Supplemental Information for 'Neural Substrates of Over-Generalized Conditioned Fear in Posttraumatic Stress Disorder'

Methods

Participants. Demographic, psychological, and psychiatric characteristics of participants are reported in Tables S1 and S2. Psychiatric diagnoses other than PTSD were determined via Structured Clinical Interview for DSM-IV (SCID-I)[1]. The following exclusion criteria were applied to the tested sample of combat veterans: 1) pre-deployment history of Axis I psychopathology; 2) current or past history of bipolar depression, psychosis, or delusional disorders; 3) history of substance abuse or dependence (other than nicotine) within 6 months of study start; 4) inability to refrain from nicotine or caffeine on day of testing; 5) current use of anti-psychotics, mood-stabilizers, anti-parkinsonian agents, anticonvulsants, anti-hypertensives, and alpha/beta adrenergic agents. Further, participants taking medications on an 'as needed' basis (i.e., benzodiazepines, sleep medication, stimulants, pain medications) were excluded unless they could refrain 12 hours prior to testing without causing either: a) undue worsening of symptoms, as determined by their treating physician/psychiatrist, or b) impeded performance on study measures; 6) current Axis I psychiatric disorders in trauma controls; 7) significant suicidal ideation or behavior; 4) any medical condition, implant, or device not safe for MRI; 8) major medical conditions that interfered with the objectives of the study (e.g., history of organic mental disorders, seizure, or mental retardation); and 9) current use of illicit drugs; As can be seen in Table S1, subjects displayed very low rates of psychiatric comorbidity, a sample characteristic likely attributable to restricting recruitment to combat veterans with no pre-deployment psychopathology. In terms of cognitive functioning, groups did not differ on such subtests of the Wechsler Adult Intelligence Scale as digit span (p=.39), coding (p=.68) or information (p=.96), suggesting equal cognitive abilities in the areas of working memory, processing speed, and verbal comprehension across PTSD, Sub-PTSD and TC groups. Additionally, no group differences were found on intellectual functioning as assessed by the Wechsler Test of Adult Reading (p=.56) and verbal learning and memory as assessed by the California Verbal Learning Test (p=.76). The study was approved by IRBs at both the Minneapolis VA and the University of Minnesota, and informed consent was obtained from all participants prior to testing. All participants received reimbursement for their time.

Stimuli. As can be seen in Figure S1, stimuli serving as conditioned stimuli (CS: CS+, oCS-) and generalization stimuli (GS: GS_1, 2 GS_2, 2 GS_3) consisted 5 checkerboard textured rings of parametrically increasing size, and one "V-shaped" stimulus (vCS-) of the same checkerboard texture, all presented on a rear-projection viewing screen mounted to the scanner. The paradigm includes one CS+ and the following two CS-: 1) either the largest or smallest ring, referred to as oCS-, and 2) a "V" shaped stimulus, referred to as vCS-. Extreme sizes served as oCS- and CS+ with big and small sizes of oCS- and CS+ counterbalanced across subjects. The three intermediately-sized rings served as GSs (GS_1, GS_2, GS_3) and formed a continuum-of-size between oCS and CS+. The vCS- was included to test the degree to which conditioned-generalization accrues to all "ringed" stimuli following reinforcement of the ring-shaped CS+. Further, inclusion of the vCS- allowed for assessment of brain responses to CS+ (vs. vCS-) that are independent of putative generalization effects to all ringed-stimuli. The unconditioned stimulus (US) was a 100ms electric shock (3-5mA) delivered to the right ankle.

The checkerboard patterned conditioned and generalization stimuli counterphase-flickered at a rate of 10 Hz. Such stimuli were designed to activate the calcarine sulcus along a continuum of visual eccentricity[2] as part of a longer-range goal to use this generalization paradigm to retinotopically map representations of CSs and GSs in sensory cortex. Important for the purposes of the current paper is the size and shape of these stimuli rather than their retinotopic-mapping characteristics, as retinotopy was unsuccessful in the current study.

Design. CSs and GSs were presented for 4s in quasi-random order (ITI=2.4-4.8s), across three phases: 1) *Pre-acquisition:* 20 of each stimulus (CS+, GS_1, GS_2, GS_3, oCS-, vCS-) without shock; 2) *Acquisition:* 15 CS+, 15 oCS-, and 15 vCS-, with 12 of 15 CS+ co-terminating with shock; and 3) *Generalization-test:* 20 of each stimulus (unreinforced CS+, GS_1 ,GS_2, GS_3, oCS-, vCS-), and an additional 10 CS+ co-terminating with shock to prevent extinction during generalization, while leaving 20 unreinforced CS+ to index responses uninfluenced by US. CS and GS trials for all 3 phases of the study were arranged in quasi-random order such that no more than two stimuli of the same class occurred consecutively. An additional constraint for the generalization sequence was the arrangement of trials into 10 blocks of 13 trials (2 unreinforced CS+, 1 reinforced CS+, 2 oCS-, 2 vCS-, 2 GS_1, 2 GS_2, 2 GS_3) to ensure an even distribution of trial types throughout runs. During all phases, a behavioral task developed to maintain visual gaze at the center of the visual field[3] was applied. This task consists of a stream of colored crosshairs (blue, yellow, red, green, purple) presented serially for a duration of 800 ms each in the center of the screen for the duration of each CS/GS, with 5 cross-hairs of different color during each 4 second CS/GS. Participants were instructed to monitor the stream and quickly rate their perceived risk for shock following each red-cross using a 3-button response pad (Lumina LP-404 by Cedrus), where 0='no-risk', 1='moderate-risk', and 2='high-risk'. These online behavioral risk ratings were recorded with Presentation software (Neurobehavioral Systems). For half of CS/GS trials during all 3 phases of the study, 1 of 5 crosshairs was red, and the remaining trials included no red crosshairs (i.e., behavioral ratings were collected on half of trials). Additionally, on reinforced CS+ trials, the red crosshair never appeared in the fourth or fifth position to avoid interference from shock on behavioral responses. Thus, for all stimuli other than shock reinforced CS+, risk ratings were assessed at either 0 ms, 800 ms, 1600 ms, 2400 ms, or 3200 ms post-stimulus onset, while risk ratings to reinforced stimuli were assessed at 0 ms, 800 ms, or 1600 ms post-stimulus onset.

Procedure. Participants were not instructed of the CS/US contingency but were told they might learn to predict the shock if they attend to the presented stimuli. Shock electrodes were then attached and a shock workup procedure was completed. During this workup, participants received between 1-3 sample shocks, and levels of shock were adjusted to achieve a level rated by participants as 'highly uncomfortable or mildly painful'. Participants next practiced using the button box to respond to red crosshairs appearing both at the center of CSs and GSs. Participants were then placed in the magnet with foam pads placed to limit head movement. Structural scans were acquired followed by preacquisition, acquisition, and generalization test. Self-reported anxiety to CS+, oCS-, and vCS- was assessed following each of three phases (10-point-scales).

fMRI Data Acquisition. A Siemens 3T with twelve-channel receive-only head coil was used to acquire T2*-weighted echo-planar images (EPIs) of the BOLD contrast, as well as high-resolution T1-weighted anatomical-references (MP-RAGE). Parameters for functional EPIs included: TR: 2300 ms; TE: 23 ms; and flip: 90°. These EPIs consisted of whole-brain acquisitions of axially-oriented slices of 3.5 mm thickness and 1.745x1.745mm in-plane

resolution (matrix: 128×128, FOV: 22 cm). The high-resolution T1-weighted anatomical scans were magnetization-prepared rapid acquisition gradient-echo sequences (MP-RAGE) and were obtained to serve as anatomical reference.

fMRI Data Analysis. Image analysis was completed with Analysis of Functional Neural Images (AFNI)[4]. Echo-planar time series data was time corrected, registered, spatially smoothed (FWHM= 4 mm), normalized, and concatenated. During individual-level analyses, functional activation maps were computed by regressing each voxel's fMRI response time-course onto an ideal response function consisting of a Gamma-variate function convolved with the time-series of each of 6 stimulus types (i.e., vCS-, oCS-, GS_1, GS_2, GS_3, unreinforced CS+) at pre-acquisition and generalization test separately. The acquisition phase was used to condition participants to CS+, oCS-, and vCS- and was not intended for image analysis due to the majority of CS+ trials being contaminated by US administrations, and because such data were not critical for testing central hypotheses of interest. Modeled as covariates of no interest were baseline drift, participant-specific movement, response time course (button presses), and the time-course of shock delivery. Three participants (2 PTSD and 1 TC) were removed due to excessive head-motion, defined as motion >3mm in any direction between consecutive EPI volumes.

Group-level analyses of generalization-test data were completed in two stages. First, brain areas sensitive to the conditioning manipulation were identified as functional regions of interest (fROI). Specifically, whole brain analyses of the contrast between responses to the 20 unreinforced CS+ versus the 20 vCS- were conducted using a voxelwise probability of $p \leq .00003$ and a cluster probability of $p \leq .05$. A stringent voxelwise probability was necessary to achieve adequate demarcation between clusters. The probability of obtaining clusters of a particular size was estimated with the AFNI program 3dClustSim. The vCS- rather than oCS- was contrasted against unreinforced CS+ because the CS+ versus vCS- contrast, but not CS+ versus oCS-, yields a measure of conditioning independent of fear generalization that may occur to all circular stimuli. That is brain activations to CS+ versus vCS- were chosen as fROIs in which to test gradients of fear generalization, because they are relatively orthogonal to the generalization process. In the second stage, beta weights averaged across voxels within these fROIs were plotted across conditioned and generalization stimuli and analyzed for effects of generalization as well as interactions between group and generalization. Such analyses began with one-way, repeated measures ANOVAs with 5 levels (oCS-, GS_1, GS_2, GS_3, unreinforced CS+). fROIs significantly instantiating generalization gradients were then analyzed with 3 (Group: PTSD, Sub-PTSD, TC) x 5 (Stimulus-type: oCS-, GS_1, GS_2, GS_3, unreinforced CS+) repeated measure ANOVAs. Because these 3 x 5 ANOVAs may not adequately detect important gradient-shape differences across any two subject groups, Group x Stimulus-type ANOVAs were also computed with Group defined as PTSD vs. TC, Sub-PTSD vs. TC, and PTSD vs. Sub-PTSD. Interactions were followed by tests of linear and quadratic components, as well as Hochberg corrected paired sample *t*-tests[5] contrasting specific stimulus-types in each group.

Functional connectivity analysis. Inter-relations between brain activations associated with generalization were tested using psychophysiological-interaction (PPI)[6], with functionally defined seed regions in the hippocampus—the central node of the theorized generalization-network. Generalization-related modifications in connectivity between seed and targets were assessed by contrasting responses to all safe circular-stimuli (oCS-,GS_1-GS_3) against responses to the non-circular control stimulus (vCS-), to assess broad generalization from the circular CS+ to all things circular. Following previous PPI work[7], criterion alpha was set at $p \leq .001$,

uncorrected. When applying more stringent adjustments for multiple comparisons (e.g., adding a required cluster probability of $p \leq .05$), no PPI effects were significant. Results of PPI analyses should thus be interpreted with caution.

Behavioral data analysis. At pre-acquisition, acquisition, and generalization test, levels of conditioning were assessed with paired sample *t*-tests comparing risk ratings to CS+ versus oCS-and CS+ versus vCS- (results reported in this supplement). Additionally, risk ratings at pre-acquisition and generalization test were analyzed with one-way, repeated measures ANOVAs with 5 levels (oCS-, GS_1, GS_2, GS_3, unreinforced CS+), and were followed, when appropriate, by tests of linear and quadratic components (results reported in this supplement). Next, group effects on generalization gradients were analyzed with 3 (Group: PTSD, Sub-PTSD, TC) x 5 (Stimulus-type: oCS-, GS_1, GS_2, GS_3, unreinforced CS+) repeated measures ANOVA. Because these 3 x 5 ANOVAs may not adequately detect important gradient-shape differences across any two subject groups, Group x Stimulus-type ANOVAs were also computed with Group defined as PTSD vs. TC, Sub-PTSD vs. TC, and PTSD vs. Sub-PTSD. Interactions were followed by tests of linear and quadratic components as well as Hochberg corrected paired sample *t*-tests[5] contrasting specific stimulus-types in each group. All analyses other than follow-up paired sample *t*-tests were tested with a criterion alpha of $p=.05$.

Quantifying levels of generalization from the steepness of gradients. For each subject, the shape of generalization gradients was assessed by calculating the linear deviation score (LDS), reflecting the degree to which each gradient departed from linearity, using the equation: $LDS = ([CS+, CS-]/2) - ([GS_1, GS_2, GS_3, GS_4]/4)$. Here $[CS+, CS-]/2$ reflects the theoretical, linear midpoint of the gradient, and $[GS_1, GS_2, GS_3, GS_4]/4$ reflects the average response to GSs which could fall above the linear midpoint (positive departure), on the linear midpoint (zero departure), or below the linear midpoint (negative departure). This equation thus provides a single number reflecting the steepness of generalization gradients, with positive versus negative values reflecting shallow convex-gradients versus steep concave-gradients, respectively. LDS scores also indicate the strength of generalization, with more positive versus negative values indicating stronger versus weaker generalization, respectively.

Results

Behavioral Findings

Pre-acquisition. Neither main effects of stimulus-type, nor Stimulus-type x Group interactions were significant for any measure ($ps \geq .20$).

Acquisition. Online risk-ratings and retrospective-anxiety were greater to CS+ versus oCS- and vCS- ($ps < .0001$). No main effects of group or Group x Stimulus interactions were found for either online risk-ratings or retrospective anxiety ($ps \geq .26$).

Generalization-test. Online risk-ratings and retrospective-anxiety continued to be were greater to CS+ versus oCS- and vCS- ($ps < .0001$) at generalization. Additionally, risk-ratings across stimulus-types formed robust downward gradients of generalization from CS+ to GSs to CS-, $F(4,57)=69.53$, $p<.0001$ (see Figure 1). Group effects on generalization gradients are reported in the main paper.

Of note, at both acquisition and generalization those with PTSD versus TC displayed significantly higher risk ratings to CS+ ($ps \leq .02$). Elevated risk ratings to CS+ were at the trend level in Sub-PTSD versus TC ($ps \leq .09$). While this might seem to indicate that those with PTSD

(and to some degree Sub-PTSD) more accurately predicted risk of shock during CS+, this may not be the case. Specifically, throughout the generalization test, the CS+ is paired with shock on 33.33% of CS+ trials. As such, it may be most accurate to rate the CS+ as indicating 'some risk of shock' rather than 'high risk of shock'. As described above, risk ratings were assessed on a 3-point scale from 0-2, where 0 = *no risk of shock*, 1 = *some risk of shock*, and 2 = *high risk of shock*. The mean risk rating to CS+ for PTSD, sub-PTSD, and TC groups was 1.61, 1.51, and 1.21 respectively. Thus the TC group endorsed 'some' risk of shock during CS+, whereas PTSD and Sub-PTSD groups endorsed more than "some" risk for shock (but not quite 'high' risk). Given the relatively low reinforcement rate of CS+ throughout the generalization test (33%), it seems the assessment made by those in the TC group, that CS+ indicates 'some' risk, is more accurate than the assessment made by those in the PTSD and Sub-PTSD groups, that CS+ indicates more than 'some' risk. That is, the levels of risk reported by those in the PTSD and Sub-PTSD groups indicate a perception of risk that is somewhat inflated relative to the degree to which the CS+ was actually paired with shock.

fMRI Correlates of Generalization

Pre-acquisition. No fROIs showed significant generalization-gradients.

Generalization-test. Table S3 lists fROIs forming generalization-gradients across all subjects. Replicating past generalization findings[8], *positive* generalization-gradients were found in bilateral AI, dmPFC (BA 6/32), bilateral IPL (BA40), right middle frontal gyrus (MFG: BA9), and right superior frontal gyrus (SFG: BA10). Additional positive gradients were found bilaterally in caudate-nucleus, fusiform-gyrus (BA18), premotor-cortex, and thalamus. Replicating past findings[8], *negative* generalization-gradients were instantiated in vmPFC, bilateral VH, and precuneus. Further negative gradients of activation were found at the border of the left VH-amygdala interface and right caudate-head.

Reduced Brain Responses to CS+ (vs. oCS-) Among those with PTSD

As can be seen in Figure 2, the overall increase in brain responses from oCS- to CS+ seems to have been constrained in PTSD versus TCs in several fROIs. Indeed increases from oCS- to CS+ were significantly smaller in PTSD vs. TC in right-AI and right-BA9 ($ps<.05$). This is in contrast to behavioral results showing larger increases in perceived threat from oCS- to CS+ among those with PTSD versus TCs ($p=.03$). This pattern of results is hard to interpret. One possibility is that constrained brain responses to CS+ (vs. oCS-) in PTSD resulted from elevated anxiety before and during the threat-of-shock portion of the study, which may have increased 'baseline' levels of activity in fear-related fROIs such as AI. In the current study, percent signal-change in brain voxels due to CS+ presentations was computed relative to the overall average signal in corresponding voxels across EPI runs. Thus, if those with PTSD were more anxious before and during the threat-of-shock portion of the study, as would be predicted by past threat-of-shock-studies in PTSD[9,10], enhanced activity in fear-related brain areas at baseline among those with PTSD may have constrained percent-signal increases to the CS+.

As part of a post-study, clinical assessment of subjects' emotional response to the experiment, we collected self-reported levels of anxiety experienced before, during, and after the fMRI scans. This rating was on a 0-100 scale where 0 = *No Anxiety*, 50 = *Moderate Anxiety*, and 100 = *Extreme Anxiety*. As can be seen in Figure S2, before the study began, PTSD and Sub-PTSD versus TCs reported elevated levels of anxiety ($p=.03$). Additionally, increased anxiety

during the study (relative to before the study) was found in TCs (p=.03) but not PTSD (p=.62) or Sub-PTSD (p=.18). These findings are consistent with the idea that elevated anxiety before the study among those with PTSD constrained anxiety increases during the study.

Controlling Potential Effects of Psychotropics

Nearly all PTSD-effects on behavioral and neural indices of generalization remained significant after either rerunning main analyses with subjects on psychotropics removed (n=8), or statistically covarying effects of medication-status. The few exceptions include: 1) the Sub-PTSD vs. TC effect on the generalization gradient became significant in vmPFC (p=.02) after removing the 8 medicated participants, and 2) the correlation between CAPS scores and gradient steepness in dmPFC became significant after partialling out medication status (ps<.005).

Controlling Potential Effects of Current Psychiatric Comorbidities

There were very low rates of comorbidity in our sample, likely due to the applied exclusion of combat veterans with any pre-combat history of psychopathology. That said, 7 participants (5 PTSD, 2 Sub-PTSD) had a current depressive disorder, and 3 participants (2 PTSD, 1 Sub-PTSD) had current generalized anxiety disorder (GAD). We therefore recomputed all primary analyses with current depressive disorder and current GAD entered as covariates. Results remained largely the same, with Group x Stimulus type interactions continuing to be significant for risk-rating data, as well as data in all previously significant fROIs (dmPFC, R-AI, L-AI, R-dlPFC, L-caudate body, L-VH/Am), with the exception that significance of Group x Stimulus-type interactions for right-caudate-body and left-VH were reduced to trend levels of significance (both ps≤.09). Additionally, correlations between CAPS scores and generalization gradients in right AI and left VH/Amyg remained significant after covarying for current depressive disorder and GAD (both ps<.01). Thus, psychiatric comorbidities in the current sample exerted little influence on results.

References

1. First MB, Gibbon M, Spitzer RL, Williams JBW. Structured Clinical Interview for DSM-IV Axis I Disorders (SCID-I) Research version. . New York: New York State Psychiatric Institute.; 2001.
2. Murray SO, Boyaci H, Kersten D. The representation of perceived angular size in human primary visual cortex. *Nature neuroscience.* 2006;9(3):429-434.
3. Schwartz S, Vuilleumier P, Hutton C, Maravita A, Dolan RJ, Driver J. Attentional load and sensory competition in human vision: modulation of fMRI responses by load at fixation during task-irrelevant stimulation in the peripheral visual field. *Cerebral cortex.* 2005;15(6):770-786.
4. Cox RW. AFNI: software for analysis and visualization of functional magnetic resonance neuroimages. *Computers and biomedical research, an international journal.* 1996;29(3):162-173.
5. Hochberg Y. A sharper Bonferroni procedure for multiple tests of significance. *Biometrika.* 1988;75(4):800-802.
6. Friston KJ, Buechel C, Fink GR, Morris J, Rolls E, Dolan RJ. Psychophysiological and modulatory interactions in neuroimaging. *NeuroImage.* 1997;6(3):218-229.
7. Passamonti L, Rowe JB, Schwarzbauer C, Ewbank MP, von dem Hagen E, Calder AJ. Personality predicts the brain's response to viewing appetizing foods: the neural basis of a risk factor for overeating. *J Neurosci.* 2009;29(1):43-51.
8. Lissek S, Bradford DE, Alvarez RP, et al. Neural substrates of classically conditioned fear-generalization in humans: a parametric fMRI study. *Soc Cogn Affect Neurosci.* 2014;9(8):1134-1142.
9. Grillon C, Morgan CA, Davis M, Southwick SM. Effects of experimental context and explicit threat cues on acoustic startle in Vietnam veterans with posttraumatic stress disorder. *Biological Psychiatry.* 1998;44:1027-1036.
10. Grillon C, Baas JMP. A review of the modulation of the startle reflex by affective states and its application in psychiatry. *Clinical Neurophysiology.* 2003;114:1557-1579.

Table S1. Sample characteristics.

Variable	PTSD (*n* = 20)		Subthreshold (*n* = 21)		Trauma Control (*n* = 20)		Significance[a]
	Mean	SD	Mean	SD	Mean	SD	
Age	33.50	9.63	35.57	9.00	33.45	9.76	$p = .72$
Level of Education	5.10	1.07	5.24	1.61	5.25	1.59	$p = .94$
STAI-State	47.60	11.81	42.38	10.67	32.90	9.30	$p < .001$
STAI-Trait	50.45	10.45	45.14	11.75	37.15	11.71	$p = .002$
BDI	17.75	8.10	13.29	7.80	9.20	7.51	$p = .004$
CAPS-Total	59.60	15.17	31.05	7.81	13.95	6.40	$p < .001$
CAPS-B	16.55	7.02	7.90	3.49	3.05	2.33	$p < .001$
CAPS-C	21.25	6.48	9.90	3.24	3.95	3.10	$p < .001$
CAPS-D	21.80	5.15	13.71	4.93	6.95	4.95	$p < .001$
GAF	61.67	13.02	58.67	12.15	68.13	15.03	*p = .16*
mTBI	1.33	1.50	2.46	2.33	2.64	2.62	*p = .23*
	N	**%**	**N**	**%**	**N**	**%**	**Significance[a]**
Ethnicity							
African American	2	10.0%	1	4.8%	1	5.0%	--
Caucasian	18	90.0%	20	95.2%	16	80.0%	--
Hispanic	0	0.0%	0	0.0%	1	5.0%	--
Asian Pacific	0	0.0%	0	0.0%	1	5.0%	--
Other	0	0.0%	0	0.0%	1	5.0%	--

[a]*p* values reflecting the significance of group differences derived from one-way ANOVAs using Tukey's correction for multiple comparisons; STAI = Spielberger State/Trait Anxiety Inventory; BDI = Beck Depression Inventory; CAPS = Clinician-Administered-PTSD-Scale for the DSM-IV; GAF = Global Assessment of Functioning via SCID; mTBI = mild traumatic brain injury assessed via the Minnesota Blast Exposure Screening Tool; Dx = disorder.

Table S2. Psychiatric diagnosis and current use of psychotropics.

Variable	PTSD (*n* = 20)		Subthreshold (*n* = 21)		Trauma Control (*n* = 20)	
	N	%	N	%	N	%
PTSD Diagnosis						
Delayed Onset	1	5.0%	3	14.0%	0	0.0%
Chronic	19	95.0%	21	100.0%	0	0.0%
Past but not current	0	0.0%	6	28.0%	3	15.0%
Fear/horror/helplessness[a]	20	100.0%	20	90.5%	14	70.0%
Current Comorbidities						
Depressive Disorder	5	25.0%	2	9.5%	0	0.0%
GAD	2	10.0%	1	4.8%	0	0.0%
Anxiety Dx NOS	1	5.0%	2	9.5%	0	0.0%
Sub Abuse/Depend	0	0.0%	0	0.0%	0	0.0%
Past Comorbidities						
Depressive Disorder	10	50.0%	7	33.3%	4	20.0%
Anxiety Dx NOS	1	5.0%	0	0.0%	1	5.0%
Sub Abuse/Depend	14	70.0%	9	42.9%	9	45.0%
Current Psychotropics						
Antidepressant	4	20.0%	3	14.3%	1	5.0%

[a]Number of participants endorsing fear/horror/helplessness during combat-related trauma via the Clinician-Administered-PTSD-Scale for the DSM-IV. PTSD = posttraumatic stress disorder; GAD = generalized anxiety disorder; Dx = diagnosis; NOS = not otherwise specified.

Table S3. Functional regions of interest (fROIs) instantiating positive or negative generalization gradients in all subjects (*N*=61).

fROI	Volume (µl)	Peak[a]				Generalization Gradient	
		X	Y	Z	*β*	Linear (*F*)	Quad (*F*)
CS+ > vCS-							
(Positive gradients)							
dmPFC	10438.88	1.5	16.5	35.5	0.29	97.25***	39.88***
R-AI	7074	52.5	16.5	1.0	0.34	165.66***	31.28***
L-AI	5936.63	-48.0	16.5	-.50	0.30	138.47***	53.80***
R-dlPFC (BA9)	3918.38	45.0	6.0	34.0	0.25	103.94***	1.15
R-dlPFC (BA10)	651.38	34.5	51.0	23.5	0.19	60.70***	8.47*
R-caudate-body	1434.38	7.5	3.0	8.5	0.34	78.03***	22.07***
L-caudate-body	1589.63	-6.0	4.5	7.0	0.34	61.23***	22.06***
R-IPL	567	61.5	-42.0	22.0	0.20	51.69***	34.95***
L-IPL	33.75	-57.0	-45.0	28.0	0.19	53.53***	16.72**
L-IPL	256.5	-61.5	24.0	-23.5	0.24	19.79***	24.35***
R-IPL	77.63	48.0	-43.5	25.0	0.18	23.94***	33.81***
R-LG/FG (BA18)	803.25	13.5	-82.5	-9.5	0.45	0.68	17.39***
R-thalamus	1586.25	1.5	-21.0	1.0	0.29	65.19***	14.45**
L-thalamus	833.63	-6.0	-16.5	11.5	0.26	49.40***	13.26**
L-culmen	290.25	-31.5	-49.5	-27.5	0.20	29.18***	9.38*
R-PMC	60.75	13.5	-6.0	64.0	0.22	13.13**	9.71*
vCS- > CS+							
(Negative gradients)							
vmPFC	4053.38	-6.0	31.5	-8.0	0.31	85.43***	6.09*
PCu	1701	-1.5	-51.0	17.5	0.22	82.17***	0.95
R-VH	135	22.5	-16.5	-11.0	0.35	29.67***	0.30
L-VH	114.75	-21.0	-16.5	-9.5	0.35	31.46***	1.81
L-VH/Am	128.25	-27.0	-7.5	-14.0	0.22	54.14***	.68
R-caudate-head	30.38	7.5	18.0	5.5	0.24	18.24***	8.18*
R-MTG	236.25	48.0	-66.0	23.5	0.19	29.65***	0.65
L-MTG	1761.75	-42.0	-72.0	34.0	0.22	52.52***	1.88
R-ITG (BA19)	722.25	54.0	-64.5	-0.5	0.27	9.62*	14.00**
L-SFG (BA 9)	280.13	-9.0	60.0	29.5	0.20	50.44***	1.06

Positive-gradients reflect strongest responding to CS+ with decreases as rings differentiate from CS+. Negative-gradients reflect strongest responding to oCS- with decreases as rings differentiate from oCS-. [a]XYZ=LPI; fROI= functional region of interest defined as brain loci responding differentially to CS+ versus vCS-; CS+ = conditioned danger-cue; vCS- = 'v-shaped' conditioned safety cue; Linear = linear component of generalization gradient; Quad= quadratic component of gradient; L = left; R = right; dmPFC = dorsomedial prefrontal cortex; AI = anterior-insula, dlPFC = dorsolateral-prefrontal-cortex; BA = Brodmann area; IPL = inferior-parietal-lobule; LG = lingual-gyrus; FG = fusiform-gyrus; PMC = premotor-cortex; vmPFC = ventromedial-prefrontal-cortex; PCu = precuneus; VH = ventral-hippocampus; Am = amygdala; MTG = middle-temporal-gyrus; ITG = inferior-temporal-gyrus; SFG = superior-frontal-gyrus; *p<.05, ** p<.001, *** p<.0001.

Table S4. Results from psychophysiological interaction (PPI) analyses with left and right ventral-hippocampus as seed regions.

Brain Loci		Target Coordinates[a]			Between Group Effect		Within Group Effects			
							PTSD		TC	
Seed	**Target**	**X**	**Y**	**Z**	***t***	***Direction***	***t***	***Direction***	***t***	***Direction***
L-VH/Am	vmPFC	3.0	43.5	-3.5	3.40***	PTSD>TC	2.89**	All>V	2.24*	V>All
	L-AI	-27.0	24.0	13.0	3.57***	PTSD>TC	2.67*	All>V	3.00**	V>All
	L-BA10	-40.5	40.5	20.5	3.64***	TC>PTSD	2.76*	V>All	3.16**	All>V
	R-IPL	48.0	-39.0	29.5	4.18***	PTSD>TC	3.09**	All>V	3.68**	V>All
L-VH	R-IPL	55.5	-24.0	29.5	4.36***	PTSD>TC	2.67*	All>V	4.21***	V>All
	BA47	37.5	34.5	-6.5	3.82***	TC>PTSD	3.47**	V>All	3.11**	All>V
	Precuneus	1.50	-48.0	25	3.73***	PTSD>TC	2.30*	All>V	2.46*	V>All
R-VH	vmPFC	0.0	33.0	-0.5	3.60***	PTSD>TC	2.49*	All>V	3.49**	V>All
	L-AI	-25.5	16.5	-3.5	3.85***	PTSD>TC	2.79*	All>V	3.86***	V>All
	dmPFC	-4.5	4.5	43.0	4.05***	PTSD>TC	2.61*	All>V	2.40*	V>All
	R-Am	19.5	-3.0	-23.0	3.92***	PTSD>TC	2.56*	All>V	1.59	ns
	L-BA10	-33.0	43.0	9.0	3.59***	TC>PTSD	3.13**	V>All	2.70*	All>V

[a]XYZ=LPI; TC = trauma control; L = left; R = right; VH = ventral-hippocampus; Am = amygdala; vmPFC = ventromedial-prefrontal-cortex; AI = anterior-insula; BA = Brodmann area; IPL = inferior-parietal-lobule; dmPFC = dorsomedial-prefrontal-cortex; All = average of all circular stimuli except the conditioned danger-cue (i.e., oCS-, GS_1, GS_2, GS_3); V = 'v-shaped conditioned safety-cue; ns = non-significant; *$p \leq .05$, **$p \leq .01$, ***$p \leq .001$.

Figure S1. Conditioning and generalization stimuli. Counterbalancing groups are designated by A and B (vCS- = v-shaped conditioned safety cue; oCS- = ring-shaped conditioned safety cue; GS_1, GS_2 and GS_3 = 3 classes of generalization stimuli; CS+ = conditioned danger cue). Half of participants were assigned to counterbalancing group A and the other half to B. For both counterbalancing groups A and B, GS_3 was the closest in size to CS+, with GS_2 and GS_1 becoming increasingly dissimilar to CS+.

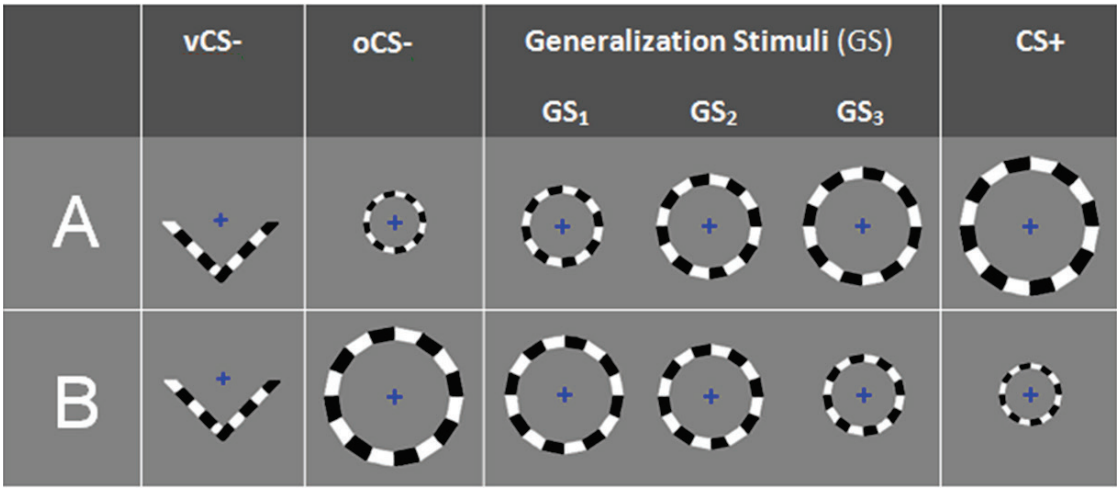

Figure S2. Reported anxiety before, during, and after the study.