

Cortico-limbic connectivity changes following fear extinction and relationships with trait anxiety

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Abstract

Fear extinction is a powerful model of adaptive and anxiety-related maladaptive fear inhibition. This learning process is dependent upon plastic interactions between the amygdala, the anterior midcingulate cortex (aMCC), the hippocampus, and the ventromedial prefrontal cortex (vmPFC). With regard to the amygdala, the basolateral (BLA) and centromedial amygdala (CMA) serve unique roles in fear extinction. In a large sample ($N = 91$), the current study examined pre- to post-extinction changes in resting state functional connectivity (RSFC) of fear inhibition and expression pathways. We also examined how trait anxiety and extinction performance were associated with extinction-related changes within these neural pathways. We found stronger pre- to post-extinction RSFC in pathways known to play a role in the down-regulation of fear responses (BLA-hippocampus, aMCC-hippocampus, CMA-hippocampus, CMA-aMCC). We also found that trait anxiety was associated with strengthening of a BLA-aMCC circuit supporting fear expression following extinction learning. Furthermore, we found that physiological indices of poorer extinction learning were linked to weaker pre- to post-extinction RSFC of a BLA-hippocampus pathway important for fear extinction consolidation. Our results highlight the network changes that occur during extinction, the separable role of CMA and BLA-based circuitry and a key pathway linked to risk for anxiety pathology.

Key words: fear extinction; resting state connectivity; anxiety; amygdala; plasticity

Introduction

Fear extinction is a prominent model of adaptive fear inhibition and fear inhibition problems that are central to anxiety disorders (Graham and Milad, 2011). Research in laboratory animals has demonstrated that the acquisition, consolidation, and retention of fear extinction are dependent upon plasticity of a neural network involving the amygdala, the anterior midcingulate cortex (aMCC), the ventromedial prefrontal cortex (vmPFC) and the hippocampus (Quirk and Mueller, 2008).

With respect to the amygdala, the basolateral amygdala (BLA) subregion has been shown to be involved in fear and extinction learning (Orsini and Maren, 2012). Neuronal connections between the BLA and aMCC (BLA-aMCC) support the expression of fear (Sotres-Bayon et al., 2012; Senn et al., 2014), whereas connections between the BLA and vmPFC (BLA-vmPFC) and the BLA and hippocampus (BLA-hippocampus) support the inhibition of fear (Orsini and Maren, 2012; Senn et al., 2014). Thus, increased interactions between BLA-vmPFC and BLA-hippocampus and

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decreased interactions between BLA–aMCC may mediate successful inhibition of fear responses. On the other hand, the centromedial amygdala (CMA) has largely been shown to be involved in fear expression. Although growing evidence indicates that the CMA may also be involved in the inhibition of fear via its connection with the vmPFC (Keifer *et al.*, 2015).

In addition to amygdala-based networks, laboratory animal and some human neuroimaging work have shown that connections between the aMCC, vmPFC and hippocampus also play a role in fear extinction. The vmPFC–hippocampus network has been implicated in the consolidation and retention of fear extinction (Kalisch *et al.*, 2006; Milad *et al.*, 2007). Further, increased connectivity between the aMCC and vmPFC, as well as the aMCC and hippocampus during extinction learning, likely gates the expression of fear to facilitate fear extinction (Lang *et al.*, 2009; Sotres-Bayon, *et al.*, 2012).

While animal studies have provided a foundational understanding of neural plasticity supporting extinction learning, few human studies have examined this extinction-related plasticity. One approach to this issue is to examine changes in functional connectivity of targeted networks measured at rest before and after learning (Schultz *et al.*, 2012; Feng *et al.*, 2014). These changes in connectivity likely foster the consolidation of new learning and reflect coordinated patterns of activity within defined circuits. Indeed, these resting state functional connectivity (RSFC) changes following learning have been linked to learning-related behavioral performance (unconditioned stimulus (UCS) probability ratings, skin conductance, fear ratings; Schultz *et al.*, 2012; Feng *et al.*, 2014). This resting state paradigm has not been applied to fear extinction learning. Additionally, no one has published pre- to post-extinction-related connectivity changes of distinct amygdala subregion pathways.

Given that extinction learning is important to understanding anxiety pathology (Graham and Milad, 2011), characterizing anxiety-related differences in dynamic neural processes supporting extinction has clinical implications. Trait anxiety is a core prospective risk factor for anxiety disorders (Barlow *et al.*, 2014). Thus, delineating trait anxiety-related aberrations in neural changes underlying fear extinction would aid in understanding the risk for pathological anxiety.

Fear extinction and resting state studies have generally demonstrated that individuals with high anxiety show increased activity within, and connectivity between, brain structures involved in the expression of fear including the amygdala and aMCC (Barrett and Armony, 2009; Milad *et al.*, 2009; Bijsterbosch *et al.*, 2014; Vytal *et al.*, 2014). While no one has investigated associations between trait anxiety and connectivity of amygdala subregion pathways, one study found that individuals with post-traumatic stress disorder (PTSD) show greater BLA–aMCC connectivity, a pathway linked to the expression of fear (Brown *et al.*, 2014). Further, individuals with high levels of anxiety show reduced activity in the vmPFC (Milad *et al.*, 2009) and reduced connectivity of the amygdala–vmPFC pathway central to downregulating fear responses (Kim *et al.*, 2011; Bijsterbosch *et al.*, 2014).

The purpose of the current study was to examine changes in amygdala subregions and associated vmPFC and hippocampal pathways by measuring resting state connectivity before and after fear extinction. Furthermore, given the strong link between anxiety and aberrant fear extinction, we investigated whether trait anxiety and extinction learning behavioral performance were associated with altered pre- to post-extinction neural changes in fear inhibition and expression circuits. Across the

sample, we hypothesized an enhanced pre- to post-extinction connectivity within networks involved in fear inhibition, as well as the consolidation and retention of extinction learning, including the BLA–vmPFC, BLA–hippocampus, CMA–vmPFC, vmPFC–aMCC, vmPFC–hippocampus, and hippocampus–aMCC. In contrast, we expected a reduced pre- to post-extinction connectivity within the BLA–aMCC network involved in the expression of fear. We predicted that higher levels of trait anxiety and poorer extinction learning performance would be associated with increased pre- to post-extinction connectivity within a pathway that supports the expression of fear (BLA–aMCC pathway) and decreased connections in pathways subserving the regulation of fear (BLA–vmPFC, CMA–vmPFC).

Materials and methods

Participants

One hundred and ten right-handed young adult participants from the University of Wisconsin-Milwaukee student body and local community were scanned. The study was approved by the Institutional Review Board, and participants gave written informed consent according to the Declaration of Helsinki. Participants were excluded if they endorsed a history of head trauma, neurological disorders, psychosis or mania. Participants completed the Mini-International Neuropsychiatric Interview (Sheehan *et al.*, 1998) to assess for current Axis I disorders and history of psychosis or mania. Within this sample, 19.8% were diagnosed with an anxiety disorder, 6.6% with major depressive disorder, 24.2% with an alcohol/substance use disorder and 13.2% were on psychotropic medication. Nineteen participants were excluded for the following reasons: the presence of manic episodes ($n = 1$), technical problems with the scanner ($n = 2$) or electrical stimulation ($n = 2$), excessive motion during scanning ($n = 12$) and a failure to show signs of implicit and explicit learning ($n = 2$) during fear conditioning. This resulted in a final sample of 91 participants (53 F, mean age: 22.05, s.d. = 3.94).

Quantifying trait anxiety

Participants completed the State-Trait Anxiety Inventory-Trait (STAI-T; Spielberger *et al.*, 1983), a 20-item measure of dispositional anxiety. The STAI-T has high internal consistency ($\alpha = 0.89$) and test-retest reliability ($r = 0.88$) (Barnes *et al.*, 2002). For our sample, trait anxiety scores ranged from 21 to 66, with a mean score of 40.21 and an s.d. of 11.13.

Fear conditioning and extinction paradigm

While in the scanner, participants completed a differential fear conditioning and extinction paradigm (see [Supplementary Materials](#)). The conditioned stimuli (CS) were two different fractal displays projected onto a screen (see Schultz *et al.*, 2016, for examples). The UCS was a 500-ms shock delivered to the right ankle and tailored to each individual's tolerance level. Participants then underwent a conditioned fear acquisition protocol that included five presentations of the CS+ (unique display co-terminated with an aversive shock) and five presentations of the CS− (similar image not associated with a shock). For all five CS+ trials, the UCS co-terminated with the CS+. Participants were not given any explicit instruction about CS–UCS associations. Following acquisition, participants completed a 5-min resting state scan during which they were

instructed to close their eyes but remain alert. Participants then completed a fear extinction protocol in which they received five presentations each of the CS+ and CS-, with no presentation of the UCS. After extinction, another 5-min resting state scan was completed. The CSs were presented for 8 s in a quasi-random order followed by a 16–24 s intertrial interval (mean = 20 s). Skin conductance responses (SCRs) and UCS expectancy ratings were collected throughout the task.

Data collection and reduction

Skin conductance. SCRs were recorded from the sole of the left foot (Schultz et al., 2012, 2016). To calculate the SCR amplitude for each trial, the average SCR (in micro Siemens) 2 s prior to the onset of the CS was subtracted from the highest SCR level during the duration of the CS (Milad et al., 2009; Schultz et al., 2012).

Expectancy ratings. Throughout fear acquisition and extinction, participants used a button box to make ratings using a visual analog scale regarding their perceived likelihood of receiving shock on a scale from 0 to 100 (0 = certain that I am not going to get shocked, 100 = certain that I am going to get shocked). The UCS expectancy measure was operationalized as the mean self-reported ratings of the likelihood of getting a shock during the last 4 s of the CS period for each trial for each participant (Schultz et al., 2012). Participants failing to show any differential ratings and < 0.05 μ S SCR difference between CS+ vs CS- were excluded.

MRI pre-processing. Whole brain imaging was conducted using a 3 T short bore GE Signa Excite magnetic resonance imaging system (Waukesha, WI, USA) equipped with an eight-channel head coil. Functional images were acquired using a T2*-weighted gradient-echo, echo-planar pulse sequence. We collected 41 interleaved sagittal slices [Repetition Time (TR) = 2 s; Echo Time (TE) = 25 ms; Field Of View (FOV) = 24 cm; flip angle = 77°; voxel size: 3.5 mm \times 3.5 mm \times 3.5 mm]. High-resolution spoiled gradient recalled (SPGR) images were acquired in a sagittal orientation (TR = 8.2 ms; TE = 3.2 ms; FOV = 24 cm; flip angle = 12°; voxel size = 0.9375 \times 0.9375 \times 1 mm) and served as an anatomical map for the functional images. Analysis of Functional Neuroimages (AFNI) (Cox, 1996) was used to conduct image reconstruction and pre-processing steps for both the task data and the resting state data: (i) slice time correction; (ii) remove first three images to account for scanner equilibration; (iii) rigid-body motion correction in three translational and three rotational directions with all volumes registered to the first volume of each functional run; (iv) T₁-weighted datasets were registered to the MNI152 template via FMRIB's linear image registration tool (FLIRT) and FMRIB's nonlinear image registration tool (FNIRT) (<http://fsl.fmrib.ox.ac.uk>). The transformation matrices were concatenated and applied to the Echo-Planar Imaging (EPI) data in a single step; (v) spatial smoothing with a 4-mm full-width half-maximum; and (vi) motion-censoring images based on the Euclidian norm of the first time differences of motion estimates (based on a Euclidian norm threshold of 0.3 mm) and censoring images when 10% of the automasked brain were outliers. Participants who exhibited excessive head motion (greater than an average value of 2.5 mm translational and/or 2.5° rotation) were excluded from further analysis. For

the conditioning and extinction task data, the blood oxygen level dependent (BOLD) response was modeled 0–14 s after the onset of the CS stimulus using eight tent functions. For the resting state analyses, despiking to remove extreme time series outliers was also conducted. Following these pre-processing steps with AFNI, the CONN toolbox (Whitfield-Gabrieli and Nieto-Castanon, 2012) was used for additional denoising and first-level analysis of the resting state data. In a single first-level regression model, the following steps were conducted simultaneously: (i) detrending; (ii) outlier censoring and motion regression based on estimates obtained from AFNI; (iii) component based noise correction method correction (Behzadi et al., 2007), a component based noise correction method that performs a principal component analysis to estimate physiological noise from white matter and cerebrospinal fluid from each participant; and (iv) bandpass filtering to attenuate signal above 0.1 Hz and below 0.01 Hz

Data analysis

Since the main focus of this study was to understand neural processes supporting fear extinction learning, the fear acquisition imaging analysis information and results can be found in the [Supplementary Materials](#) section. Additionally, given that our hypotheses centered on activation in and connectivity between the BLA, CMA, aMCC, vmPFC and hippocampus, we focused our analysis on these structures.

Skin conductance and expectancy ratings. A separate CS Type by Trial analysis of variance was conducted on the skin conductance and expectancy ratings. All follow-up simple effects tests were Bonferroni corrected for multiple comparisons. To examine associations with trait anxiety, we subtracted mean SCR to the CS- from mean SCR to the CS+. We repeated the same process to generate a CS+ - CS- difference score for expectancy ratings. These difference scores were then correlated with trait anxiety scores. Four participants were not included in the skin conductance analyses due to technical issues with recording skin conductance during scanning.

General imaging analyses: multiple comparison corrections. To correct for multiple comparisons, cluster thresholding via Monte Carlo simulations was conducted using AFNI's updated and improved 3dClustSim program for all imaging analyses (Cox et al., 2017).

Fear extinction imaging. A whole brain CS+ vs CS- t-test was conducted. Then, a whole brain CS+ - CS- difference score was created and correlated with trait anxiety. Given our a priori hypotheses about the role of the CMA, BLA, vmPFC, aMCC, and hippocampus in extinction learning, small volume correction was applied to these structures during extinction learning. The hippocampus/parahippocampus and aMCC masks used for small volume correction were derived from the Eickhoff et al.'s maximum probability maps (Eickhoff et al., 2006). The vmPFC mask was derived from an architectonic parcellation of the human vmPFC in Montreal Neurological Institute (MNI) space (Mackey et al., 2014). Using a voxel-based threshold of $P < 0.005$ and nearest-neighbor selection criteria, accounting for spatial correlation, clusters greater than 344 voxels for the hippocampus/parahippocampus, 332 voxels for the vmPFC and 294 voxels for the aMCC achieved a corrected P -value of < 0.05. BLA and CMA masks were determined using stereotaxic, probabilistic maps of cytoarchitectonic

Participants showed fear conditioning and extinction learning

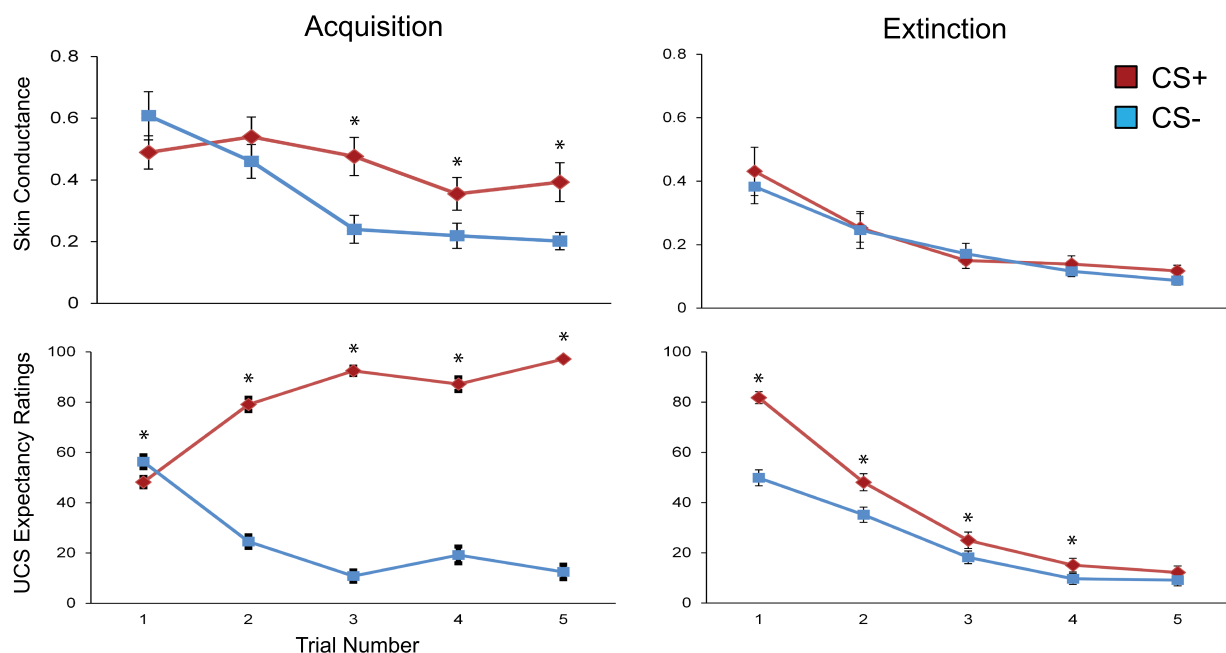


Fig. 1. Behavioral measures of fear acquisition and extinction learning. Top panel on left: there was a significant Type X Trial interaction for SCR during acquisition, $P < 0.001$. During trials 3–5, participants showed greater responses to the CS+ vs CS–, $P_s < 0.002$. Bottom panel on left: for UCS expectancy ratings during acquisition, there was a significant Type X Trial interaction, $P < 0.001$. By trials 2–4, participants showed greater UCS expectancy ratings to the CS+ compared to the CS–, $P_s < 0.001$. Top panel on right: for SCR, there was no significant CS Type main effect or CS Type X Trial interaction, $P > 0.30$ during extinction. Bottom panel on right extinction: average UCS expectancy ratings during extinction showed a significant CS Type X Trial interaction, $P < 0.001$. During the first four trials, participants' expectancy ratings were higher to the CS+ vs the CS–, $P < 0.03$. However, by the last trial, participants no longer showed differential CS+ vs CS– expectancy ratings, $P = 0.12$. Together, this suggests that the participants successfully learned to acquire and extinguish fear responses.

boundaries (Amunts et al., 2005) in AFNI (Eickhoff et al., 2005). BOLD activation from the entire BLA and CMA masks was extracted separately. With the exception of the BLA and CMA, which were derived via probabilistic maps, significant clusters from the extinction imaging analyses served as seeds for the RSFC analyses. See supplemental materials for further information.

Generation of BLA, CMA, aMCC, vmPFC and hippocampus-whole brain connectivity maps. Correlation maps between each of the seeds and the time series from every other voxel in the brain were derived for the pre-extinction resting state scan and the post-extinction resting state scan. The individual r statistics were normalized using a Fisher's z transformation and were resampled to 1 mm^3 voxels (original voxel size: $3.5 \times 3.5 \times 3.5$). Group level statistics were based on the normalized and resampled data.

RSFC. We conducted t -tests to compare each of the normalized seed (BLA, CMA, vmPFC, aMCC, hippocampus/parahippocampus)-whole brain correlation maps during the pre-extinction resting state scan with the normalized seed-whole brain correlation maps during the post-extinction learning resting state scan. In order to examine how changes in amygdala subregion RSFC relate to trait anxiety and extinction performance, each amygdala subregion-whole brain normalized correlation map during the pre-extinction resting state scan was subtracted from the corresponding amygdala subregion-normalized whole brain

correlation map during the post-extinction resting state scan. These difference scores were then correlated with trait anxiety and extinction behavioral measures. Given our a priori hypotheses centered on the amygdala, vmPFC, aMCC and hippocampus, we conducted small volume corrections using the same masks generated for the extinction task imaging analyses. Using a voxel-based threshold of $P < 0.005$ and nearest-neighbor selection criteria, accounting for spatial correlation, clusters greater than 425 voxels for the hippocampus/parahippocampus, 396 voxels for the vmPFC, 360 voxels for the aMCC and 94 voxels for the amygdala (a combined BLA and CMA mask) achieved a corrected $P < 0.05$.

Results

SCRs and UCS expectancy ratings

Across the sample, the behavioral measures showed evidence of fear acquisition and extinction learning. Fear acquisition learning was evident by trials 3–5 (SCR) and 2–5 (Expectancy) as demonstrated by significant CS+ vs CS– comparisons, $P_s < 0.002$ (see Supplemental Materials). For SCR during extinction, there was a significant main effect of Trial, $F(4,344) = 20.35$, $P < 0.001$. There was no significant main effect of CS Type, $F(1,86) = 0.91$, $P = 0.34$ or CS Type X Trial interaction, $F(4,344) = 0.32$, $P = 0.86$. Expectancy ratings showed a significant CS Type X Trial interaction, $F(4,360) = 22.04$, $P < 0.001$. During the first four trials of extinction learning, participants' expectancy ratings were higher to the CS+ compared to the CS–, $P <$

Differential CS+ vs. CS- activation in fear extinction circuitry

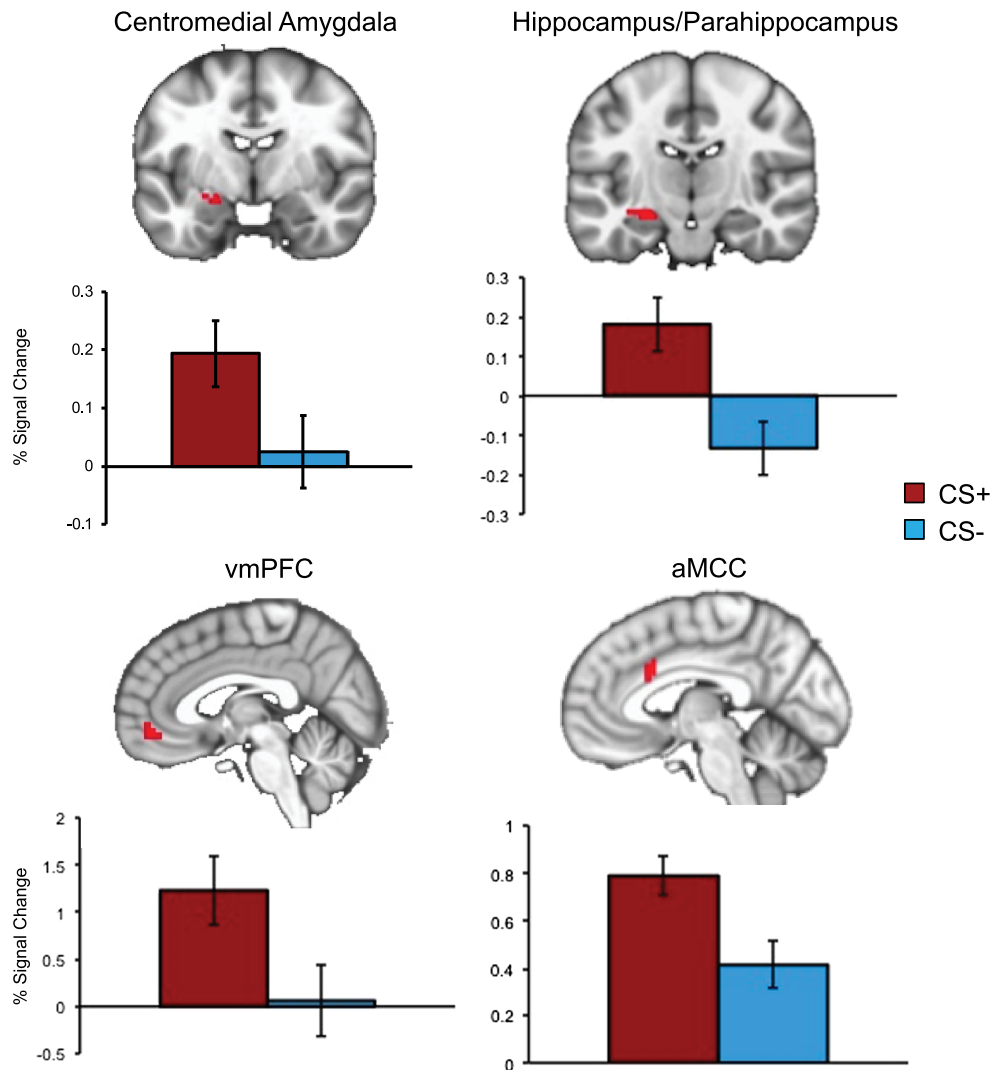


Fig. 2. Participants showed greater activation of the CMA, hippocampus/parahippocampus, vmPFC and aMCC to the CS+ compared to the CS- during fear extinction.

0.03. However, by the last trial, participants no longer showed differential expectancy ratings to the CS+ compared to the CS-, $P = 0.12$ (Figure 1).

Extinction task fMRI

A whole brain paired sample t -test revealed greater BOLD activation to the CS+ compared to the CS- in a priori regions including the bilateral aMCC (left: 395 voxels, 3, -21.5, 26; right: 658 voxels, -10, -21.5, 29), bilateral vmPFC (456 voxels, -3, -46.5, -13) and hippocampus/parahippocampus (left: 1034 voxels, 27, 14, -12; right: 1977 voxels, -24, 25, -13). With respect to the amygdala, the bilateral CMA showed greater activation to the CS+ vs the CS- ($P_s < 0.01$) but not the BLA, $P_s > 0.06$ (Figure 2). Other whole brain corrected results falling outside our a priori regions of interest can be found in the [supplementary materials](#).

Pre- to post-extinction changes in RSFC

Paired sample t -tests were conducted to compare pre- vs post-fear extinction correlation maps between the bilateral BLA, CMA, aMCC, vmPFC and hippocampus/parahippocampus

Region Of Interests (ROI) and the rest of the brain. The right and left hemisphere time courses for each of the seed ROIs were averaged. Beginning with the amygdala, the BLA showed greater post- compared to pre-extinction RSFC with the hippocampus/parahippocampus (left: 832 voxels, 20, 7, -23; right: 592 voxels, -31, 13, -29; Figure 3). The CMA also showed stronger post- vs pre-extinction RSFC with the hippocampus/parahippocampus (left: 741 voxels, 8, 6, -11; right: 624 voxels, -20, 22, -15) and aMCC (left: 410 voxels, 9, -38, 17; right: 1184 voxels, -9, -36, 18). In addition to amygdala subregion connectivity changes, the aMCC also showed predicted increases in pre- to post-extinction RSFC with the hippocampus/parahippocampus (left: 576 voxels; 13, 19, -20). See the supplementary materials for additional whole brain corrected results.

Task and RSFC relationships with trait anxiety and behavioral performance

During fear extinction, there were no significant correlations between trait anxiety scores and skin conductance or

Pre- to Post-extinction strengthening in networks supporting consolidation of learning and downregulation of fear

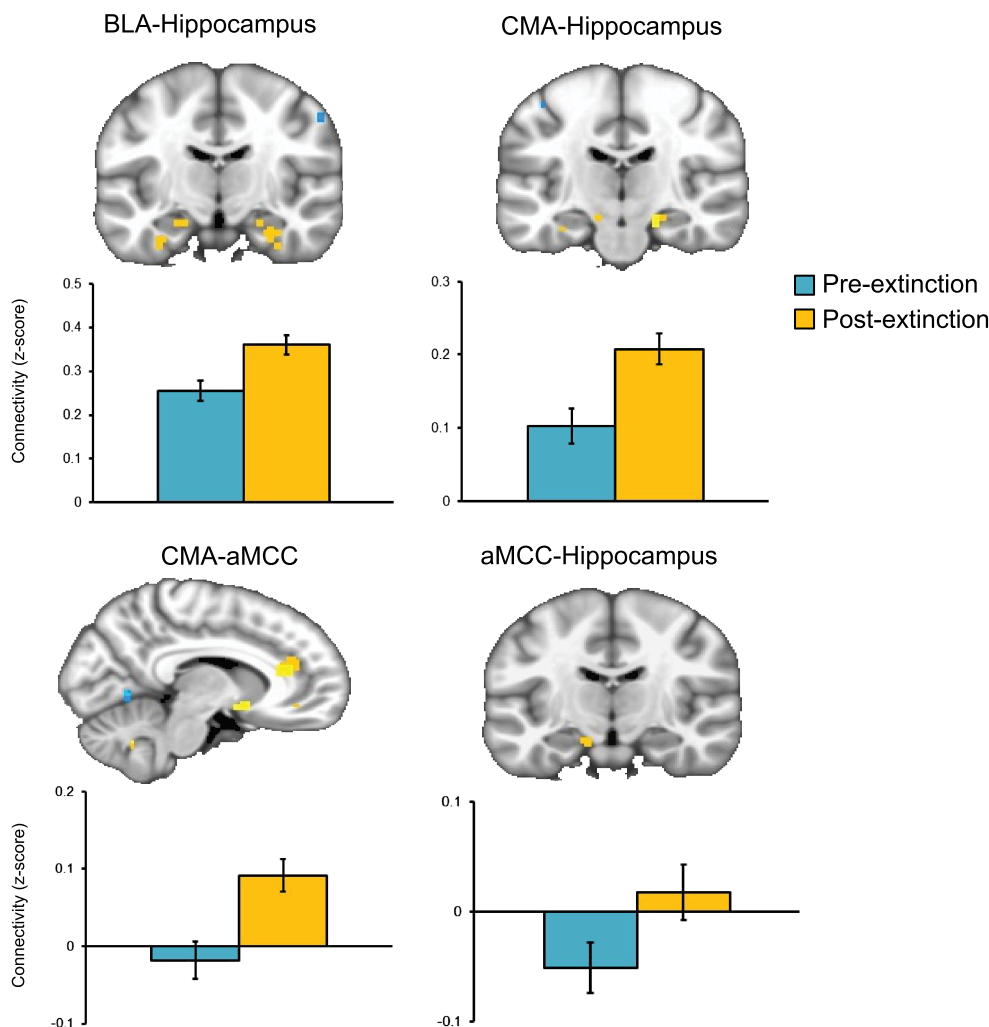


Fig. 3. Top panel: participants showed pre- to post-fear extinction strengthening of resting state connectivity in the BLA-hippocampus/parahippocampus pathway and CMA-hippocampal pathway. Bottom panel: participants demonstrated strengthening in connectivity of the CMA-aMCC and aMCC-hippocampal/parahippocampal pathways from pre- to post-fear extinction learning.

expectancy rating CS+ – CS– difference scores or CS+ and CS– alone, all $P > 0.400$. Additionally, with respect to extinction-related CS+ vs CS– brain activation, there were no significant associations between trait anxiety and a CS+ – CS– difference score for each of the amygdala subregions, a whole brain CS+ – CS– difference score or with significant clusters from the CS+ vs CS– extinction contrast. Thus, trait anxiety was not associated with the relative difference in CS+ vs CS– activation during extinction.

However, when analyzing the CS+ and CS– separately, we did find significant associations between trait anxiety and BOLD activation. Trait anxiety was positively correlated with greater activation to the CS+ in a priori regions including the left vmPFC (1398 voxels, 11, –56.5, –20; $r = 0.34$, $P = 0.001$), right vmPFC (893 voxels, –13, –46.5, –16, $r = 0.32$, $P = 0.002$) and right dorsal anterior cingulate cortex/aMCC (1157 voxels, –10, –39.5, –6.0, $r = 0.39$, $P < 0.001$) (Figure 4). For the correlation between trait anxiety and vmPFC activity to the CS+, a significant out-

lier was found (3 s.d.s above mean BOLD activation). Therefore, these results were examined again without this outlier. The results remained significant without the outlier participant (left vmPFC: $r = 0.32$, $P = 0.002$; right vmPFC: $r = 0.28$, $P = 0.007$). Higher trait anxiety was also significantly correlated with greater activation to the CS– in the left aMCC (984 voxels, 22, –39.5, 22, $r = 0.33$, $P = 0.001$) (Figure 4). Thus, the lack of relationship between trait anxiety and the CS+/CS– difference score may be due to the tendency for anxious individuals to show greater reactivity to CSs representing both threat and safety.

With respect to RSFC changes, as expected, higher trait anxiety was associated with stronger post- vs pre-extinction RSFC between the BLA and the aMCC (left: 656 voxels; 12, –7, 30, $r = 0.38$, $P < 0.001$), a pathway known to be involved in the expression of fear (Figure 5). However, contrary to our predictions, trait anxiety was not associated with decreased RSFC of pathways important to the inhibition of fear responses. Behavioral indi-

Trait anxiety is associated with greater BOLD activation in the aMCC and vmPFC during fear extinction

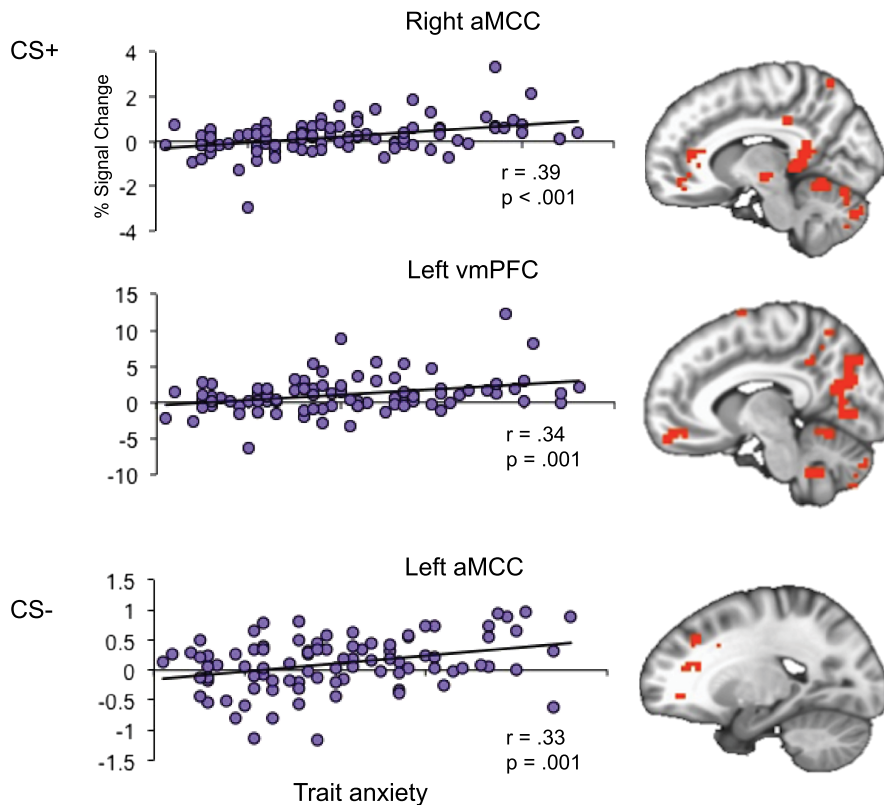


Fig. 4. Higher levels of trait anxiety were associated with greater activation within the right aMCC, the left vmPFC and the right vmPFC (not shown) to the CS+. Higher levels of trait anxiety were also associated with greater activation of the left aMCC to the CS-.

cators of extinction learning were also linked to RSFC changes within a fear inhibition pathway. Specifically, weaker post- vs pre-extinction BLA–parahippocampus/hippocampus connectivity (right: 840 voxels, 13, 17, -9, $r = -0.520$, $P < 0.001$; 576 voxels, -32, 31, -16, $r = -0.40$, $P < 0.001$) was associated with greater CS+ vs CS- SCR differences (i.e. poorer extinction learning, see Figure 6). There were no significant associations between CS+ - CS- UCS expectancy differences during extinction with amygdala RSFC changes. All of the above BOLD activation and RSFC results survived when removing participants taking medications and those with substance use disorders, as well as anxiety disorders, and when controlling for individual differences in percentage of motion-related outliers removed, all $P < 0.05$. Other whole brain corrected results that were outside the regions of interest can be found in the [supplementary materials](#).

Additional multiple comparison corrections

Given that RSFC correlation maps were generated from five ROIs, we also conducted more stringent multiple comparison corrections to correct for these additional tests. This was done by conducting a Bonferroni correction with our voxel-based threshold of $P < 0.005$ ($0.005/5$). Thus, the results had to survive a P threshold of $P < 0.001$. Indeed, all of the findings survived this more conservative threshold.

Discussion

Our primary aim was to examine how extinction learning affected connectivity in fear expression and inhibition pathways and how these changes in connectivity were associated with dispositional anxiety. Using a novel resting state paradigm, we found that a fear extinction training strengthened connectivity in hippocampal pathways thought to subservise adaptive regulation of fear. Although, somewhat surprisingly, there were no significant pre- to post-changes in vmPFC associated pathways. This may be due to the critical role of the vmPFC in extinction recall, not the acquisition of fear extinction learning *per se* (Milad et al., 2007; Quirk and Mueller, 2008). Moreover, trait anxiety and extinction performance were associated not with weakening of fear inhibition pathways but with strengthening of fear expression pathways as a function of extinction. Thus, these results help characterize RSFC changes that support extinction learning in humans and highlight aberrations in these network changes associated with one of the key risk factors for anxiety pathology.

Consistent with our predictions, there was an increased pre- to post-extinction connectivity of the BLA–hippocampus pathway. Supporting animal work (Baldi et al., 2015) suggests that strengthening of the BLA–hippocampus network following extinction learning may reflect plastic processes that mediate the consolidation of this learning. While we

Trait anxiety is associated with stronger pre- to post-extinction BLA-aMCC connectivity

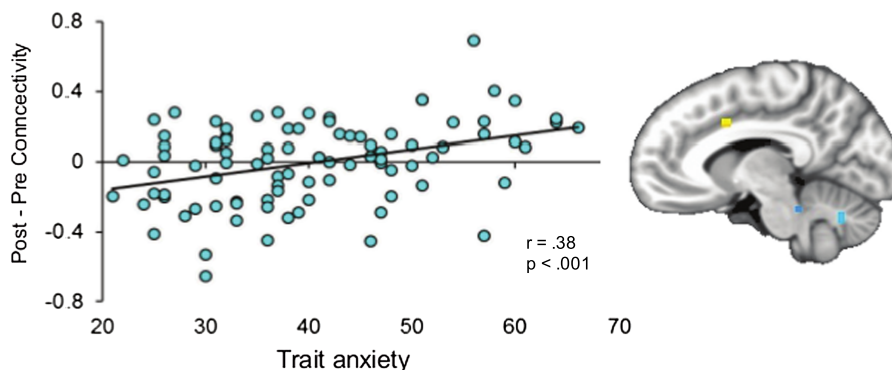


Fig. 5. Higher trait anxiety was associated with stronger post- minus pre-fear extinction connectivity of a BLA-aMCC pathway.

Greater CS+ vs. CS- SCR during extinction is associated with weaker pre- to post-extinction BLA-hippocampus/parahippocampus connectivity

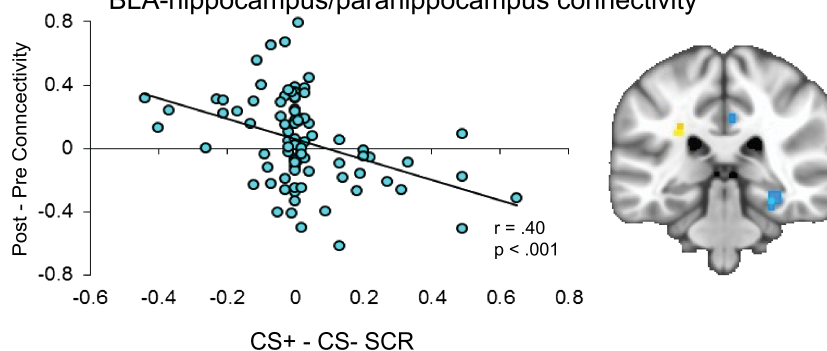


Fig. 6. Poorer extinction learning (a greater CS+ – CS– SCR difference score) was associated with weaker pre- to post-extinction.

did not find a pre- to post-extinction dampening of a BLA-aMCC pathway, changes in this pathway were linked to trait anxiety (discussed below). Additionally, as hypothesized, we found an increased pre- to post-extinction connectivity of a hippocampus-aMCC pathway. The hippocampus has been shown to inhibit activation of the aMCC, (Etkin et al., 2011; Baldi et al., 2015), a brain region critical to the expression of fear responses (Milad et al., 2009). Taken together, these findings and ours suggest that fear extinction promotes strengthening of neural networks that are critical for adaptive regulation of fear responses.

With respect to trait anxiety, we did not find any anxiety-related differential neural responses to the CS+ compared to the CS–, which is inconsistent with prior research (Barrett and Armony, 2009; Sehlmeier et al., 2011). Unlike prior studies (Barrett and Armony, 2009; Sehlmeier et al., 2011), we used a 100% CS+–UCS reinforcement schedule, which may have been a manipulation strong enough to impede the ability to detect differences associated with anxiety (Lissek et al., 2006). However, when examining activation to CS+ and CS– separately during extinction, we found that trait anxiety was associated with greater aMCC activation to both the CS+ and CS– and vmPFC activation to the CS+. Previous research has shown that anxiety is associated with aberrant responding to the CS– (Gazendam et al., 2013; Duits et al., 2015);

therefore, examining difference scores may obscure trait anxiety relationships. One possible mechanism that may explain these findings is fear generalization (Lissek et al., 2008; Dunsmoor et al., 2011; Haddad et al., 2012). Studies of fear generalization have demonstrated that successful discrimination learning is associated with declines in aMCC and amygdala activity, and increases in vmPFC activity as the generalized stimulus becomes less perceptually similar to the CS+ (Lissek et al., 2008; Dunsmoor et al., 2011). However, the neural correlates of this process are altered in those with anxiety problems (Greenberg et al., 2013; Cha et al., 2014; Morey et al., 2015). Although fear generalization was not directly examined in our study, the results may reflect deficient discriminatory learning.

In addition to anxiety-related activation differences during extinction, we found that those with higher trait anxiety had stronger pre- to post-extinction connectivity between the BLA and aMCC. This BLA-aMCC pathway has been implicated in the expression of fear responses (Senn et al., 2014), and individuals with PTSD show greater RSFC within this circuit (Brown et al., 2014). Thus, more robust BLA-aMCC connectivity likely contributes to the sustained fear that characterizes individuals with problematic anxiety.

Surprisingly, trait anxiety was not associated with less robust pre- to post-extinction strengthening of connectivity within

our hypothesized fear inhibition pathways, the BLA–vmPFC and CMA–vmPFC. Our predictions were based on previous findings, but this earlier work focused on associations between trait anxiety and connectivity in a single resting state scan (Kim et al., 2011; Bijsterbosch et al., 2014) and not within the context of extinction learning. Based on an integration of numerous studies, Admon et al. (2013) developed a model in which dysfunction in the amygdala and aMCC serve as predisposing factors to PTSD, whereas vmPFC deficits are acquired after the full development of the disorder. Thus, it is possible that pre- to post-extinction strengthening in the BLA–aMCC pathway seen in high trait anxious individuals serves as a vulnerability marker for anxiety pathology. However, deficits in vmPFC fear inhibition circuits may be more likely to be evident in those with clinically significant anxiety.

With respect to behavioral indices of fear extinction learning performance, less pre- to post-extinction connectivity of a fear inhibition pathway was also linked to poorer extinction learning. Specifically, a greater SCR to the CS+ compared to the CS– during extinction was linked to weaker pre- to post-extinction BLA–hippocampal/parahippocampal connectivity. Given the importance of the BLA–hippocampus pathway to fear extinction consolidation (Orsini and Maren, 2012), weaker pre- to post-extinction connectivity within this pathway likely reflects poorer ability to consolidate fear extinction learning.

Our study had some notable strengths, including the use of a novel paradigm to study plastic processes linked to fear extinction learning in a relatively large sample stratified on trait anxiety and theory-based examination of amygdala sub-region pathways. However, the addition of a baseline resting state scan before fear acquisition or a control task would have helped to clarify which neural changes were uniquely associated with extinction learning. That said, the neural changes found in this study differ from prior studies examining resting state changes following fear acquisition learning (Feng et al., 2014; Schultz et al., 2012) and are more consistent with plastic changes associated with extinction learning (Kalisch et al., 2006; Milad et al., 2007; Lang et al., 2009). Additionally, RSFC changes were linked to extinction-related behavioral indices, which suggest that these neural changes are related to extinction learning processes.

In sum, our study provides novel evidence for fear extinction-related plasticity of networks thought to be critical for the consolidation and retention of this learning, as well as the down-regulation of fear. These changes were also linked to extinction-related behavior (e.g. SCR to CS+ vs CS–). Moreover, our data suggest that trait anxiety is linked to aberrant strengthening of fear expression circuits, which likely undermines the fear inhibition network's attempts to downregulate fear responses. This study is a first step toward understanding plastic neural processes supporting adaptive fear regulation. Future work should clarify how these extinction-related resting state connectivity changes may be related to aberrant retention learning in those with problematic anxiety. The results may inform research examining biomarkers of anxiety disorders as well as ways to optimize treatment for these disorders via techniques that impact connectivity in these fear inhibition and expression pathways.

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