Neural changes in extinction recall following prolonged exposure treatment for PTSD: A longitudinal fMRI study ☆

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ABSTRACT

Background: Neurobiological models of posttraumatic stress disorder (PTSD) implicate fear processing impairments in the maintenance of the disorder. Specific deficits in extinction recall, the retention of learned extinction, have been demonstrated. While deficient extinction recall, and the associated activation pattern of prefrontal and hippocampal regions, distinguishes individuals with PTSD from controls, research has not yet examined changes following treatment. We examined the behavioral and neural correlates of extinction recall before and after cognitive behavioral treatment of PTSD.
Methods: Fifty-eight participants (30 with PTSD, 28 trauma-exposed matched controls) underwent a 2-day behavioral fear conditioning, extinction, and recall paradigm during functional magnetic resonance imaging (fMRI). The same procedures were repeated 10 weeks later, after PTSD patients had completed prolonged exposure treatment. We analyzed fMRI data from 32 subjects (16 PTSD; 16 controls) and skin conductance response (SCR) data from 33 subjects (16 PTSD; 17 controls). Neural activity during extinction recall, SCR, and PTSD symptoms were compared across groups and over time.
Results: PTSD patients exhibited pre- to post-treatment reduction in rostral anterior cingulate cortex (rACC) activation during extinction recall, and increase in functional coherence between the rACC and the ventromedial prefrontal cortex (vmPFC) and subgenual anterior cingulate cortex (sgACC). Reduced PTSD symptom severity from pre- to post-treatment was significantly associated with reduced subgenual ACC and parahippocampal activation during this task. SCR during the extinction recall phase did not significantly change with treatment in the PTSD group, but change in SCR was associated with reduction in PTSD symptom severity.
Conclusions: Prolonged exposure treatment appears to alter neural activation in PTSD patients during recall of fear extinction, and change in extinction recall (measured by SCR) is associated with symptom reduction. We discuss results in the context of neural systems involved in response to affective stimuli.

1. Introduction

Posttraumatic stress disorder (PTSD) is a chronic and serious disorder associated with suicidal risk, functional impairment, and medical comorbidity (Breslau et al., 1998; Kessler, 1995; Shvil et al., 2014). Fearful responses to trauma reminders in PTSD include intrusive thoughts and feelings, avoidance of such reminders, and various alterations in cognitions, mood, arousal, and reactivity (Diagnostic and Statistical Manual of Mental Disorders, 5th Edition, 2013).

Neurobiological models of PTSD implicate fear and safety learning processes in the persistence of, and recovery from, PTSD. Among these are impaired fear inhibition (Jovanovic et al., 2010), excessive fear generalization (Lissek and Grillon, 2012), and difficulty in the maintenance of learned fear extinction, also termed extinction recall (Milad et al., 2008). Such impairments have been shown to distinguish PTSD from non-PTSD controls (Jovanovic et al., 2013; Milad et al., 2008), but

research has yet to assess the relationship between changes in these impairments and PTSD treatment. Testing whether PTSD treatments that target fear processing deficits enhance neural functioning in brain regions associated with fear processing may meaningfully improve our understanding of the underlying mechanisms and neural targets of PTSD and its treatments. The current study examined whether prolonged exposure (PE; Foa et al., 2007), a therapy targeting fear processing through gradual habituation to traumatic memories and their reminders through repetitive imaginal and in vivo exposure within a safe context, enhanced extinction retention and associated neural activations.

1.1. Neural underpinnings of extinction

Fear extinction refers to the ability to learn to suppress a fear response to a previously conditioned stimulus (i.e., a stimulus previously repeatedly presented paired with a fear eliciting cue, such as an electric shock or a loud sound) through gradually reducing the fear-eliciting capacity of that stimulus by repeatedly presenting it with no paired fear-eliciting cue (Sotres-Bayon et al., 2004). The neural circuits in fear learning have been well established through animal and human research. They involve the amygdala, arguably the main subcortical structure involved in emotional processing (Gallagher and Chiba, 1996; LeDoux, 2007; Phelps and LeDoux, 2005); several prefrontal cortical regions (PFC), and the hippocampus. These areas, unsurprisingly, are involved in fear extinction and extinction recall: medial prefrontal cortex (mPFC), and specifically its ventral aspect (vmPFC), appear key to extinction learning. Neural activity in mPFC increases during extinction learning (Milad and Quirk, 2002; Rosenkranz et al., 2003), direct electrical stimulation of mPFC facilitates extinction learning (Garcia, 2002), and vmPFC damage alters rats' capacity for extinction learning (Morgan et al., 1993, 2003; Morgan and LeDoux, 1995; Quirk et al., 2000). Research specifically implicates hippocampus in context learning (Maren and Fanselow, 1995), with hippocampal inputs to vmPFC apparently performing a gating function for extinction by providing contextual information (Sotres-Bayon et al., 2004). Accumulating evidence indicates the more specific involvement of vmPFC in the memory or retention of learned extinction (i.e., extinction recall) (Lissek et al., 2013; Milad et al., 2007).

1.2. Impaired extinction recall in PTSD

PTSD patients exhibit impaired capacity to recall extinction memory, as evinced by increased skin conductance responses (SCR) when previously extinguished fear stimuli are re-presented the next day (Milad et al., 2009b). In functional magnetic resonance imaging (fMRI) studies, PTSD patients have reduced hippocampal and vmPFC activation, and greater dorsorostral ACC (drACC) activation, during extinction recall (Milad et al., 2009a,b; Shvil et al., 2014), possibly indicating lower capacity for regulation of fear response.

1.3. Extinction-based treatment for PTSD

Several behavioral therapies for anxiety disorders purportedly rely on extinction via habituation through repetitive exposure to threatening cues, memories, or events within a safe context (Rothbaum and Davis, 2003), and an extinction of conditioned fear task may be considered a laboratory analog of exposure procedures in psychotherapies such as PE, a first line psychotherapy for PTSD (Blechert et al., 2007; Bouton et al., 2001). Although PE benefits many patients, almost half exhibit persistent PTSD (Ponniah and Hollon, 2009). Elucidating whether PE treatment reverses extinction deficits and normalizes activity in associated neural circuitry would advance understanding of the neural basis of PTSD and provide a clinically meaningful biological marker of treatment response.

1.4. Functional neural changes following treatment of PTSD

Several studies examined functional brain changes after treatment among patients with PTSD. Findings include increased activation in varied fear-related structures: lateral (Farrow et al., 2005; Felmingham et al., 2007; Lansing et al., 2005; Peres et al., 2007b) and medial (Peres et al., 2007b, 2011) prefrontal regions; anterior cingulate cortex (ACC) (Felmingham et al., 2007; Peres et al., 2007b), thalamus (Peres et al., 2007b; Peres et al., 2011), and hippocampus (Felmingham et al., 2007; Peres et al., 2007b). Decreased activation following psychotherapy was found in lateral prefrontal structures (Lindauer et al., 2007), insula (Aupperle et al., 2013; Peres et al., 2007a, 2011), thalamus (Lansing et al., 2005), and amygdala (Felmingham et al., 2007; Peres et al., 2007b, 2011). Although highly informative in defining medial and lateral prefrontal regions, as well as emotion-related subcortical structures, as ROIs in PTSD treatment, these studies have limitations. Treatments and activation tasks have varied across studies; only a few studies used fMRI (Farrow et al., 2005; Felmingham et al., 2007; Peres et al., 2011), and none to date employed a fear learning-extinction-retention task before and after treatment, or prolonged exposure therapy.

1.5. Current study aims

This study sought to address gaps in knowledge by examining whether: 1) PE improves extinction recall and related neural changes in circuitry associated with fearful emotional response (e.g., mPFC, ACC, amygdala, and hippocampus), and 2) neural and psychophysiological changes in extinction recall are associated with clinical improvement. We hypothesized that: 1) Physiological response during extinction recall (skin conductance) would decrease following treatment; 2) Hippocampal and vmPFC activation would increase, and rostral ACC activation would decrease, during extinction recall; and 3) all of these changes would be associated with reduction in PTSD symptoms. To test these hypotheses we used fMRI and an established 2-day extinction learning and recall paradigm (Graham and Milad, 2013; Milad et al., 2005, 2009a,b, 2013) to assess psychophysiological and neural changes post PE treatment. Functional MRI data were acquired simultaneously with fear extinction recall quantified physiologically by skin conductance response (SCR). All PTSD, and control subjects repeated these procedures 10 weeks later, after PTSD subjects had completed 10 weeks of PE treatment.

2. Materials and methods

2.1. Participant recruitment and assessments

PTSD patients and trauma-exposed, medically healthy controls (TE-HC) matched on gender, age at exposure to trauma, trauma type (interpersonal vs. non-interpersonal) and duration, race, and ethnicity, were recruited via advertisement and fliers. All participants met DSM-IV PTSD criterion A1 for adult traumatic events, including vehicular accidents, sexual or physical assaults, and witnessing serious injuries or deaths. Medical history, review of systems, physical examination, and laboratory tests determined all participants' health status. Three trained raters administered the Structured Clinical Interview for DSM-IV Axis I Disorders (SCID), Hamilton Depression Rating Scale (HAM-D-17; Hamilton, 1960), and Clinician-Administered PTSD Scale (CAPS; Blake et al., 1995) for DSM-IV to assess PTSD diagnosis and clinical severity. A senior clinician trained raters to administer these instruments, and interrater reliability scores, obtained by pairs of the three participating raters, ranged between 0.92 and 0.99 for clinician-administered instruments.

PTSD subjects, included only following clinician diagnosis of PTSD and CAPS score of ≥50, were excluded with diagnosis of psychosis, substance/alcohol dependence within the past six months or abuse within past two months, use of any psychotropic medication in the 4 weeks

preceding participation (6 weeks for fluoxetine), or HAM-D-17 score > 24. TE-HC exclusion criteria were any current or past Axis I disorders, and CAPS score > 19 (considered symptomatic) (Blake et al., 1995). The New York State Psychiatric Institute Institutional Review Board approved all procedures, and all participants provided written informed consent for this clinicaltrials.gov-registered trial (identifier NCT01576510). Eighty-five participants consented (PTSD = 48), including 57 women, with a mean age of 35.73 years (SD = 10.20) and a primary trauma (on which treatment would focus) that occurred a mean of 7.80 (SD = 7.60) years prior to treatment. Participants had been exposed to a mean 3.20 (SD = 3.50) traumatic events.

Dropout yielded a completer sample of 58 subjects: 30 patients with PTSD and 28 TE-HCs (Fig. 1). PTSD and TE-HC participants were matched for age, gender, and ethnicity, and the completer samples also did not differ on these variables; see Table 1. Dropouts and completers did not differ significantly on any clinical or demographic variables.

2.2. Treatment

PTSD patients entered treatment with one of two trained therapists who adhered to the 10-week standard PE protocol (Foa et al., 2007), wherein patients repeatedly recount the traumatic experience (imaginal exposure) and confront previously avoided trauma reminders (in vivo exposure) to extinguish fear responses. Therapists had treated two pilot cases under supervision to confirm expertise and were continuously monitored and supervised by PE experts for adherence and competence. To this end, independent assessors used the PE integrity measure (Foa et al., 2005) to rate audiotaped sessions.

2.3. Task procedures

An established computer-based paradigm, completed in the MR scanner, was used to assess fear conditioning and extinction on day 1 and extinction recall on day 2 (Milad et al., 2007). The three conditioned stimuli (CSs), presented on a screen, were differentiated by the color of a lamp (red, blue, or yellow), which turned on and off in two different

Table 1
Demographic and clinical characteristics of completers.

	TEHC (N = 28)		PTSD (N = 30)		F(1, 58)	p
	M	SD	M	SD		
Age (years)	35.61	9.36	35.31	9.89	0.06	0.81
Education (years)	16.11	1.77	14.53	2.06	9.65	0.00
CAPS	5.15	6.39	78.53	16.31	463.97	0.00
HAM-D	2.42	2.60	16.07	5.46	135.64	0.00
Primary trauma age (years)	26.43	9.45	28.90	10.02	0.93	0.34
Life events-count of all events	2.46	2.47	3.80	4.40	1.99	0.16
Reduction in CAPS over the course of 10 weeks (%)	12.56	136.30	64.00	29.99	3.84	0.06
	N	%	N	%	χ^2	p
Sex					0.03	0.55
Female	20	71.43%	22	73.33%		
Male	8	28.57%	8	26.67%		
Ethnicity					4.41	0.33
White	10	35.71%	9	30.00%		
Asian or Pacific Islander	1	3.57%	1	3.33%		
Hispanic	9	32.14%	14	46.67%		
Black	8	28.57%	4	13.33%		
Other	0	0.00%	2	6.67%		

Note: CAPS = Clinician Administered PTSD Scale; HAM-D = Hamilton Depression Rating Scale 17 item score; CAPS and HAM-D scores indicate severity of symptoms (e.g., higher score corresponds to worse symptoms); Questions regarding trauma adapted from the PTSD module used in the National Comorbidity Study (Kessler et al., 1995).

rooms. A 500 ms shock delivered via electrodes attached to the second and third fingers of the dominant hand served as the unconditioned stimulus (US). Participants selected the US intensity, ranging from 0.8 to 4.0 mA, following instructions to choose a level that was highly annoying but not painful. Shock levels chosen ranged from 1.1 to 4 mA, averaging 2.7 mA (SD = 1.2) for the pre-treatment session and 2.7 mA (SD = 1.1) for the post-treatment session. Shock levels were compared using t-tests and did not differ between the two sessions (p = 0.93) or between the PTSD and TE-HC groups (p = 0.17 pre-treatment, p = 0.98 post-treatment). Following the US calibration, a habituation phase comprised 12 counterbalanced CS trials (4 of each of the three CS types).
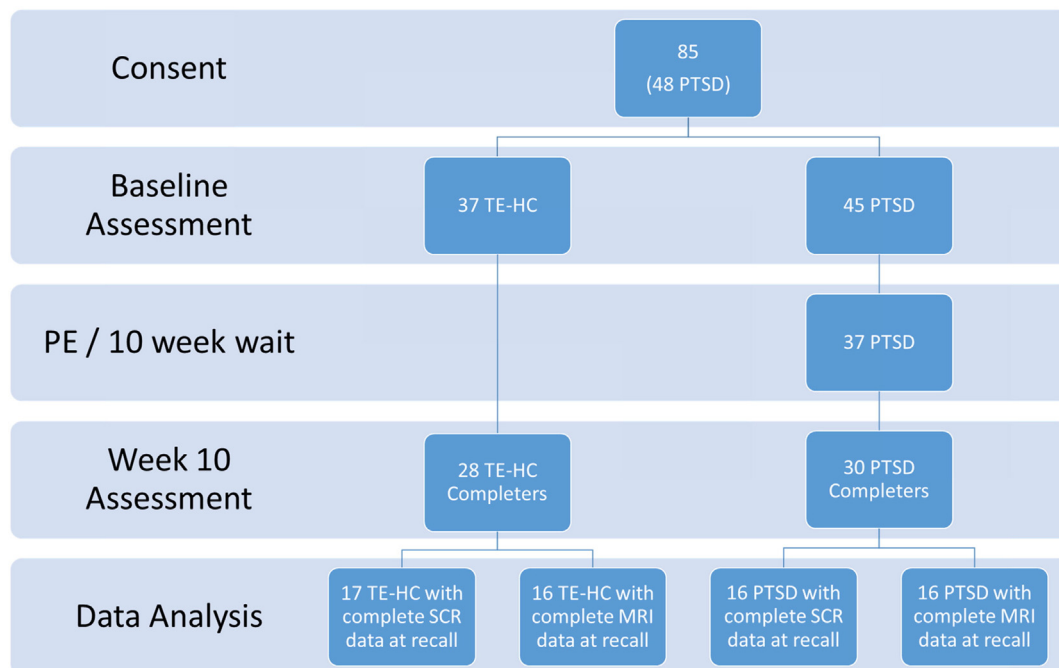
Fig. 1. Participant retention throughout the study. Note: PTSD = Posttraumatic stress disorder; TE-HC = trauma exposed healthy controls; PE = prolonged exposure; SCR = skin conductance response; MRI = magnetic resonance imaging. *Differences in numbers between stages due to drop out (3 after consent, 8 after baseline assessment, 6 during treatment/wait period, and 2 did not complete end of treatment assessment). **Note: SCR and fMRI data overlap included: 11 TE-HC and 6 PTSD participants.

During the conditioning phase, each of the two CS + s (red and blue lights) was paired with the US at a partial reinforcement rate of 62.5%. One of the CS + s was extinguished during the subsequent extinction phase on Day 1 (CS + E), while the other was not extinguished (CS + NE). The two CS + s were presented in blocks of eight trials (8 CS + E, and 8 CS + NE) intermixed with eight CS − (yellow light) presentations that were never paired with the US, for a total of 16 trials (8 for each CS). After conditioning, a 5-min gap preceded the onset of extinction training, which consisted of 16 unreinforced presentations of CS + E intermixed with 16 presentations of the CS −. On day 2, participants were tested for extinction recall with blocks of 8 trials of the extinguished CS + E, and 8 trials of the non-extinguished CS + NE intermixed with an equal number of CS − trials in each block, counterbalanced between subjects. Shock electrodes remained attached to the subject's fingers through the entire experiment, and subjects were told, "You may or may not receive an electric shock." The mean inter-trial interval was 15 s (range: 12–18 s).

2.4. Skin conductance response (SCR) assessment

Skin conductance responses (SCR) for each CS trial were calculated by subtracting the mean skin conductance level (SCL) during the 2 s period before CS onset from the peak SCL during the 6 s CS duration (Milad et al., 2009a,b). Further, the raw SCR were square root transformed prior to analysis.

Conditioning was assessed by comparing the mean SCR amplitude of all CS + trials with the mean SCR amplitude of all CS − trials, and extinction was assessed by comparing the mean SCR amplitude of the last 8 CS + trials with the mean SCR amplitude of the last 8 CS − trials for the extinguished stimulus only (Marin et al., 2016; Milad et al., 2013). Extinction recall was assessed by subtracting the mean SCR amplitude of the first 4 CS + E (not extinguished CS+) trials from the first 4 CS + NE (extinguished CS+) trials (Milad et al., 2009a,b).

2.5. fMRI assessment

All images were acquired on a 1.5 T GE Twin Speed MR Scanner operating on the Excite 3 12.0 M4 HD platform using an 8-channel head coil. An Integrated Functional Imaging System (IFIS, MRI Devices Corp.) synchronized the behavioral paradigm with scanning and stimuli presentations. A high-resolution T1-weighted 3D MPRAGE sequence was acquired for each subject for spatial normalization and anatomical localization (repetition time = 7.25 ms, echo time = 3 ms, Flip angle = 7°, field of view = 25.6 cm, 256 × 256 pixel matrix, slice thickness = 1 mm). Functional MRI images (using blood oxygenation level dependent [BOLD] contrast) were acquired using gradient echo T2*-weighted sequence (TR/TE/Flip angle = 3 s/30 ms/90° [Kwong et al., 1992]). The functional images were collected in the same plane (45 coronal oblique slices parallel to the anterior-posterior commissure line, tilted 30 anterior, 3 × 3 × 3 mm voxels). Identical scanning procedures were conducted on Day 1 and Day 2.

2.6. Statistical analyses

Quality control assessment was performed on the skin conductance data. Due to data corruption (4 PTSD, no TE-HC) and lack of response to the UC stimulus (SCR < 0.05) during the conditioning phase (10 PTSD, 11 TE-HC), a total of 25 participants were excluded from the SCR analyses (for exclusion of data points at different study stages, see Fig. 1). Analyses examining potential differences between SCR responders and non-responders revealed no group differences (see Table S1 in Supplementary Materials).

To examine whether participants exhibited intact conditioning and extinction, separate analyses were conducted for each phase at baseline [N = 47 (24 PTSD)] and follow-up [N = 42 (20 PTSD)] with mixed-effects ANOVAs used to test the effect of Group, Stimulus, and Group-by-

Stimulus interaction. To gauge SCR pre-to-post-treatment changes during the recall phase, a mixed-effects ANOVA was conducted with Time as a within-subject repeated measure, Group as a between-subject variable, and Recall (defined above) as the dependent variable [N = 46 (24 PTSD) at baseline, N = 39 (18 PTSD) at follow-up], with Time-by-Group interactions included in the model for the 33 participants (16 PTSD) with complete data for both time points. Difference between pre- and post-treatment SCR, operationalized as pre-to-post change in recall, was then correlated with change in PTSD symptom severity (CAPS total score) to gauge the relationship between changes in recall and changes in symptoms following treatment.

Prior to analyses, all fMRI images were preprocessed using standard procedures in SPM8. All images were slice-time and motion corrected, and coregistered to their own structural images. The coregistered images were warped into MNI space and smoothed with an 8 mm full-width half-max kernel. All analyses were performed on the smoothed images. A general linear model (GLM) was fit separately for each subject, with task-related regressors created using SPM's canonical double-gamma hemodynamic response function. Each subject-specific GLM included regressors to control for head motion (24 per run, including 6 motion regressors derived from realignment parameter estimates, squared motion estimates, and squared derivatives), as well as dummy regressors to account for outlier images identified as having a significantly greater Mahalanobis distance compared to the other images (FDR-corrected p < 0.05). Within-subject contrasts were generated for each subject separately for extinction recall (first four CS + E vs. first four CS + NE). These contrasts parallel those used in the SCR analyses. Paired t-tests were performed for each group separately, TEHC and PTSD, to compare differences between baseline and follow-up activations. For all fMRI analyses, an initial threshold of p < 0.005 with a minimum of 10 contiguous voxels was used to identify significant activations within the nodes of the fear extinction network. Small volume corrections were then performed, and only results surviving family-wise error (FWE) corrections of p < 0.05 are reported.

Seventeen participants (11 PTSD, 6 TEHC) were excluded from the baseline fMRI analyses, and 15 participants (9 PTSD, 6 TEHC) were excluded from the follow-up fMRI analyses, leaving 16 TE-HC and 16 PTSD participants with complete pre/post data. Participants were excluded due to artifacts in images caused by a radiofrequency noise leak in the shock-delivery equipment, which was subsequently addressed. They were thus not excluded for reasons related to group status, symptom severity, or treatment effects, and available evidence indicates that they are missing at random. We compared demographic, clinical, and SCR data between subjects included (n = 32) and those omitted from the analysis (n = 26), finding no differences. We conducted psychophysiological interactions (PPI) analyses for the PTSD cohort, including only subjects who had fMRI data for the recall phase at both baseline and follow-up (n = 16). As a seed, we used the region that showed a significant change from baseline to follow-up during recall (contrast CS + E vs CS + NE) in that same cohort (right rACC, see the Results section). The seed was defined as a 5 mm sphere centered around the peak voxel resulting from that contrast (MNIxyz = 16, 42, 20). We then performed PPI analyses using the generalized PPI toolbox (McLaren et al., 2012) for each subject at baseline and at follow-up during recall for the contrast CS + E vs eCS + NE. These first-level maps for each individual subject were used to build a second-level map, where positive activations and negative activations relative to the seed were performed at baseline vs. follow-up contrast. We used an initial threshold of p < 0.005 with a minimum of 10 contiguous voxels to identify significant activations within the nodes of the fear extinction network. Small volume corrections were then performed, and only results surviving family-wise error (FWE) corrections of p < 0.05 are reported. All contrasts were tested within predefined ROIs including amygdala, hippocampus, insula, subcallosal cortex, medial prefrontal cortex (mPFC), OFC, ACC, thalamus, and vmPFC. ROIs were created with the anatomical

70% FSL Harvard-Oxford maximum likelihood cortical and subcortical atlases.

3. Results

3.1. Change in PTSD symptoms following treatment

A mixed-effects ANOVA of PTSD symptom severity assessed by CAPS at baseline and week 10 (posttreatment for PTSD group) showed a significant effect of *Group* (F(1, 49) = 186.80, $p < 0.001$), *Time* (F(1, 49) = 89.00, $p < 0.001$), and a *Group*-by-*Time* interaction (F(1, 49) = 87.72, $p < 0.001$). Pairwise LSD tests between the groups showed a significant difference in CAPS total scores at baseline (mean difference = 73.95, SE = 3.68, $p < 0.001$) and at follow-up (mean difference = 24.20, SE = 5.13, $p < 0.001$). Pairwise LSD tests between time points showed significant reduction in CAPS total score in the PTSD group (mean difference = 49.93, SE = 3.49, $p < 0.001$), but not the TEHC group (M difference = 0.18, SE = 4.01, $p = 0.96$).

3.2. SCR measures of fear acquisition and extinction

At baseline, both groups conditioned to the stimuli paired with shock, demonstrating significantly higher mean SCRs across the 16 trials of both CS+ relative to CS− (F(1, 45) = 57.48, $p < 0.001$). There were no significant effects of *Group* ($p > 0.56$), or *Group*-by-*Stimulus* interaction ($p = 0.42$), suggesting similar group conditioning.

At follow-up, both groups again conditioned to the stimuli paired with shock, demonstrating significantly higher mean SCRs across the 16 trials of both CS+ relative to CS− (F(1, 40) = 18.78). There were no significant effects of *Group* ($p = 0.24$), or *Group*-by-*Stimulus* interaction ($p = 0.37$), again suggesting similar conditioning between the groups. Across both time points there was a significant effect of *Stimulus* (F[1, 34] = 45.81, $p < 0.001$), and a nonsignificant trend of reduced conditioning over *Time* (F[1, 34] = 3.81, $p = 0.06$). Neither an effect of *Group* (F[1, 34] = 0.80, $p = 0.38$) nor significant interactions were observed (all $p > 0.08$). Thus, the two groups apparently acquired fear

conditioning equally well before and after treatment, with no discernable change (Fig. S1a).

At baseline, a comparison of the last eight extinction trials of the CS + E versus the last eight trials of the CS− showed no effect of *Stimulus* ($p = 0.12$), *Group* ($p = 0.98$), or *Group*-by-*Stimulus* interaction ($p = 0.28$), suggesting comparable SCR levels following extinction across both groups. At follow-up there was no significant effect of *Stimulus* ($p = 0.13$), or *Group* ($p = 0.66$), or a *Group*-by-*Stimulus* interaction ($p = 0.44$). Across time points there was a significant effect of *Stimulus* (F[1, 34] = 5.16, $p = 0.03$). No effect of *Group* ($p = 0.48$), *Time* ($p = 0.08$), or any interactions (all $p > 0.29$) were observed. Thus, the two groups extinguished equally well before and after treatment, without discernable change (Fig. S1b).

3.3. Fear extinction recall

3.3.1. SCR measures

Although repeated-measures ANOVA of change in extinction recall over time by group revealed no significant *Time* ($p = 0.637$), *Group* ($p = 0.178$) or *Time*-by-*Group* ($p = 0.620$) effects, the PTSD group showed non-significantly higher recall index at pre-treatment than did TE-HCs ($p = 0.130$), and the two groups were comparable at post-treatment ($p = 0.420$), indicating potential normalization of extinction recall over time (Fig. 2).

3.3.2. Change in neural activation during extinction recall

Analyses of BOLD data showed greater baseline activation of the right rACC in the PTSD group at baseline relative to follow-up [MNIxyz = 16, 42, 20, t(15) = 3.79, cluster size = 23, $p = 0.021$, FWE corrected]. The TEHC group showed less activation in the left vmPFC at baseline relative to follow-up [MNIxyz = −4, 52, −10, t(15) = 3.76, cluster size = 61, $p = 0.019$, FWE corrected]; see Fig. 3.

3.3.3. Relationships between physiological activation during extinction recall and change in PTSD symptoms

The PTSD group showed a significant positive correlation between decrease in the extinction recall index and the percent decrease in
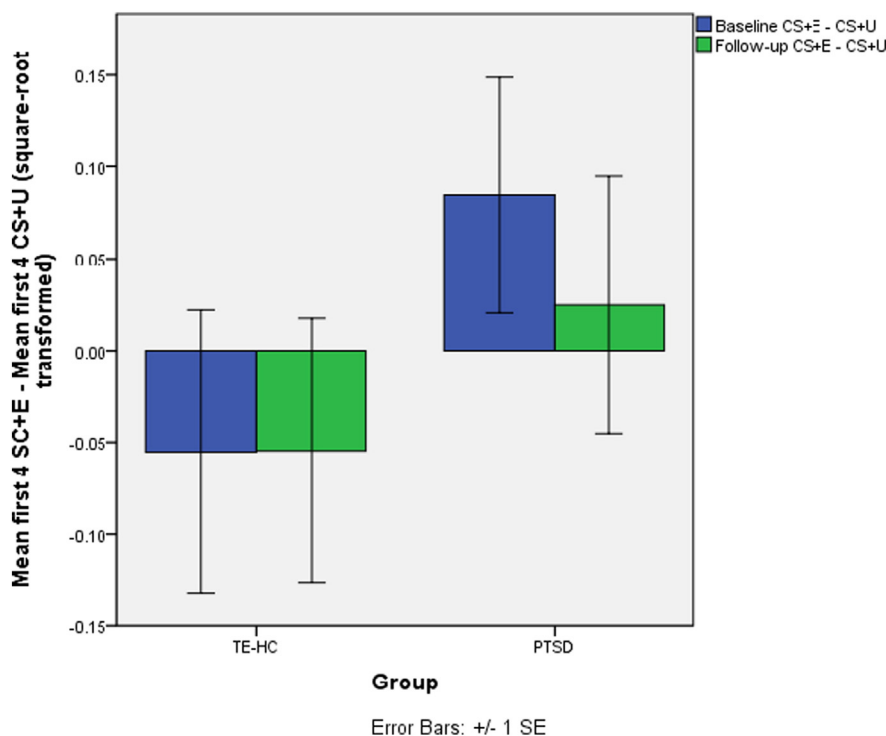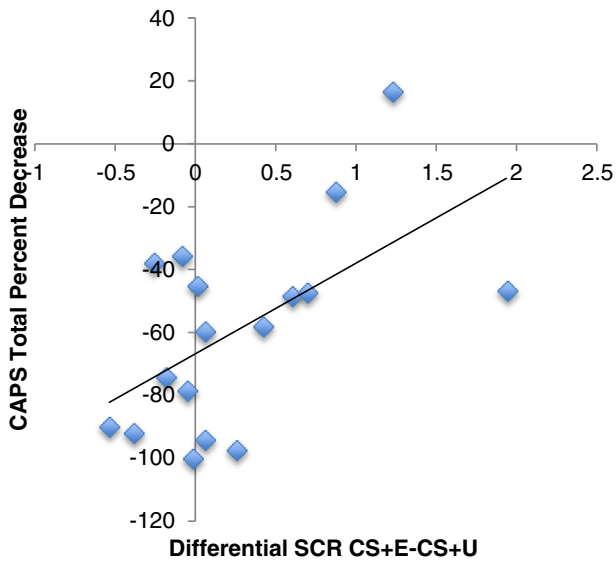

Fig. 2. Psychophysiological activation during extinction recall at baseline and after 10 weeks in PTSD (treated) and TE-HC groups.

Fig. 3. Change in neural activation during extinction recall following treatment among PTSD and TE-HC groups. Note: PTSD = posttraumatic stress disorder; TE-HC = trauma exposed healthy controls; rACC = rostral anterior cingulate cortex; mPFC = medial prefrontal cortex. Images represent pre-post contrasts and mean beta weights represent the average activation in the specified ROI of the entire cluster surviving the 0.005 threshold.

CAPS ($r = 0.57$, $p = 0.02$; Fig. 4), indicating significant association between improvement in psychophysiological indicator of extinction retention and symptom reduction.

3.3.4. Relationships between neural activation during extinction recall and change in PTSD

Relationships between pre- vs. post-treatment changes in neural activation during recall and CAPS changes among PTSD group members appeared in several regions: Decreased activation from pre- to post-treatment in the right subgenual ACC (sgACC, MNIxyz = 6, 6, −16), and in left hippocampal and parahippocampal region (MNIxyz = −16, 16, −12, −14), were significantly associated with percent decrease in CAPS score [$t(15) = 4.29$, cluster size = 84, $p = 0.017$ (FWE corrected) for sgACC and $t(15) = 4.00$, cluster size = 45 $p = 0.011$ (FWE corrected) for the hippocampus/parahippocampus].

Secondary analyses correlating symptom change and change in activation associated with the rACC seed (region noted above to significantly change during extinction recall) did not significantly relate to CAPS (see supplemental materials).

3.3.5. Functional neural connectivity

Comparing activation during extinction recall at baseline vs. follow-up within the PTSD group, we found that the rACC seed exhibited greater functional coherence at follow-up with the following regions: left rACC [MNIxyz = −2, 30, −8, $t(15) = 4.10$, cluster size =52, $p = 0.008$ (FWE corrected)], vmPFC [MNIxyz = −14, 48, −18, $t(15) = 5.25$, cluster size = 154, $p = 0.004$ (FWE corrected)], and sgACC [MNIxyz = −10, 20, −20, $t(15) = 4.87$, cluster size = 217, $p = 0.01$ (FWE corrected)].

These findings suggest that rACC regions showing significant change in activation from pre- to post-treatment also exhibited significant increases in functional coherence with ventral prefrontal regions (namely an additional seed in the ventral rACC, sgACC and vmPFC) following treatment (see Fig. 5). For more information regarding functional connectivity at pre- and post-treatment, see supplementary materials: Functional connectivity of ROIs and Table S2.

4. Discussion

This study demonstrates, for the first time, changes in neural circuits and psychophysiology related to fear extinction recall following PE treatment for PTSD. Findings partially support our hypotheses, indicating a PTSD group specific post-treatment decrease in right rACC activation during extinction recall. The TEHC group showed a reduction in vmPFC activation at 10-week follow-up, possibly indicating habituation to the task. This change was not seen in the PTSD group. The finding of
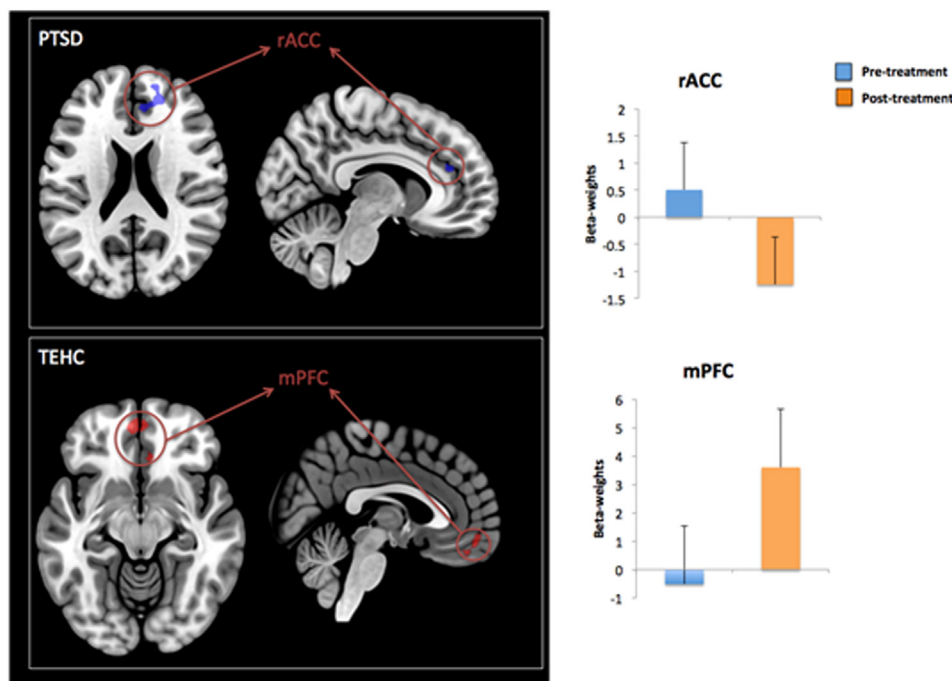
**Fig. 4.** Correlation between change in SCR and change in CAPS score. Note: SCR = skin conductance response; CS + E = extinguished conditioned stimulus; CS + U = unextinguished conditioned stimulus; CAPS = clinician administered posttraumatic symptom scale. Summary: individuals with the largest change in extinction recall SCR (CS + E − CS + U) from pre- to post-treatment showed the largest decrease in CAPS.

change in rACC activation during PE accords with our previous findings of decreased cortical thickness and volume in rACC among remitters following PE, in a partly overlapping sample (Helpman et al., 2016). Together, these findings suggest that PE treatment of PTSD may affect rACC structure and function, evoking the possibility of treatment mechanism working through this region. The change in rACC activation during recall found here correlated with enhanced functional coherence with additional ventral prefrontal areas such as the vmPFC and sgACC. This pattern suggests, that during recall, rACC may work in synchrony with ventral aspects of prefrontal cortex downregulating fear circuitry, possibly in coordination with hippocampus: hippocampal activity was associated with post-treatment symptom change. However, we were not able to directly demonstrate treatment-related changes in activation of the vmPFC and hippocampus during extinction recall. Our findings further suggest that both physiological (SCR) and neural (fMRI activation)

changes during recall following treatment significantly were correlated with reduction in PTSD symptoms, indicating potential mechanistic connections between these changes and clinical improvement. This echoes findings by Felmingham and colleagues (Felmingham et al., 2007) who also found increased rACC activation and decreased amygdalar activation during processing of fearful vs. neutral faces after exposure-based cognitive behavioral therapy for PTSD. Although coupling with amygdala failed to reach significance in our sample, our findings of changes in prefrontal activation and its synchrony with hippocampus may indicate improvement in discrimination, using contextual information from hippocampus, and further illuminate the mechanisms behind this change.

The masked whole brain analyses linking post-treatment changes with symptom improvement support the relationship between neural mechanisms related to extinction recall and posttraumatic symptoms:
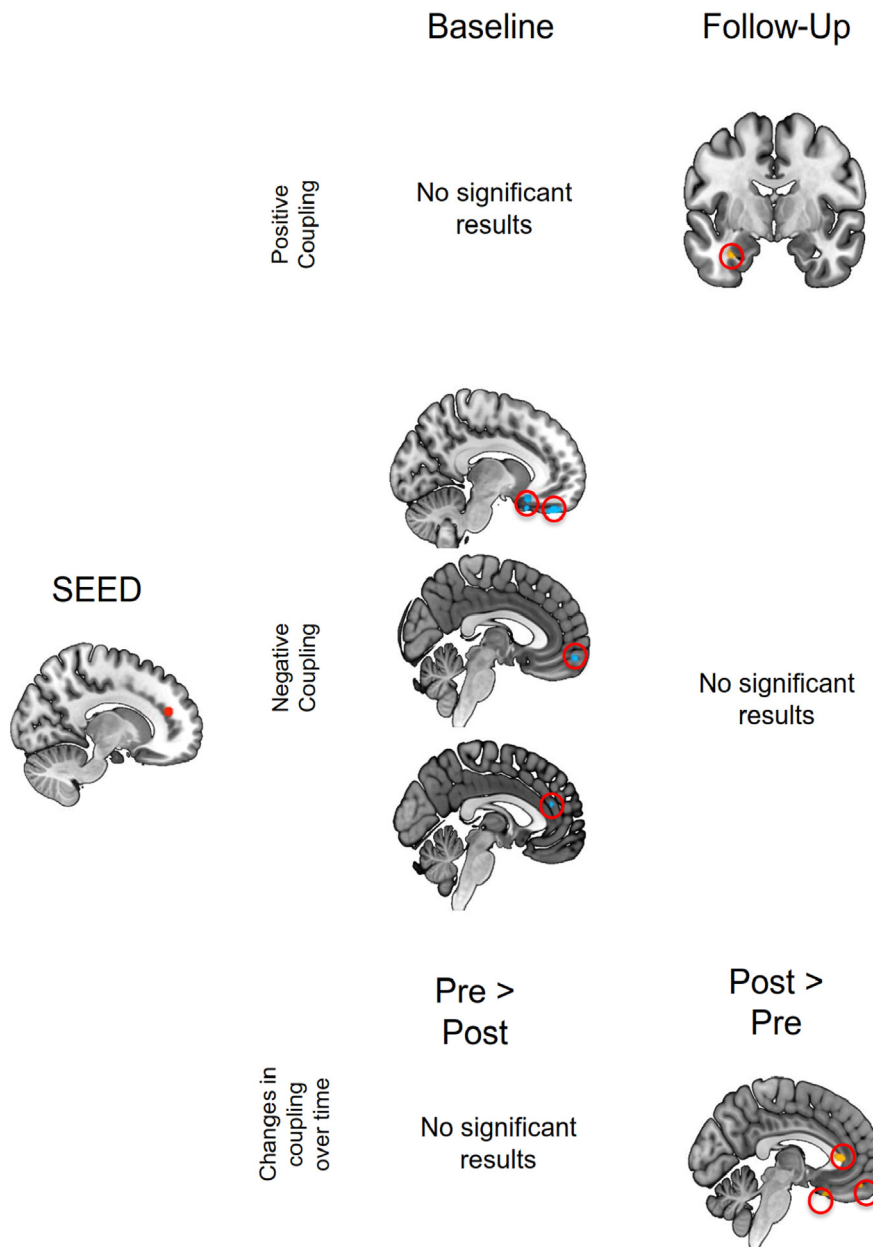
Fig. 5. Psychophysiological interactions during extinction recall phase between seed region with identified pre-to-post-treatment changes and additional regions of interest. Note: Seed = rostral anterior cingulate seed identified as decreasing in activation during extinction recall phase from pre-to-post-treatment; Positive coupling = marked region evinces increased activation when activation in the seed region increases during extinction recall; Negative coupling = marked region evinces reduction in activation when activation in the seed region increases during extinction recall; Pre > Post — coupling between marked areas and seed decrease from pre-to-post-treatment; Post > Pre — coupling between marked areas and seed increase from pre-to-post-treatment.

sgACC, parahippocampal, and hippocampal region decreases in activation correspond to decreases in symptoms, which in turn correspond to improvement in physiological measures of extinction recall.

Our rACC and connectivity findings are congruent with findings showing consistent resting-state ACC hyperactivity in PTSD patients compared to healthy controls (Koch et al., 2016) and data indicating reduced default mode network intra-network connectivity in PTSD (Sripada et al., 2012). They also dovetail with previous accounts of two neural systems, ventral and dorsal, acting in emotional perception and response (Phillips et al., 2003). We demonstrate here that these patterns normalize with treatment, showing reduced rACC activation and increase in its functional coherence with other regions of the default mode network. Additionally, we demonstrate that ventral ("identification and reactivity") system decreases in activation during recall correlate with symptom reduction. Thus, changes in neural activation during extinction recall following treatment may reflect decreased "flagging" of stimuli as dangerous or affect-laden, thus lowering reactivity to threat cues, and enhancing ability to retain extinction memory, as treatment gains. We did not include dorsal ("regulation") system activation in the ROIs in this study. Thus, we conclude that, although prolonged exposure treatment produces significant changes in neural activation patterns associated with the ability to retain extinction of fear, the relationship between this ability and posttraumatic symptomatology is complex and may involve changes in additional neural systems beyond the scope of this investigation.

Our findings, while robust in demonstrating significant changes in psychophysiology corresponding to symptoms and in neural function during extinction recall following PE treatment for PTSD, have limitations. Due to attrition and technical issues, our final sample for fMRI pre-post contrasts included only 16 individuals per group, limiting generalizability of findings. Replication is also needed. This small sample size precluded sufficient power for interaction analyses (see supplementary materials) and additional analyses of subgroups, such as remitters vs. non-remitters (Helpman et al., 2016) or males vs. females, which have shown divergent patterns in fear acquisitions, extinction and recall (Hwang et al., 2015; Milad et al., 2009a,b; Shvil et al., 2014). Future studies should address these issues, as well as test for specificity of findings for PE (in comparison to a different treatments). Nonetheless, our findings support the idea that prolonged exposure treatment may produce a host of neural and psychophysiological changes leading to clinical improvement of PTSD.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.nicl.2016.10.007.

References

Aupperle, R.L., Allard, C.B., Simmons, A.N., Flagan, T., Thorp, S.R., Norman, S.B., Paulus, M.P., Stein, M.B., 2013. Neural responses during emotional processing before and after cognitive trauma therapy for battered women. Psychiatry Res. Neuroimaging 214, 48–55.

Blake, D.D., Weathers, F.W., Nagy, L.M., Kaloupek, D.G., Gusman, F.D., Charney, D.S., Keane, T.M., 1995. The development of a clinician-administered PTSD scale. J. Trauma. Stress. 8, 75–90.

Blechert, J., Michael, T., Vriends, N., Margraf, J., Wilhelm, F.H., 2007. Fear conditioning in posttraumatic stress disorder: evidence for delayed extinction of autonomic, experiential, and behavioural responses. Behav. Res. Ther. 45, 2019–2033.

Bouton, M.E., Mineka, S., Barlow, D.H., 2001. A modern learning theory perspective on the etiology of panic disorder. Psychol. Rev. 108, 4–32.

Breslau, N., Kessler, R.C., Chilcoat, H.D., Schultz, L.R., Davis, G.C., Andreski, P., 1998. Trauma and posttraumatic stress disorder in the community: the 1996 Detroit Area Survey of Trauma. Arch. Gen. Psychiatry 55, 626–632.

Diagnostic and Statistical Manual of Mental Disorders. 5th Edition. American Psychiatric Publishing.

Farrow, T.F.D., Hunter, M.D., Wilkinson, I.D., Gouneea, C., Fawbert, D., Smith, R., Lee, K.-H., Mason, S., Spence, S.A., Woodruff, P.W.R., 2005. Quantifiable change in functional brain response to empathic and forgivability judgments with resolution of posttraumatic stress disorder. Psychiatry Res. Neuroimaging 140, 45–53.

Felmingham, K., Kemp, A., Williams, L., Das, P., Hughes, G., Peduto, A., Bryant, R., 2007. Changes in anterior cingulate and amygdala after cognitive behavior therapy of posttraumatic stress disorder. Psychol. Sci. 18, 127–129.

Foa, E.B., Hembree, E.A., Cahill, S.P., Rauch, S.A.M., Riggs, D.S., Feeny, N.C., Yadin, E., 2005. Randomized trial of prolonged exposure for posttraumatic stress disorder with and without cognitive restructuring: outcome at academic and community clinics. J. Consult. Clin. Psychol. 73, 953–964.

Foa, E.B., Hembree, E.A., Rothbaum, B.O., 2007. Prolonged Exposure Therapy for PTSD: Emotional Processing of Traumatic Experiences – Therapist Guide. Oxford University Press, New York, NY, US.

Gallagher, M., Chiba, A.a., 1996. The amygdala and emotion. Curr. Opin. Neurobiol. 6, 221–227.

Garcia, R., 2002. Postextinction of conditioned fear: between two CS-related memories. Learn. Mem. 9, 361–363.

Graham, B.M., Milad, M.R., 2013. Blockade of estrogen by hormonal contraceptives impairs fear extinction in female rats and women. Biol. Psychiatry 73, 371–378.

Hamilton, M., 1960. A rating scale for depression. J. Neurol. Neurosurg. Psychiatry 23, 56–62.

Helpman, L., Papini, S., Chhetry, B.T., Shvil, E., Rubin, M., Sullivan, G.M., Markowitz, J.C., Mann, J.J., Neria, Y., 2016. PTSD remission after prolonged exposure treatment is associated with anterior cingulate cortex thinning and volume reduction. Depress. Anxiety 8 n/a-n/a.

Hwang, M.J., Zsido, R.G., Song, H., Pace-Schott, E.F., Miller, K.K., Lebron-Milad, K., Marin, M.-F., Milad, M.R., 2015. Contribution of estradiol levels and hormonal contraceptives to sex differences within the fear network during fear conditioning and extinction. BMC Psychiatry 15, 295.

Jovanovic, T., Norrholm, S.D., Blanding, N.Q., Davis, M., Duncan, E., Bradley, B., Ressler, K.J., 2010. Impaired fear inhibition is a biomarker of PTSD but not depression. Depress. Anxiety 27, 244–251.

Jovanovic, T., Ely, T., Fani, N., Glover, E., 2013. Reduced neural activation during an inhibition task is associated with impaired fear inhibition in a traumatized civilian sample. Cortex 49, 1884–1891.

Kessler, R.C., 1995. Posttraumatic stress disorder in the National Comorbidity Survey. Arch. Gen. Psychiatry 52, 1048.

Kessler, R.C., Sonnega, A., Bromet, E., Hughes, M., Nelson, C.B., 1995. Posttraumatic stress disorder in the National Comorbidity Survey. Arch. Gen. Psychiatry 52, 1048.

Koch, S.B.J., van Zuiden, M., Nawijn, L., Frijling, J.L., Veltman, D.J., Olff, M., 2016. Aberrant resting-state brain activity in posttraumatic stress disorder: a meta-analysis and systematic review. Depress. Anxiety 14 n/a-n/a.

Kwong, K.K., Belliveau, J.W., Chesler, D.A., Goldberg, I.E., Weisskoff, R.M., Poncelet, B.P., Kennedy, D.N., Hoppel, B.E., Cohen, M.S., Turner, R., 1992. Dynamic magnetic resonance imaging of human brain activity during primary sensory stimulation. Proc. Natl. Acad. Sci. U. S. A. 89, 5675–5679.

Lansing, K., Amen, D.G., Hanks, C., Rudy, L., 2005. High-resolution brain SPECT imaging and eye movement desensitization and reprocessing in police officers with PTSD. J. Neuropsychiatr. Clin. Neurosci. 17, 526–532.

LeDoux, J., 2007. The amygdala. Curr. Biol. 17, 868–874.

Lindauer, R.J., Booij, J., Habraken, J.B., van Meijel, E.P., Uylings, H.B., Olff, M., Carlier, I.V., den Heeten, G.J., van Eck-Smit, B.L., Gersons, B.P., 2007. Effects of psychotherapy on regional cerebral blood flow during trauma imagery in patients with post-traumatic stress disorder: a randomized clinical trial. Psychol. Med. 38, 543–554.

Lissek, S., Grillon, C., 2012. Learning models of PTSD. The Oxford Handbook of Tramatic Disorders, pp. 175–190.

Lissek, S., Glaubitz, B., Uengoer, M., Tegenthoff, M., 2013. Hippocampal activation during extinction learning predicts occurrence of the renewal effect in extinction recall. NeuroImage 81, 131–143.

Maren, S., Fanselow, M.S., 1995. Synaptic plasticity in the basolateral amygdala induced by hippocampal formation stimulation in vivo. J. Neurosci. 15, 7548–7564.

Marin, M.-F., Song, H., VanElzakker, M.B., Staples-Bradley, L.K., Linnman, C., Pace-Schott, E.F., Lasko, N.B., Shin, L.M., Milad, M.R., 2016. Association of resting metabolism in the fear neural network with extinction recall activations and clinical measures in trauma-exposed individuals. Am. J. Psychiatry, appiajp201514111460.

McLaren, D.G., Ries, M.L., Xu, G., Johnson, S.C., 2012. A generalized form of context-dependent psychophysiological interactions (gPPI): a comparison to standard approaches. NeuroImage.

Milad, M.R., Quirk, G.J., 2002. Neurons in medial prefrontal cortex signal memory for fear extinction. Nature 420, 70–74.

Milad, M.R., Quinn, B.T., Pitman, R.K., Orr, S.P., Fischl, B., Rauch, S.L., 2005. Thickness of ventromedial prefrontal cortex in humans is correlated with extinction memory. Proc. Natl. Acad. Sci. U. S. A. 102, 10706–10711.

Milad, M.R., Wright, C.I., Orr, S.P., Pitman, R.K., Quirk, G.J., Rauch, S.L., 2007. Recall of fear extinction in humans activates the ventromedial prefrontal cortex and hippocampus in concert. Biol. Psychiatry 62, 446–454.

Milad, M.R., Orr, S.P., Lasko, N.B., Chang, Y., Rauch, S.L., Pitman, R.K., 2008. Presence and acquired origin of reduced recall for fear extinction in PTSD: results of a twin study. J. Psychiatr. Res. 42, 515–520.

Milad, M.R., Igoe, S.A., Lebron-Milad, K., Novales, J.E., 2009a. Estrous cycle phase and gonadal hormones influence conditioned fear extinction. Neuroscience 164, 887–895.

Milad, M.R., Pitman, R.K., Ellis, C.B., Gold, A.L., Shin, L.M., Lasko, N.B., Zeidan, M.A., Handwerger, K., Orr, S.P., Rauch, S.L., 2009b. Neurobiological basis of failure to recall extinction memory in posttraumatic stress disorder. Biol. Psychiatry 66, 1075–1082.

Milad, M.R., Furtak, S.C., Greenberg, J.L., Keshaviah, A., Im, J.J., Falkenstein, M.J., Jenike, M., Rauch, S.L., Wilhelm, S., 2013. Deficits in conditioned fear extinction in obsessive-compulsive disorder and neurobiological changes in the fear circuit. JAMA Psychiat. 70, 608.

Morgan, M.A., LeDoux, J.E., 1995. Differential contribution of dorsal and ventral medial prefrontal cortex to the acquisition and extinction of conditioned fear in rats. Behav. Neurosci. 109, 681–688.

Morgan, M.A., Romanski, L.M., LeDoux, J.E., 1993. Extinction of emotional learning: contribution of medial prefrontal cortex. Neurosci. Lett. 163, 109–113.

Morgan, M.A., Schulkin, J., LeDoux, J.E., 2003. Ventral medial prefrontal cortex and emotional perseveration: the memory for prior extinction training. Behav. Brain Res. 146, 121–130.

Peres, J.F.P., Moreira-Almeida, A., Nasello, A.G., Koenig, H.G., 2007a. Spirituality and resilience in trauma victims. J. Relig. Health 46, 343–350.

Peres, J.F.P., Newberg, A.B., Mercante, J.P., Simão, M., Albuquerque, V.E., Peres, M., Nasello, A.G., 2007b. Cerebral blood flow changes during retrieval of traumatic memories before and after psychotherapy: a SPECT study. Psychol. Med. 37.

Peres, J.F.P., Foerster, B., Santana, L.G., Fereira, M.D., Nasello, A.G., Savoia, M., Moreira-Almeida, A., Lederman, H., 2011. Police officers under attack: resilience implications of an fMRI study. J. Psychiatr. Res. 45, 727–734.

Phelps, E.A., LeDoux, J.E., 2005. Contributions of the amygdala to emotion processing: from animal models to human behavior. Neuron 48, 175–187.

Phillips, M.L., Drevets, W.C., Rauch, S.L., Lane, R., 2003. Neurobiology of emotion perception I: the neural basis of normal emotion perception. Biol. Psychiatry 54, 504–514.

Ponniah, K., Hollon, S.D., 2009. Empirically supported psychological treatments for adult acute stress disorder and posttraumatic stress disorder: a review. Depress. Anxiety 26, 1086–1109.

Quirk, G.J., Russo, G.K., Barron, J.L., Lebron, K., 2000. The role of ventromedial prefrontal cortex in the recovery of extinguished fear. J. Neurosci. 20, 6225–6231.

Rosenkranz, J.A., Moore, H., Grace, A.A., 2003. The prefrontal cortex regulates lateral amygdala neuronal plasticity and responses to previously conditioned stimuli. J. Neurosci. 23, 11054–11064.

Rothbaum, B.O., Davis, M., 2003. Applying learning principles to the treatment of post-trauma reactions. Ann. N. Y. Acad. Sci. 1008, 112–121.

Shvil, E., Sullivan, G.M., Schafer, S., Markowitz, J.C., Campeas, M., Wager, T.D., Milad, M.R., Neria, Y., 2014. Sex differences in extinction recall in posttraumatic stress disorder: a pilot fMRI study. Neurobiol. Learn. Mem. 113, 101–108.

Sotres-Bayon, F., Bush, D.E., LeDoux, J.E., 2004. Emotional perseveration: an update on prefrontal-amygdala interactions in fear extinction. Learn. Mem. 11, 525–535.

Sripada, R.K., King, A.P., Welsh, R.C., Garfinkel, S.N., Wang, X., Sripada, C.S., Liberzon, I., 2012. Neural dysregulation in posttraumatic stress disorder: evidence for disrupted equilibrium between salience and default mode brain networks. Psychosom. Med. 74, 904–911.