administered to determine presence and severity of PTSD symptoms. Given the objectives of the present study, and the fact that high rates of trauma exposure and PTSD have been previously observed in this population (Schwartz, Bradley, Sexton, Sherry, & Ressler, 2005), TEI total score and PSS total score were used as covariates in statistical analyses. Secondarily, PSS total and subscale scores (re-experiencing, avoidance/numbing, and hyperarousal) were used in exploratory correlational analyses with structural connectivity.

2.3 Fear-potentiated startle paradigm

Fear-potentiated startle was defined as the increase in startle response to the CS compared to noise alone (NA) trials of baseline startle as in our previous studies (Fani, Tone, et al., 2012). Startle response data were acquired at a sampling rate of 1 kHz using the electromyography (EMG) module of the BIOPAC MP150 for Windows (Biopac Systems, Inc., Aero Camino, CA). The eyeblink component of the startle reflex was measured by EMG recordings of the right orbicularis oculi muscle using two 5-mm Ag/AgCl electrodes filled with electrolyte gel, placed 1 cm below the pupil of the right eye and 1 cm below the lateral canthus. The acquired data were filtered between 28 and 500 Hz, rectified, and smoothed using the MindWare software (MindWare Technologies, Ltd., Gahanna, OH). The maximum amplitude of the EMG 20–200ms after presentation of the startle probe was used as a measure of the acoustic startle response. The startle probe was a 106-dB (A) SPL, 40-ms burst of broadband noise delivered binaurally through headphones.

The startle session began with a habituation phase to reduce startle reactivity and familiarize the subjects to the CSs. The fear acquisition phase immediately followed habituation and consisted of three blocks with four trials of each type (a reinforced conditioned stimulus, CS +; a non-reinforced conditioned stimulus, CS-; and the 40 ms noise probe alone (NA), for a total of 36 trials. Figure 1 shows a diagram of the FPS session and trial definitions. Both CSs were colored shapes presented on a computer monitor for 6 seconds. The 106-dB startle probe was delivered on every trial 0.5 seconds prior to the aversive US. The US was a 250ms airblast with an intensity of 140 p.s.i. directed at the larynx. The CS+ was reinforced on 100% of the trials during acquisition. The extinction session was administered 10 min after fear conditioning, and consisted of four blocks with four trials of each type (CS+, CS−,

and NA), for a total of 48 trials. The stimuli were same as above, except that the CS+ was no longer reinforced with the airblast. The inter-trial intervals were randomized to be 9–22sec. FPS was calculated as a differences score between the startle magnitude to each CS minus the startle magnitude to the NA.

2.4 MRI acquisition, image processing and statistical analyses

Scanning was conducted on a research-dedicated Siemens 3-Tesla TIM-Trio scanner at Emory University Hospital. Diffusion-weighted images were acquired with maximum gradient strength of $40mTm^{-1}$ with the following parameters: 39 × 2.5 mm thick axial slices, matrix = 128 × 128, field of view (FOV) = 220 × 220 mm, voxel size = 1.72 × 1.72 × 2.5 mm. Diffusion weighting was isotropically distributed along 60 directions using a b-value of 1,000 s/mm^2. Four normalization images, with no diffusion encoding (b=0), were acquired and averaged for each direction using linear rigid body registration (FLIRT; Jenkinson & Smith, 2001). All diffusion-weighted image processing and analysis was conducted using FMRIB Software Library (FSL version 4.1; www.fmrib.ax.ac.uk/fsl; Smith et al., 2004). A high-resolution T1-weighted structural scan was also acquired for co-registration purposes using an MPRAGE sequence: 176 slices, FOV= 256 mm cubic voxels; 1mm isotropic slices; repetition time (TR)= 2600 msec; echo time (TE) = 3.02 msec; inversion time (TI)= 900msec; flip angle=8°. A total of 26 contiguous echoplanar, T2-weighted images parallel to the anterior-posterior commissure line were acquired during task administration (TR= 2530msec; TE= 30 msec; FOV= 240 mm; 64 × 64 matrix; 3.75 × 3.75 × 4.0 mm voxel). Functional MRI images were acquired using the Z-saga pulse sequence (Heberlein & Hu, 2004) to minimize susceptibility signal loss. Statistical Parametric Mapping, version 5 (SPM5, Wellcome Trust Centre for Neuroimaging, London, UK: http://www.fil.ion.ucl.ac.uk/spm/) was used for fMRI file conversion, image pre-processing and statistical analyses.

2.5 DTI Data Processing and Probabilistic Tractography

Correction for head motion and eddy current distortion was performed for data from each participant using an automated affine registration algorithm. Both diffusion-weighted and T1 images were skull-stripped using the FSL brain extraction tool (Smith, 2002). FA maps were generated using the DTIfit in the FMRIB Diffusion Toolbox. Markov Chain Monte Carlo sampling was used to calculate within-voxel probability density functions of the principal diffusion direction using FSL's BEDPOSTX tool, which also accounts for the possibility of crossing fibers within a voxel (Behrens et al., 2003). Probabilistic fiber tracking was conducted with PROBTRACKX implemented in FSL; this method repeatedly samples the distribution at each voxel to produce 'streamlines' that connect voxels from selected seed regions (5000 streamline samples, .5 mm step length, curvature threshold = .2). A mask of the cingulum, created using the JHU White Matter Tractography Atlas (Mori, Wakana, Nagae-Poetscher, & Van Zijl, 2005), was used as an anatomical waypoint for all paths; a separate exclusion mask was created to eliminate the likelihood of pathways in irrelevant white matter tracts, gray matter regions and CSF. Hippocampus, ACC and amygdala masks were created using the Harvard-Oxford Subcortical Structural Atlas (http://www.cma.mgh.harvard.edu/fsl_atlas.html). The vmPFC was defined using a 6mm sphere centered at Talairach coordinates x=4, y=42, z=–5, selected based on our previous study

which observed associations between vmPFC activation and fear-potentiated startle during extinction (Jovanovic et al., 2013). Only streamlines that passed through seed regions and the waypoint were retained; example streamlines for the two sets of tractography analyses are illustrated in Figure 2. The resulting streamlines were transformed to Montreal Neurological Institute (MNI) space. Given the large size and extent of cingulum streamlines, these paths were thresholded by 10% to reduce the likelihood of including extraneous tracts. Fractional anisotropy was used as our measure of tract integrity, given that earlier studies have indicated it to be a reliable assessment of microstructural integrity of white matter fibers (Fox et al., 2011). Individual FA maps were linearly aligned to a standard MNI brain using FLIRT; mean FA for all streamlines was then extracted and entered into statistical analyses.

All data were visually inspected for major artifacts before being included in analyses. Data from 1 participant was discarded due to motion artifacts. Among the remaining 47, 7 participants had unusable cingulum tracts (included anatomically unfeasible, deviated projections), leaving a sample size of 40 for cingulum analyses; an additional 7 had unusable UF tracts, which reduced the sample size for these analyses to 33. This sub-group of participants was similar to the overall sample in terms of age (M=40.6, SD=13.5), PTSD symptoms (PSS total: M=12.7, SD=11), overall trauma exposure (TEI total: M=4.9, SD=2.8) and trauma exposure in adulthood (TEI adult: M=4.1, SD=2.3). Using IBM SPSS version 20, partial correlations were conducted with mean FA values of all tracts and FPS variables. Age, lifetime trauma exposure and PTSD symptoms were controlled for in partial correlational analyses, as these factors have been known to influence white matter integrity (Fani, King, et al., 2012; Lebel et al., 2012; Ly et al., 2013; Wang et al., 2010). A threshold of $p<.05$, two-tailed, was used to define statistical significance.

2.6 Functional Connectivity

Functional connectivity analyses were conducted with 34 of the 40 participants who had also completed a Go/No-go task during fMRI; the task and MRI pre-processing procedures have been previously detailed (Jovanovic et al., 2013). Briefly, participants were asked to respond with a button press when presented with an “X” or an “O” (go condition) and refrain from responding when the background changed to red (no-go condition). Individuals’ responses to the no-go condition were compared to the go condition, similar to our prior analyses (Jovanovic et al., 2013), given our interest in examining functional connectivity during efforts to inhibit a prepotent response.

Connectivity analyses were conducted with the CONN toolbox (Whitfield-Gabrieli & Nieto-Castanon, 2012) implemented in SPM (http://www.fil.ion.ucl.ac.uk/spm/) using the same ROI masks utilized for structural connectivity analyses. This toolbox permits computation of temporal correlations between BOLD signals from selected ROIs to other voxels in the brain, and has been used in earlier functional connectivity studies of emotion processing and learning (Pecina et al., 2013; Powers, Hevey, & Wallace, 2012; Stevens et al., 2013). We utilized this toolbox to examine individual differences in extinction learning and psychophysiological interactions (PPIs) with connectivity for our regions of interest during no-go vs go (i.e., “inhibit”) trials. FPS values to the CS+ during extinction were entered as

subject-level regressors. Signal from subject motion was removed from the data. Seed-to-voxel bivariate correlations were computed for the no-go>go contrast; target voxels were restricted to an *a priori* selected ROI masks. AlphaSim, an application included in the REST toolbox for SPM (Song et al., 2011) was used to correct for multiple comparisons. Monte Carlo simulation was conducted for 1000 iterations, using a cluster-forming threshold of $p < .01$. A cluster of k=14 was necessary to reach a corrected threshold of $p<.05$ (voxel-wise $p = .004$).

3. Results

3.1 Fear Conditioning

Fear conditioning to the CS+ and CS− was compared with repeated-measures ANOVA with trial type as a within subjects variable and FPS (startle to each CS trial minus startle to NA) as the dependent variable (see Figure 3). This analysis showed significantly higher FPS to the CS+ than the CS− during the last block of acquisition, $F(1,40)=5.75$, $p=.02$. Extinction was analyzed comparing FPS to the CS+ across early and late extinction, with extinction phase as the within-subjects variable. This analysis showed a significant decrease in FPS across phase of extinction, $F(1,40)=16.01$, $p<.001$.

3.2 Probabilistic Tractography

Bivariate correlational analyses (illustrated in Table 2 and Figure 4) revealed a significant negative correlation between hippocampus/ACC (cingulum) connectivity and startle response during early extinction (Blocks 1 and 2; $r=-.35$, $p=.03$). No significant correlations were observed between cingulum connectivity and fear acquisition or late extinction (Blocks 3–4). After covarying age, trauma exposure (TEI total) and current PTSD symptoms, cingulum connectivity demonstrated a stronger association with early extinction ($r=-.42$, $p=.01$); however, a significant negative correlation also emerged with late extinction ($r=-.36$, $p=.03$).

Secondarily, we examined associations between cingulum connectivity and PTSD symptoms. After covarying for age and overall trauma exposure (TEI total), no significant correlations emerged between current PTSD symptoms and cingulum connectivity (all *p*s>.05), although associations with re-experiencing approached significance ($r=-.31$, $p=.057$). After statistically controlling for age and adult trauma exposure, a significant negative association was observed between cingulum connectivity and re-experiencing symptoms of PTSD ($r=-.35$, $p=.03$).

No significant correlations were observed between amygdala/vmPFC (UF) connectivity and startle response during fear conditioning or extinction, even after statistically controlling for current PTSD symptoms, trauma exposure, and age (all *p*s>.05). Similarly, no significant associations were observed between PTSD symptoms and UF connectivity after controlling for age and trauma exposure (all *p*s>.05).

3.3 Functional Connectivity

A regression analysis was conducted to examine correlations between FPS magnitude during early extinction (blocks 1 and 2) and hippocampal connectivity to the ACC, given the findings from structural connectivity analyses. The timecourse of the hippocampal seed region was extracted using the average signal across voxels within the bilateral hippocampus ROI for the no-go>go contrast. Target voxels were restricted to the *a priori* mask of the ACC ROI. Hippocampal connectivity to the ACC was negatively correlated with the startle response during early extinction (k=17, t= 2.87, MNI x,y,z =−4, 36, 8; see Figure 5). No significant associations were observed between overall hippocampal connectivity to the ACC for the no-go>go contrast. We conducted secondary analyses to examine hippocampal-ACC connectivity within participants with the lowest startle magnitudes during early extinction (n=17, defined by a median split) to the no-go>go contrast, and found significant connectivity between the ACC and hippocampus (k=3, t= 2.18, MNI x,y,z =−32, −40, −8; $p<.05_{uncorrected}$) in this subgroup, although these findings did not survive correction for multiple comparisons. We did not find significant associations between PTSD symptoms and hippocampal connectivity to the ACC.

4. Discussion

The present study investigated associations between FPS response during fear learning and extinction and white matter connectivity. Secondarily, we examined associations between FPS during extinction and patterns of functional connectivity during performance of a separate inhibition task. We specifically investigated white matter microstructure for cingulum and UF tracts, which provide connections between the hippocampus and ACC, and the amygdala and vmPFC. We hypothesized that white matter integrity would be negatively correlated with FPS response during extinction learning (which occurred outside the scanner); we expected that greater microstructural integrity of these tracts would be associated with a dampened psychophysiological response during learning to inhibit fear. Our hypotheses were partially confirmed. Structural integrity of the cingulum corresponded negatively with startle during extinction. This pattern was mirrored in the functional connectivity data; increased connectivity between the hippocampus and ACC corresponded with lower startle during extinction. Cingulum microstructure was also inversely associated with PTSD re-experiencing symptoms. However, no associations were observed between UF connectivity and FPS during either fear acquisition or extinction.

As predicted, we found that stronger white matter microstructure and increased functional connectivity in the hippocampus/ACC pathway was associated with lower FPS during learning to extinguish fear to a discrete “danger” (CS+) cue; this association was particularly strong for the earliest stages of extinction. These data indicate that the integrity of this pathway may be critical to extinction learning, especially during initial stages of the learning process. The hippocampus is thought to be involved in the encoding of cue context during fear conditioning. During extinction, signals between the hippocampus and prefrontal cortical regions, specifically the ACC, are used to evaluate whether a context is threatening or safe, therefore communicating the need to either heighten or dampen a defensive physiological response (Maren et al., 2013). Our data indicate that the integrity of a primary

tract that connects the hippocampus and the ACC is linked to extinction learning, possibly affecting efficiency of learning. Structural abnormalities in the cingulum are likely to translate into differences in function; our fMRI data also indicate that strength of connectivity between the hippocampus and ACC during performance of an inhibition task corresponded significantly with FPS during early extinction. Prior studies of healthy individuals have indicated heightened hippocampal response during extinction learning (Knight, Smith, Cheng, Stein, & Helmstetter, 2004; Lissek, Glaubitz, Uengoer, & Tegenthoff, 2013; Merz, Stark, Vaitl, Tabbert, & Wolf, 2013) and extinction recall (Kalisch et al., 2006; Milad et al., 2007). Our findings extend the observations of earlier research, and could suggest that white matter connections between the hippocampus and ACC influence extinction learning.

This pattern of white matter microstructure and functional connectivity may, in turn, affect the development of anxious psychopathology. Delayed or diminished attenuation of startle response during extinction is characteristic of individuals with anxiety disorders, such as PTSD (Graham & Milad, 2011; Norrholm et al., 2011); this phenomenon may be due, in part, to abnormalities in structural brain connectivity between the hippocampus and the ACC. Milad and colleagues (Milad et al., 2009) observed that, during extinction recall, individuals with PTSD demonstrated lower hippocampal and rostral ACC (vmPFC) activation, whereas an opposite pattern was observed in trauma-exposed controls. In this study we not only found that poorer hippocampus to ACC connectivity corresponded with heightened FPS to danger cues during extinction, but also with PTSD symptoms. After accounting for variance associated with age and adult trauma exposure, connectivity of this pathway was significantly associated with re-experiencing symptoms of PTSD. The fact that these associations were observed with re-experiencing, but not avoidance/numbing and hyperarousal symptoms, is of particular interest. Re-experiencing symptoms are exclusive to post-traumatic psychopathology, often considered to be hallmark signs of this disorder, and have been linked to alterations in hippocampal structure and function (Carrion, Haas, Garrett, Song, & Reiss, 2010; Lindauer, Olff, van Meijel, Carlier, & Gersons, 2006) and deficits in early extinction (Norrholm et al., 2011). In our previous studies, we found that exaggerated levels of FPS during early extinction are associated with anxiety-related phenomena, including attention biases to threat-relevant stimuli (Fani, Tone, et al., 2012)—we have termed this phenomenon “fear load,” which appears to be an intermediate phenotype of PTSD psychopathology. Importantly, this phenotype may be more closely associated with the underlying neurobiology than DSM-based symptoms, underscoring the importance of utilizing physiological biomarkers.

Although a number of studies (e.g., Corcoran & Maren, 2001; Holt & Maren, 1999) have supported the idea that the hippocampus is necessary for contextual aspects of extinction learning and recall, it is also important to note the presence of evidence that suggests otherwise. For example, Corcoran and colleagues (Corcoran, Desmond, Frey, & Maren, 2005) found that although inactivation of the hippocampus attenuated extinction acquisition in rats, the process was not blocked entirely. Thus, interpretations of the present data must be made with some caution.

Surprisingly, no significant associations emerged for FPS during extinction and amygdala to vmPFC (UF) connectivity; similarly, connectivity in this pathway did not correspond with PTSD symptoms. Previous studies have indicated that amygdala/vmPFC connectivity is involved with emotion regulation processes (Admon et al., 2013; Delgado et al., 2008), and both regions have been implicated in extinction (Phelps et al., 2004). Specifically, intercalated cells in the amygdala are thought to be most involved with expression of extinction; this nucleus contains a high amount of GABAergic neurons and has direct connections to the vmPFC and the central amygdala (Manko, Geracitano, & Capogna, 2011). One possible explanation for these null connectivity findings is the slightly smaller sample size, which may have limited statistical power, as well as the size of our vmPFC ROI, which may have affected tractography results. It is possible that a larger ROI would improve detection of probabilistic connections from this region to the amygdala; however, this could also increase inclusion of irrelevant white matter tracts. Similarly, our UF connectivity findings may be affected by a high variability in volume of this tract, given that variability in UF volumes has been shown to influence tractography findings (Kurki, Laalo, & Oksaranta, 2013). In addition, given what has been found earlier with regard to the role of the vmPFC in extinction recall (e.g., Lissek et al., 2013), it is possible that associations between vmPFC/amygdala connectivity and extinction recall would have emerged if we had examined recall. This is worthy of investigation in future studies.

A limitation of the present study is that we did not examine other potentially relevant white matter connections, including connections between the hippocampus and the amygdala, which have been highlighted in earlier studies (e.g., Maren & Hobin, 2007; Milad et al., 2009)) and may play a role in this network. We purposefully selected a broad ACC ROI in order to improve our ability to detect relevant connections with the hippocampus; however, dissociating dorsal and ventral ACC regions and exploring their disparate associations with hippocampus connectivity and extinction merits investigation in future studies. Further, we did not measure extinction recall, a process that may be dependent on hippocampal function and connectivity with the ACC, which also merits investigation in further studies of white matter connectivity. Finally, there are possible limitations in generalizability of our findings given our sample demographics. Most of our participants experienced at least one traumatic event, and although we statistically controlled for these effects, it is impossible to exclude the potential influence of trauma-related factors. Finally, a meditational analysis is ideal for exploring the relationships between hippocampus-ACC structural and functional connectivity and extinction learning, but we did not have the statistical power to perform this analysis, given the relatively small sample size of the fMRI subgroup.

In sum, the results of this study demonstrate that the integrity of a major white matter tract that connects the hippocampus to the ACC, the cingulum bundle, may directly influence extinction learning, and likewise, PTSD symptoms. These data highlight the importance of this pathway in extinction learning, suggesting that abnormalities in cingulum connectivity may be a biological sign of vulnerability for the development of anxious psychopathology.

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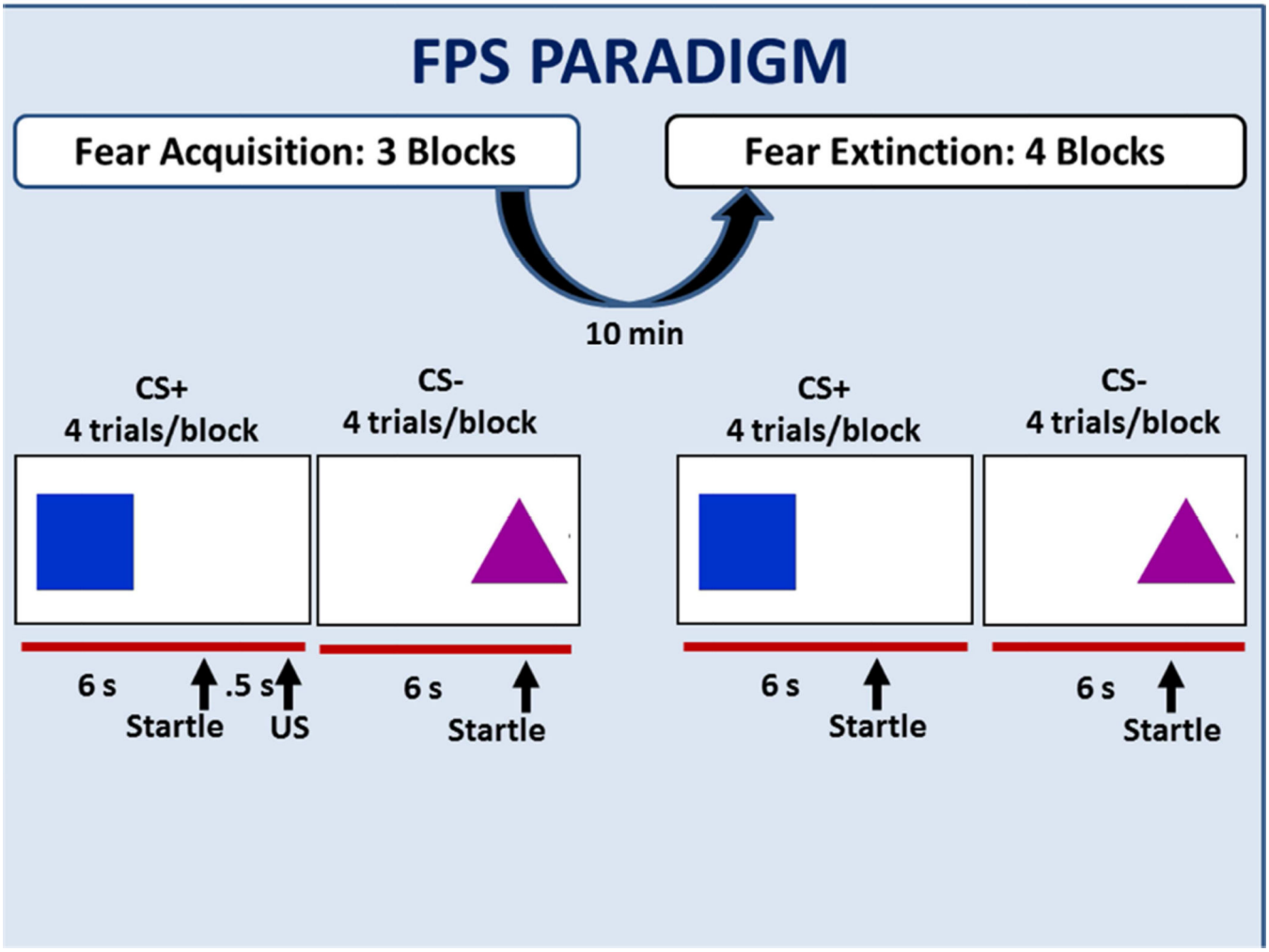

Figure 1.
Diagram of FPS session and trial definitions.

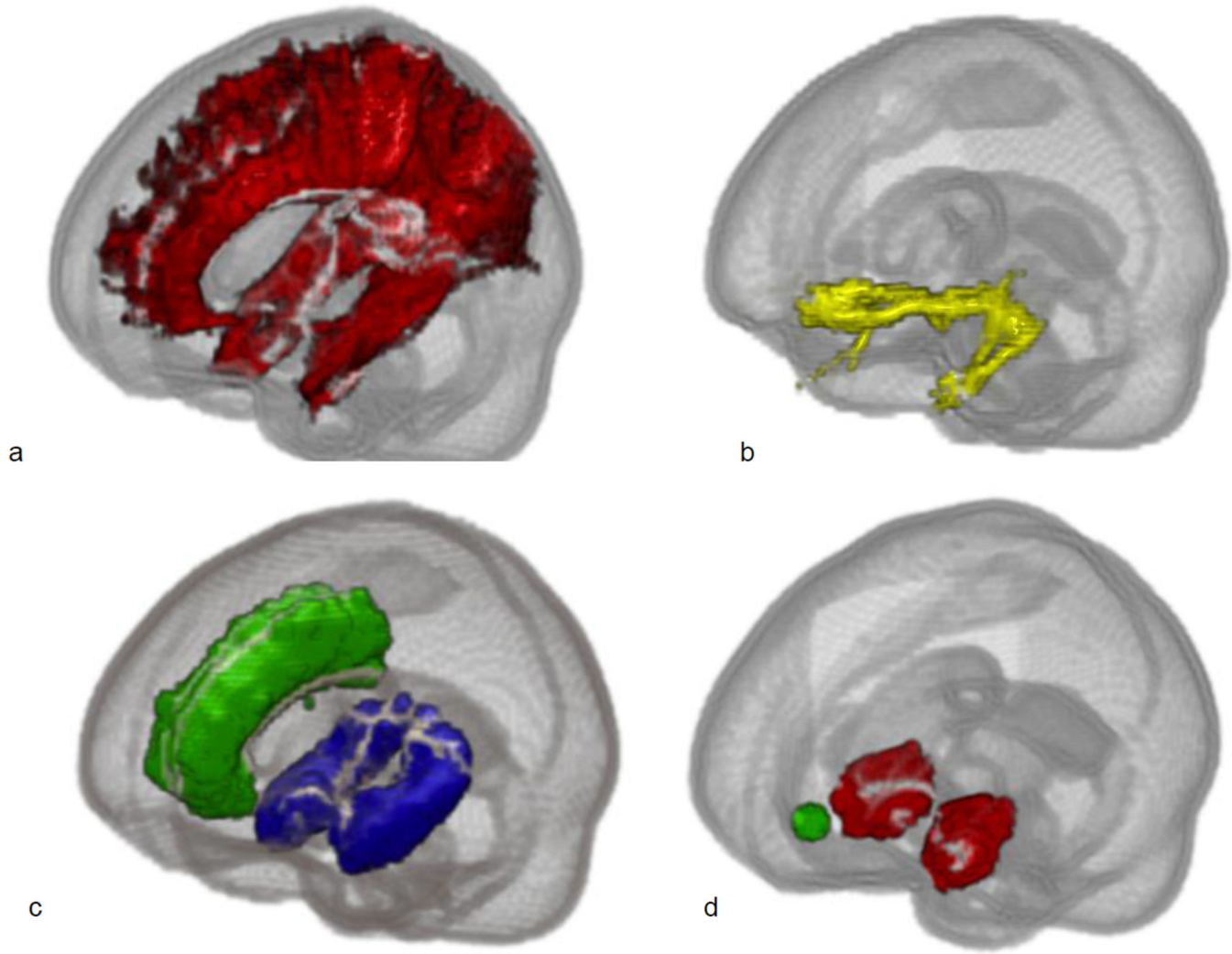

Figure 2.

Example thresholded probabilistic pathways of the (a) cingulum and (b) uncinate fasciculus. Seed regions for tractography analyses represented in the bottom panel: c) hippocampi (blue) and ACC (green); d) amygdalae (red) and vmPFC (green)

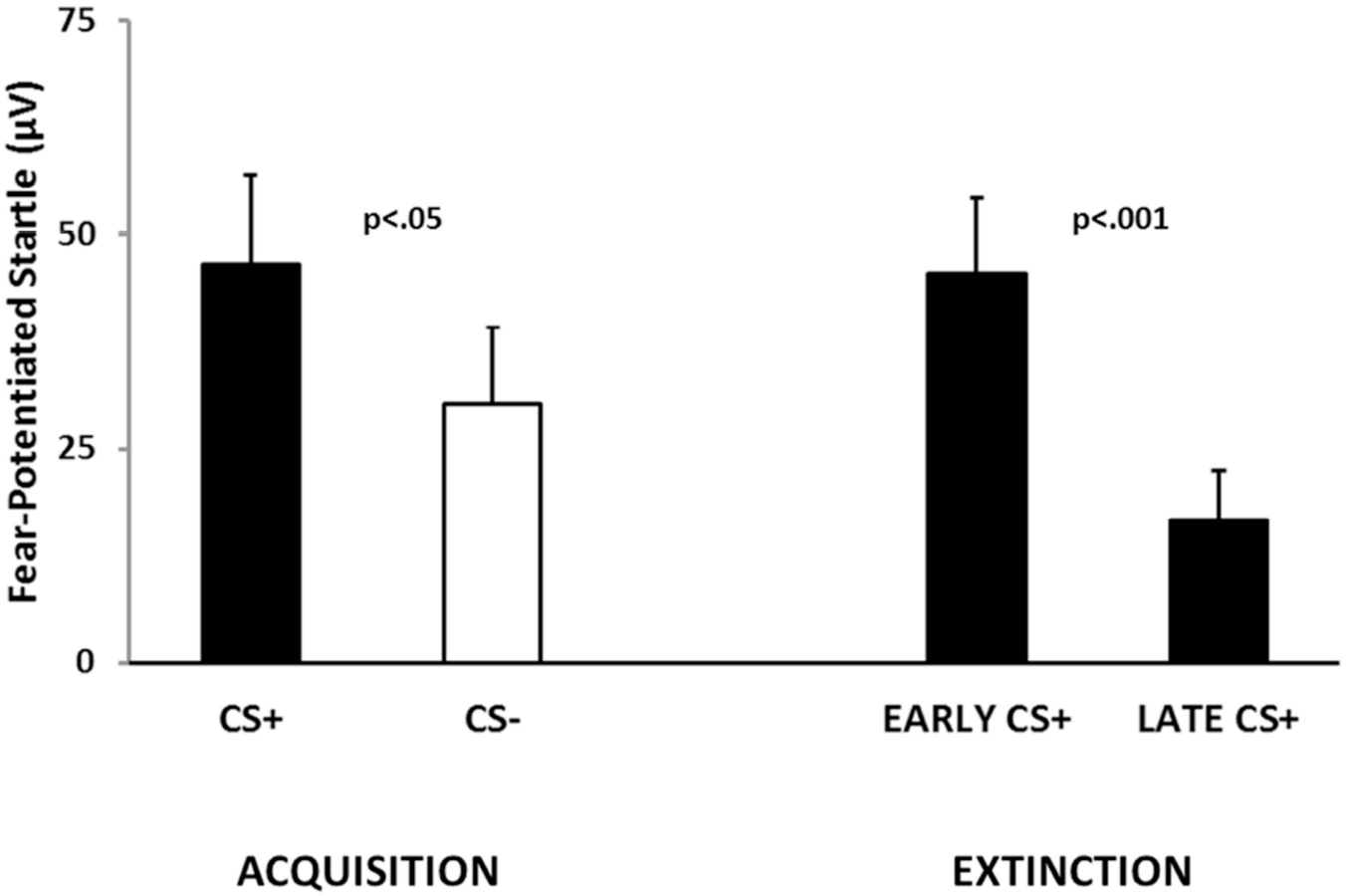

Figure 3.
Differences in FPS response during acquisition and extinction phases.

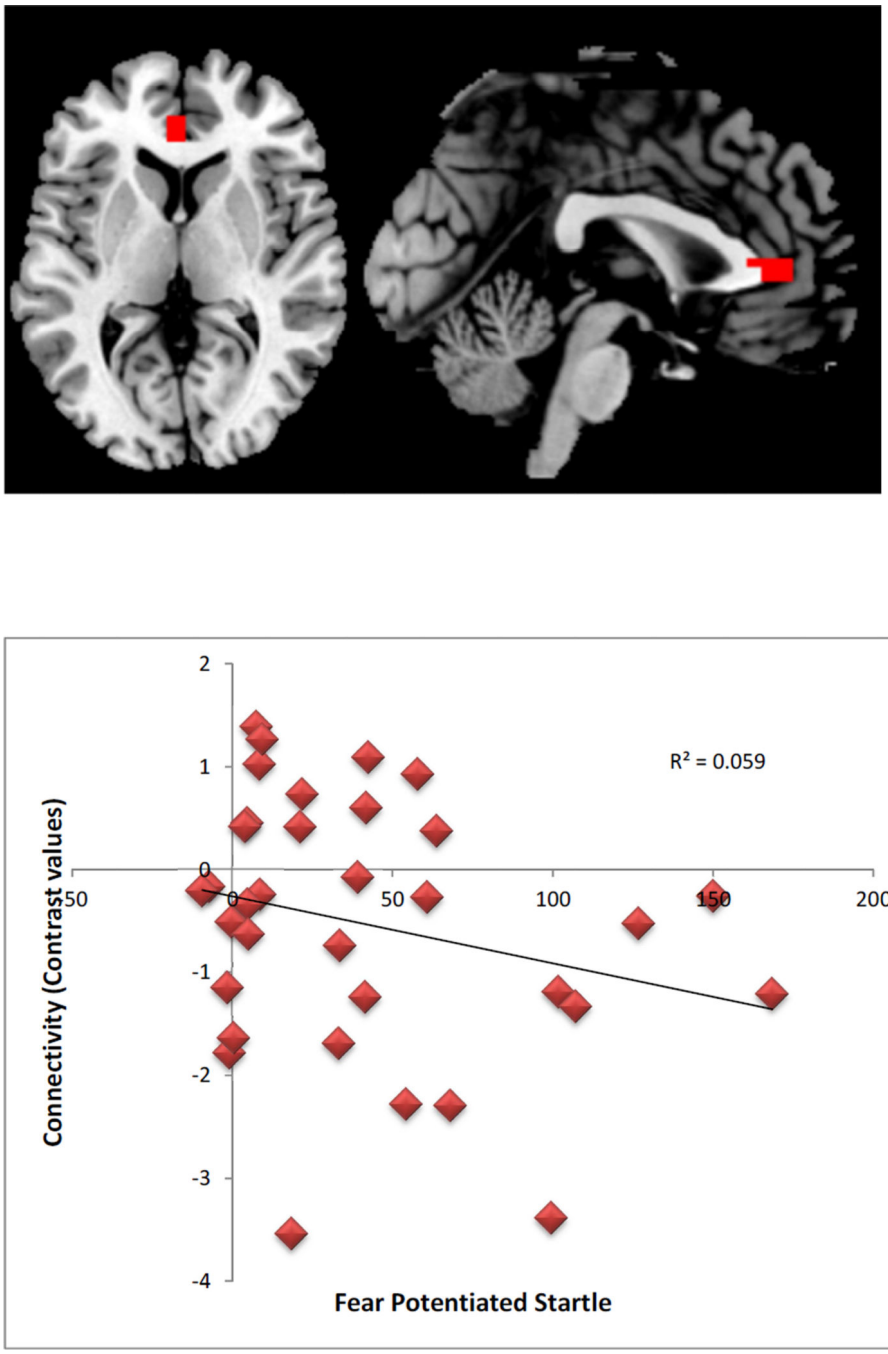

Figure 4.

Associations between FPS during early extinction and functional connectivity of the hippocampus and ACC. Scatterplot depicts negative correlation between fear potentiated startle during extinction and functional connectivity of the hippocampus to the ACC (MNI x, y, z = −4, 36, 8) to no-go > go condition.

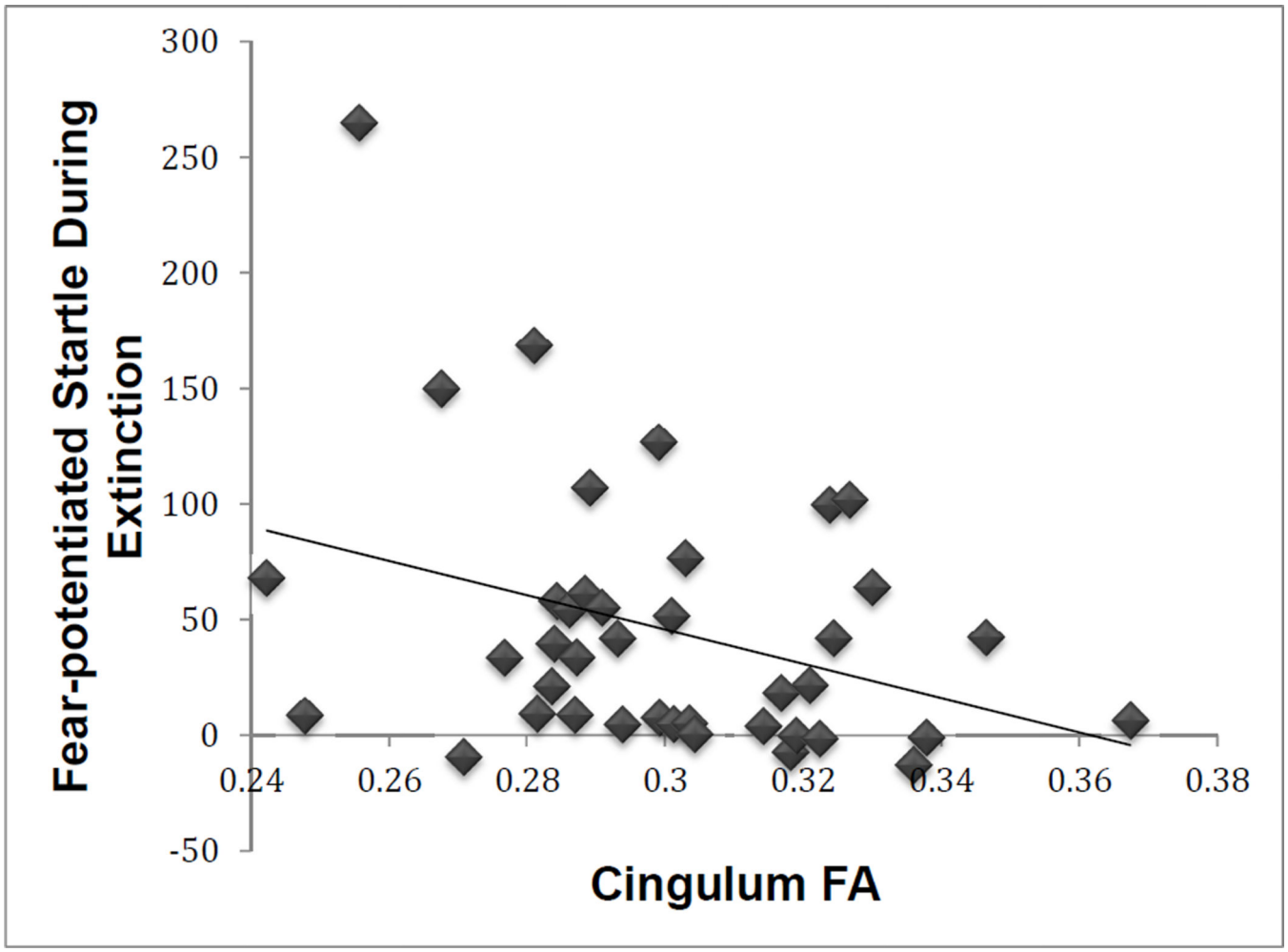

Figure 5.
Scatterplot depicting negative correlation between FPS during early extinction and fractional anisotropy of hippocampal/ACC (cingulum) pathway.

Table 1

Demographic and Clinical Characteristics (N=40)

	Mean (SD)
Age	39.4 (12.4)
PSS total	13.6 (11.1)
TEI total	4.8 (2.9)
TEI adult trauma	4 (2.4)
	%
Education	
< 12th grade	10.3
12th grade/high school graduate/GED	30.8
Some college/technical school	38.5
Tech school/College graduate	20.5
Household Monthly Income	
$0 – 249	15.8
$250 – 499	13.2
$500 – 999	39.5
$1000–1999	23.7
$2000+	7.9

* $p < .05$

PSS= PTSD Symptom Scale
TEI = Traumatic Events Inventory

Table 2

***Partial Correlations Between Cingulum FA and Fear Potentiated Startle after Controlling for Age, Trauma Exposure and PTSD Symptoms* (n=40)**

	2	**3**	**4**	**5**
1. FPS Late Acquisition (CS+)	.76**	.61**	.66**	−3
2. FPS Late Acquisition (CS−)		.63**	.61**	−.24
3. FPS Early Extinction (CS+)			.57**	−.42*
4. FPS Late Extinction (CS+)				−.36*
5. Cingulum FA				--

Partial Correlations Between Uncinate Fasciculus FA and Fear Potentiated Startle after Controlling for Age, Trauma Exposure and PTSD Symptoms (n=33)

	2	3	4	5
1. FPS Late Acquisition (CS+)	.82**	.66**	.71**	−.16
2. FPS Late Acquisition(CS−)		.66**	.62**	−.06
3. FPS Early Extinction (CS+)			.59**	−11
4. FPS Late Extinction (CS+)				−.15
5. Uncinate Fasciculus FA				--

FPS = fear potentiated startle

* p <.05

** p <.01