Estradiol Modulates Neural and Behavioral Arousal in Women with Posttraumatic Stress Disorder During a Fear Learning and Extinction Task

Supplemental Information

Estradiol Assays

Salivary specimens were obtained via passive drool between the hours of 3-8:00pm, one hour following fear conditioning and extinction training and at least two hours following eating, drinking, or smoking. Samples were stored at -20°C until analysis at the University of Wisconsin (Madison, WI). Before assaying, specimens were thawed completely, vortexed, and centrifuged at 1,500 rpm for 15 minutes. Samples were then pipetted in duplicate into a 96-well test plate and assayed using a commercially available enzyme immunoassay kit (Salimetrics, State College, PA) to determine concentration of 17β-estradiol within each sample. Before calculating skin conductance responses, individuals whose estradiol concentrations were determined to be statistical outliers (n=1, conc=4.372 pg/mL) were not included in any further analyses. Duplicate values were averaged for analyses. Sample coefficient of variation (CV) between all replicates did not exceed 20%. The estradiol assays had an intra-assay CV (n=40) of 6.12% and an inter-assay CV (n=2) of 5.46%. The standard ranged from 1 – 32 pg/mL with an assay sensitivity of approximately 0.1 pg/mL.

Fear Conditioning and Fear Extinction Task

During fMRI and SCR acquisition, participants completed a contextual fear learning paradigm modeled in two prior studies (1). The unconditioned stimulus (US) was a mild, yet aversive electrotactile stimulation administered to the subject's left shin. Conditioned stimuli (CS) were two different alternating geometric shapes, each displayed for 3s with a jittered inter-trial interval of 2-6s. Colored backgrounds distinguished acquisition and extinction contexts. Shapes serving as CS+ vs CS- and background colors distinguishing contexts were counterbalanced across participants. An initial baseline phase consisted of 6 presentations of each stimulus with no UCS onsets. The task then alternated between acquisition and

extinction phases twice each, with an acquisition phase and subsequent extinction learning phase representing one learning block, referred to from here as a "Run." During the acquisition phase, each CS was presented 18 times, with a 50% probability of offset US delivery overlapping for 2.5s following CS+ presentation. The extinction phase presented each stimulus 18 times and no US occurred. Across two Runs of Acquisition learning and Extinction learning, there were a total of 156 trials. Participants' instruction on the task was to identity the stimulus presented on each trial, and they were not informed about any specific contingencies between stimuli and shocks. A contingency awareness assessment occurred after every 12 trials (i.e., three contingency awareness assessments per context), in which participants provided a 0-10 rating of how likely they believed the shock was to follow each of the stimuli. Unfortunately, contingency assessments were administered in a way that was not explicitly linked to a context, and therefore, it cannot be known whether participants were reporting stimulus contingencies within the most recent context or overall contingency awareness throughout the task. Accordingly, all analyses focus on the SCR and imaging data collected throughout the task.

Skin Conductance Acquisition and Processing

SCR data were acquired on a BIOPAC MP150 Data Acquisition System using the EDA100C module with MECMRI-TRANS cable system. Data were acquired directly into BIOPAC AcqKnowledge 4.3 software at 1000 Hz. Stimulations were administered through the BIOPAC STM100C module using BIOPAC EL509 dry electrodes coated with SPECTRA 260 Electrode Gel on the fleshy portion of the mediolateral, left lower leg, directly over the tibialis anterior. SCR recording electrodes were placed on the medial portions of the thenar and hypothenar eminences of the left hand. Amperage on the stimulation device was set to the maximum (50 mA) to allow the greatest range of intensity selections. Participants were told to select an intensity of 7/10 on a subjective pain scale.

We excluded data from five participants from SCR analyses whose galvanic skin response showed excessive artifact or flat responding. Skin conductance preprocessing included, in order, 1) a 10ms median filter, 2) unidirectional butterworth filter with .0159hz and 5hz low and high pass frequencies, respectively,

and 3) downsampling to 10hz. Skin conductance responses were then estimated on a trial-by-trial basis by applying the well-validated forward convolution model of skin conductance responses. Resulting SCRs were normalized to each individual's max SCR per day to account for inter-individual differences in overall magnitude of SCR responding. Reinforced CS+ trials from Fear Acquisition phase blocks were not included in analyses to avoid any contamination of SCR responses to the stimulus with SCR responses to the shock.

MRI Conductance Acquisition and Processing

FMRI data were acquired on a GE MR750 3T scanner using an 8-channel headcoil. T1-weighted anatomic images were acquired with a MP-RAGE sequence (matrix = 256x256, 156 axial slices, TR/TE/FA = 8.2ms/3.2ms/12°, FOV = 25.6cm, final resolution = 1x1x1mm). EPI sequences used to collect the functional images used the following parameters: TR/TE/FA = 2000ms/ 25 ms/ 60, FOV = 24cm, matrix = 64 x 64, 40 sagittal slices, slice thickness = 4mm, original resolution was 4 x 3.75 x 3.75, and images were resampled to match the resolution of the UAMS data of 3x3x3mm.

Image preprocessing followed standard steps and was completed using AFNI software. In the following order, images underwent despiking, slice timing correction, deobliquing, motion correction using rigid body alignment, alignment to participant's normalized anatomical images, spatial smoothing using a 8 mm FWHM Gaussian filter (AFNIs 3dBlurToFWHM that estimates the amount of smoothing to add to each dataset to result in the desired level of final smoothing), detrending, low frequency bandpass filtering (.0078 Hz), and rescaling into percent signal change. Images were normalized using the MNI 452 template brain. We corrected for head motion related signal artifacts by using motion regressors derived from Volterra expansion, consisting of [R R^2 R_{t-1} R^2_{t-1}], where R refers to each of the 6 motion parameters, and separate regressors for mean signal in the CSF and WM. This step was implemented directly after motion correction and normalization of the EPI images in the image preprocessing stream. Additionally, we censored TRs from the first-level GLMs based on threshold of framewise displacement (FD) > 0.4. FD refers to the sum of the absolute value of temporal differences across the 6 motion parameters; thus, a cut-off of 0.4 results in censoring TRs where the participant moved, in total across the 6 parameters, more than

~0.4 mm plus the immediately following TR (to account for delayed effects of motion artifact). Additionally, we censored isolated TRs where the preceding and following TRs were censored, and we censored entire datasets if more than 70% of TRs within that run were censored. This led to the removal of five women from task analyses.

Additional Analysis for Within-Phase Habituation and Estradiol

An additional analysis included a linear slope regressor for each stimulus in each phase, modeling linear learning/habituation upon repeated presentation to each cue during the task. The purpose of this analysis was to test whether estradiol modulated habituation in response to each cue within a given a phase (i.e., acute learning/habituation), which differs from the effect of learning block, which models the repeated presentation of each context. This analysis was similar to the LMEs described in the main manuscript: CS x Context x Slope x learning block x Estradiol x PTSD severity, with covariates for age, education, contraceptive use, and ethnicity. This analysis again demonstrated a Run x Estradiol x PTSD severity interaction, $t(4783)=4.395$, $p<.001$, while there was no significant Slope x Estradiol x PTSD severity interaction, $t(4783)=-1.180$, $p=0.238$ nor a significant Run x Slope x Estradiol x PTSD severity interaction, $t(4783)=-1.017$, $p=.309$.

Table S1. Summary of Omnibus LME results.

Effect Tested	**t-values n=272**	**p-values**
CS	**6.930**	**<.001**
Context	**3.450**	**<.001**
Run	**4.107**	**<.001**
Estradiol	-0.024	.981
Age	1.957	.051
Ethnicity	1.226	.221
Education	-0.241	.810
Contraceptive Use	-0.709	.479
CAPS	-1.245	.214
CS x Context	**5.630**	**<.001**
CS x Run	1.729	.085
Context x Run	0.071	.943
CS x Estradiol	1.495	.136
Context x Estradiol	-0.009	.993
Run x Estradiol	-1.473	.142
CS x CAPS	-1.506	.133
Context x CAPS	-0.768	.443
Run x CAPS	**-2.033**	**.043**
Estradiol x CAPS	-0.611	.542
CS x Context x Run	1.801	.073
CS x Context x Estradiol	0.441	.660
CS x Run x Estradiol	-1.218	.224
Context x Run x Estradiol	0.173	.863
CS x Context x CAPS	-1.082	.280
CS x Run x CAPS	0.271	.787
Context x Run x CAPS	-0.096	.924
CS x Estradiol x CAPS	-0.622	.535
Context x Estradiol x CAPS	0.147	.883
Run x Estradiol x CAPS	**3.180**	**.002**
CS x Context x Run x Estradiol	0.794	.428
CS x Context x Run x CAPS	1.163	.246
CS x Context x Estradiol x CAPS	-0.237	.813
CS x Run x Estradiol x CAPS	1.149	.252
Context x Run x Estradiol x CAPS	0.495	.621
CS x Context x Run x Estradiol x CAPS	-0.386	.700

Table S2. Whole-brain voxelwise LME among only L-EST participants, determined by a median split in order to probe the three-way Estradiol x Run x PTSD severity interaction determined by the omnibus LME. Significant clusters of activation for the Run (Run 1 vs Run 2) x PTSD severity interaction from the linear mixed-effects analysis on imaging data during the fear learning and extinction paradigm. Cluster thresholding was implemented using AFNI's 3dClustSim, in which a voxel-level uncorrected $p < .001$ was used with a cluster size of k>=18 and a corrected threshold of t=3.148. Images used Orig space and RPI orientation.

Region	MNI Center-of-Mass Coordinates			Peak t	Cluster Size
	X	Y	Z		
Left temporoparietal junction	-50	-61	16	-5.23	117
Left supplementary motor rea	-7	6	56	-5.34	78
Left superior temporal gyrus	-62	-43	12	-6.53	53
Right cerebellum	38	-50	-35	-6.52	52
Left postcentral gyrus	-60	-7	31	6.42	49
Right middle temporal gyrus	47	1	-28	-6.16	48
Left posterior insular cortex	-41	-2	3	-4.82	48
Left dorsolateral prefrontal cortex (dlPFC)	-27	46	26	-5.93	47
Left frontal eye fields/precentral gyrus	-46	3	37	-5.16	47
Right postcentral gyrus	19	-32	60	-4.66	45
Right V3/Fusiform gyrus	29	-88	-4	5.12	40
Left inferior frontal gyrus	-50	16	22	-4.45	35
Left rolandic operculum	-44	-25	17	-5.89	33
Left posterior insular cortex	-44	-41	21	-4.88	28
Right lateral occipital gyrus	46	-70	18	-4.86	23
Left superior frontal gyrus	-18	14	60	-5.15	23
Left postcentral gyrus	-25	-41	61	-4.50	21
Left dlPFC	-35	44	36	-5.93	20
Right cerebellum	20	-69	-44	-5.21	19

Table S3. Whole-brain voxelwise LME among only H-EST participants, determined by a median split in order to probe the three-way Estradiol x Run x PTSD severity interaction determined by the omnibus LME. Significant clusters of activation for the Run (Run 1 vs Run 2) x PTSD severity interaction from the linear mixed-effects analysis on imaging data during the fear learning/extinction paradigm. Cluster thresholding was implemented using AFNIs 3dClustSim, in which a voxel-level uncorrected $p < .001$ was used with a cluster size of k>=18 and a corrected threshold of t=3.148. Images used Orig space and RPI orientation.

Region	MNI Center-of-Mass Coordinates			Peak t	Cluster Size
	X	Y	Z		
Left cerebellum	24.7	-63.6	-42.0	5.66	25
Left intraparietal sulcus	30.8	-76.4	39.6	5.13	20
Left angular gyrus	54.9	-63.4	25.7	5.32	18

Table S4. Impact of estradiol on SCR when considering possible confounding effect of psychotropic medication use and time since last assault.

Effect	Without accounting for SNRI use or TSLA	Covarying for psychotropic medication use	Removing participants taking an SNRI medication	Covarying for TSLA
Estradiol	*t*(272)=-0.024, *p*=.981	*t*(271)=0.092, *p*=.926	*t*(248)=0.847, *p*=.193	*t*(271)=-0.171, *p*=.864
Run	***t*(272)=4.107, *p*<.001**	***t*(271)=4.104, *p*<.001**	***t*(248)=4.037, *p*<.001**	***t*(271)=4.106, *p*<.001**
CAPS	*t*(272)=-1.245, *p*=.214	*t*(271)=-1.274, *p*=.204	*t*(248)=-0.888, *p*=.375	*t*(271)=-1.156, *p*=.249
Run x CAPS	***t*(272)=-2.033, *p*=.043**	***t*(271)=-2.025, *p*=.044**	***t*(248)=-2.523, *p*=.012**	***t*(271)=-2.035, *p*=.043**
Run x Estradiol	t(272)=-1.473, p=.142	t(271)=-1.476, p=.141	t(248)=-1.599, p=.111	*t*(271)=-1.475, *p*=.141
Estradiol x CAPS	t(272)=-0.611, p=.542	t(271)=-0.408, p=.683	*t*(248)=-0.491, *p*=.624	*t*(271)=-0.571, *p*=.568
Run x Estradiol x CAPS	**t(272)=3.180, p=.002**	***t*(271)=3.178, *p*=.002**	**t(248)=1.969, p=.050**	***t*(271)=3.182, *p*=.002**

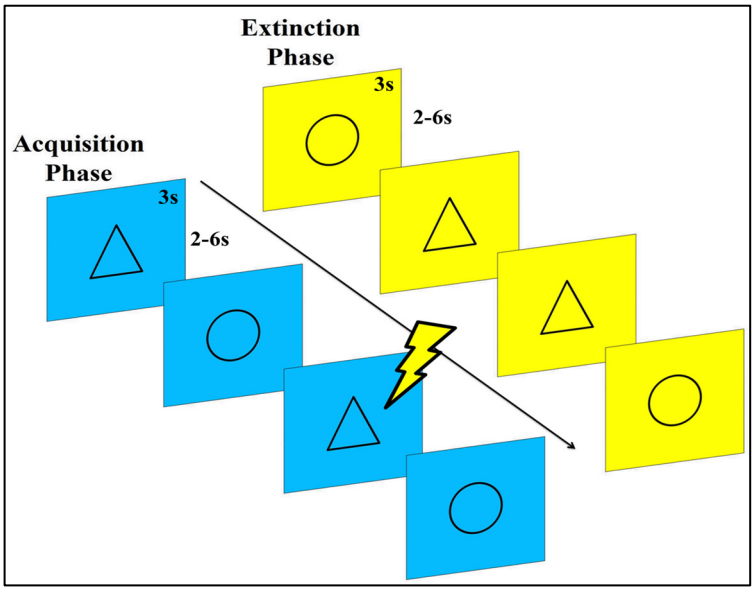

Figure S1. Fear Conditioning and Fear Extinction Task Structure. Participants completed the fear conditioning and fear extinction task during fMRI. The unconditioned stimulus (US) was an electric shock, which participants calibrated to an intensity level of 7/10 on a Likert scale. Conditioned stimuli consisted of triangles and circles, each displayed for 3s with a jittered inter-trial interval of 2-6s, and counterbalanced across participants. An initial baseline phase consisted of 6 presentations of each stimulus with no UCS onsets. The task then alternated between acquisition and extinction phases for 156 trials, with two presentations of each phase. The acquisition phase presented each CS 18 times, with a shock occurring 2.5s following CS+ presentation with a 50% reinforcement schedule. The extinction phase presented each stimulus 18 times and no shocks occurred.

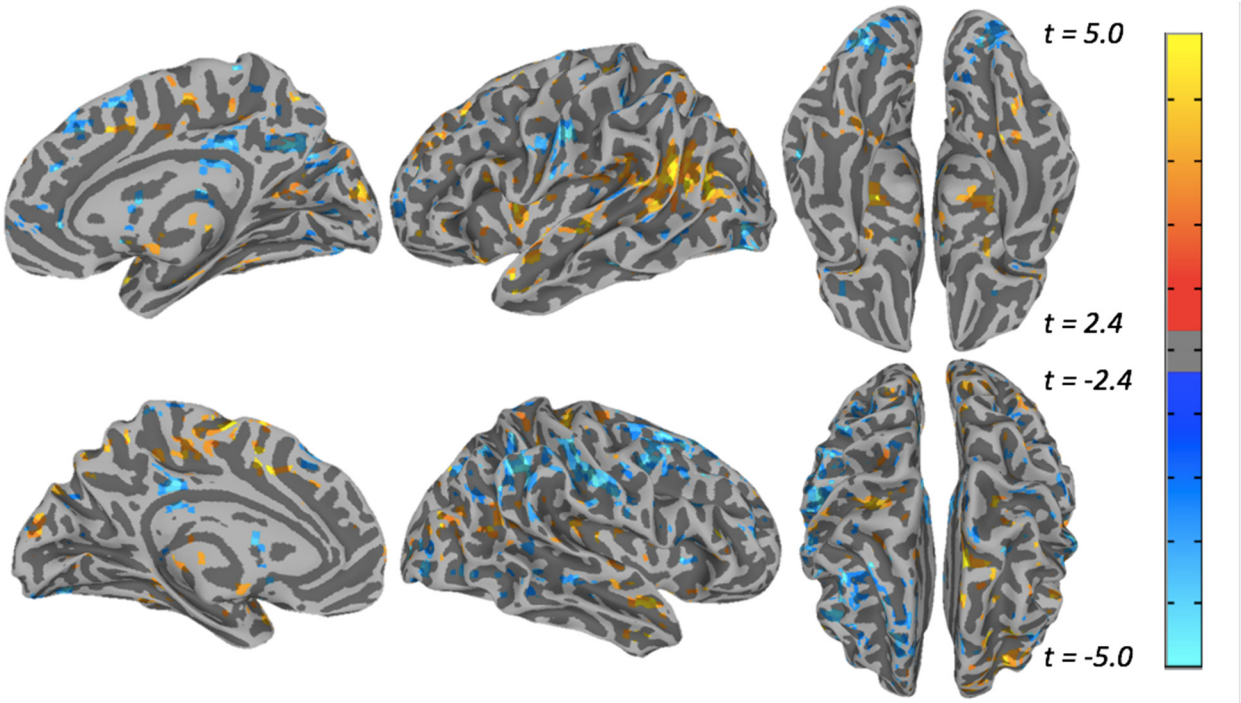

Figure S2. Voxelwise LME results for Estradiol x Run (Learning Block 1 vs Learning Block 2) x PTSD Severity interaction. Results are displayed at a voxelwise threshold of p = .01 for visualization of overall effects. Corresponds with whole-brain voxelwise cluster-threshold statistics provided in Table 2.

Supplemental Reference

1. Haaker J, Gaburro S, Sah A, Gartmann N, Lonsdorf TB, Meier K, *et al.* (2013): Single dose of l-dopa makes extinction memories context-independent and prevents the return of fear. *Proc Natl Acad Sci* 110: E2428–E2436.