Oxytocin Facilitates the Extinction of Conditioned Fear in Humans

Supplemental Information

SUPPLEMENTAL METHODS

Psychophysiological Measurement and Electrical Stimulation

The unconditioned stimulus consisted of brief electrical shocks of 2 ms duration. The electrical shock stimuli were delivered via a Biopac stimulator module STM100C and a STIMSOC adapter (Biopac Systems, Inc., Goleta CA, USA) coupled with the notebook computer presenting the fMRI paradigm. Current was passed from the generator to the subject via two MRI-compatible Ag/AgCl electrodes filled with electrolyte gel on the subject's left (non-dominant) dorsal lower arm. Before acquisition, shock intensity levels were set manually for each individual by delivering gradually more intense shocks until the subject reported the shock was "highly annoying yet not painful." During the conditioning procedure the skin conductance responses (SCRs) were sampled simultaneously with MR scans. SCRs were acquired at a sampling rate of 1000 Hz from Ag/AgCl electrodes filled with isotonic electrolyte gel on the tenar and hypotenar of the left (non-dominant) hand via Biopac Module EDA100C-MRI and acquisition module MP150 (Biopac Systems Inc., Goleta CA, USA). The SCRs were saved and analyzed with *Acqknowlege 4.3* software.

Processing of Psychophysiological Data

The SCR was defined as the maximum of the conductance signal in a 5 s time window after conditioned stimulus (CS) onset minus a baseline value (the mean conductance 1 s before the onset of the CS) (1, 2). To account for interindividual differences in physiological reactivity, SCR data were z-transformed (3) and outliers of +/- 2SD were excluded within each subject.

Acquisition of fMRI Data

The MRI data were acquired on a Siemens Avanto MRI system (Siemens, Erlangen, Germany) operating at 1.5T.T2*-weighted echoplanar (EPI) images were acquired with a blood-oxygen-level-dependent contrast (TR = 3000 ms, TE = 35 ms, matrix size: 64 x 64, pixel size: 3 x 3 x 3

mm, slice thickness = 3.0 mm, distance factor = 10%, FoV = 192, flip angle = 90°, 36 axial slices). In addition, high-resolution anatomical images were acquired on the same scanner using a T1-weighted 3D MPRAGE sequence (imaging parameters: TR = 1570 ms, TE = 3.42 ms, matrix size: 256 x 256, pixel size: 1 x 1 x 1 mm, slice thickness = 1.0 mm, FoV = 256, flip angle = 15°, 160 sagittal slices).

Preprocessing of fMRI Data

The MRI data were preprocessed and analyzed using SPM8 software (Wellcome Trust Centre for Neuroimaging, London, UK; http://www.fil.ion.ucl.ac.uk/spm) implemented in MATLAB 7 (The MathWorks Inc., Natick, MA). The first five volumes of each functional time series were discarded to allow for T1 equilibration. Images were corrected for head movement between scans by an affine registration. For realignment, images were initially realigned to the first image of the time-series and subsequently re-realigned to the mean of all images. For spatial normalization, the mean EPI image of each subject was normalized to the current Montreal Neurological Institute (MNI) template (4, 5) using the unified segmentation function in SPM8. This algorithm combines image registration, tissue classification, and bias correction within the same generative model. All images were thereby transformed into standard stereotaxic space and resampled at 3 x 3 x 3 mm voxel size. The normalized images were spatially smoothed using an 8 mm FWHM Gaussian kernel. Raw time series were detrended using a high-pass filter (cut-off period 128 s).

Analysis of fMRI Data

A two-level random effects approach based on the general linear model as implemented in SPM8 was used for statistical analyses. On the first level, the four conditions (face CS+, face CS-, house CS+, house CS-) were defined and modeled in a mini-epoch design convolved with a hemodynamic response function (7). Regressors for the early and late phase of extinction were defined as trials 1-30, the late phase as trials 31-60 of the stimulus. The movement parameters were included as confounds in the design matrix. Each experimental condition was compared relative to the low-level baseline and differences between each condition were computed separately for the oxytocin (OXT) and placebo (PLC) group. For the whole-brain a significance threshold of $P < 0.05$, corrected for multiple comparisons (family-wise error (FWE)), was used.

Specific effects of OXT on extinction were assessed using multiple repeated measures analyses of variance (ANOVAs) with the within-subject factor ‘phase’ (early, late) and the between-subject factor ‘treatment’ (OXT, PLC). Unspecific effects of OXT were assessed by means of second level 2-sample *t*-test. To further examine the specificity of the OXT effect, parameter estimates were extracted from regions showing significant between-group differences using the MarsBaR toolbox (8) (see also http://marsbar.sourceforge.net/).

Based on previous studies investigating the neural effects of OXT (9), we used structural regions of interest (ROIs) for the amygdala, the medial, and the middle frontal gyrus. ROI-based ANOVAs and two-sample *t*-tests were computed with a threshold of $P < .05$ and FWE-corrected for multiple comparisons based on the size of the ROI. Anatomical classification was done using the WFU Pick atlas, automatic anatomic labeling, or Talairach Daemon labels (10, 11).

To further address OXTs effects on the interplay of extinction-related regions, a psychophysiological interactions (PPIs) analysis was conducted using a gPPI (12). In contrast to the standard PPI implementation in SPM, gPPI analysis allows modeling of more than two task conditions in the same PPI by spanning the entire experimental space to improve model fit and to improve specificity to true-negative and sensitivity to true-positive findings. We examined modulation effects of OXT on functional connectivity of the extinction-related between-group differences in the prefrontal cortex. The seed region was defined as a 6 mm radius sphere centered at the maximum *t*-value of the between-group effect and reaching to the peak of the BOLD effect (MNI coordinates x, y, z = 24, 26, 43) as well as the structurally defined amygdala, using the WFU Pick atlas (10).

SUPPLEMENTAL RESULTS

Behavioral Results

Participants were unaware of whether they had received OXT or PLC ($\chi^2_{(1)} = 3.18$, $P > 0.05$). A repeated measures ANOVA with 'time' (pre-extinction, post-extinction) as within-subject factor, 'treatment' (OXT, PLC) as between-subject factor, and positive affect as the dependent variable showed a significant main effect of time. Positive affect increased ($F_{(1,54)} = 26.24$, $P < .01$, $\eta^2 = .33$) from pre-extinction to post-extinction for the whole sample (cf. **Table S1)**. There were no further main or interaction effects. Parallel analysis with negative affect as the dependent variable did not result in any main of interaction effects.

Physiological Parameters Results

SCR conditioning data from $n = 4$ subjects could not be analyzed due to acquisition failure. Successful conditioning is evidenced by a one-tailed paired-sample *t*-test ($t_{(57)} = -1.30$, $P < .1$) for dependent measurements, showing larger SCRs to the CS+ ($M = -0.20 \pm 0.53$) than to the CS- ($M = -0.29 \pm 0.42$) in response to the last 10 trials of each stimulus type (cf. **Figure S1A**).

fMRI Results

The Pavlovian fear conditioning procedure led to robust neural activity in several regions previously implicated in fear conditioning (6) including the insula, cingulate cortex, thalamus, caudate, and middle frontal gyrus (cf. **Table S2 and Figure S1B**), confirming successful fear acquisition.

To explore whether the effects of OXT on extinction were different for social and non-social stimuli, the individual contrasts [face CS+ > face CS-] and [house CS+ > house CS-] were subjected to a 2 x 2 repeated measures ANOVA with the within-subject factor 'sociality' [face, house] and the between-subject factor 'treatment' [OXT, PLC]. This analysis revealed no significant interaction effects, and the data from the social and non-social stimulus categories were consequently pooled to increase the statistical power.

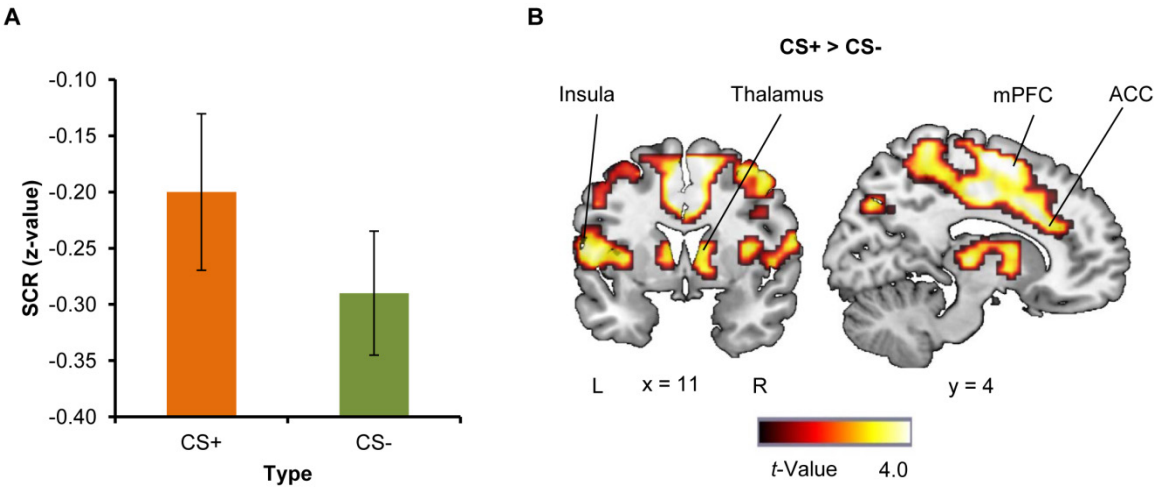

Figure S1. Effects of conditioning. (A) In the last ten trials, participants exhibited a stronger SCR to the CS+ than to the CS-. **(B)** The contrast CS+ > CS- showed a broad neural activation network comprising the cingulate cortex, insula, thalamus, and prefrontal cortex. Error bars indicate the standard error of the mean (SEM). ACC, anterior cingulate cortex; BOLD, blood-oxygen-level-dependent; CS+, fear-associated stimulus; CS-, non-fear-associated stimulus; L, left; mPFC, medial prefrontal cortex; R, right; SCR, skin conductance response.

Table S1. Ratings of State Anxiety and Positive/Negative Affect

Assessment	Test	OXT group Mean (SD)	PLC group Mean (SD)	*t*	*df*	*P*
Pre Extinction	STAI State Anxiety	-0.26 (2.48)	-0.17 (3.25)	0.12	59	0.90
Post Extinction		-0.69 (2.62)	-0.71 (2.22)	-0.32	58	0.96
Pre Extinction	PANAS Positive Affect Scale	-3.20 (4.50)	-2.80 (4.31)	0.35	58	0.73
Post Extinction		-5.00 (5.50)	-6.69 (5.75)	-1.10	56	0.26
Pre Extinction	PANAS Negative Affect Scale	0.23 (1.76)	0.57 (3.52)	0.48	58	0.63
Post Extinction		0.38 (1.76)	-0.10 (3.17)	-0.72	56	0.48

Values are corrected to a baseline assessment at beginning of the session.
STAI, Spielberger Trait State Anxiety Inventory; PANAS, Positive Affect Negative Affect Scale.

Table S2. Activation table for the GLM analysis (Conditioning [CS+ > CS-])

Side	Region	Cluster size[#]	Peak Z	MNI coordinates		
				x	y	z
R	Postcentral Gyrus	4322	6.04**	42	-31	58
R	Precentral Gyrus			30	-19	61
R	Postcentral Gyrus			21	-43	64
R	Insula	963	5.49**	2	26	4
R	Insula			42	-22	19
R	Insula			57	-16	19
L	Insula	812	5.46**	-30	17	10
L	Gyrus Supramarginalis			-54	-28	25
L	Gyrus Supramarginalis			-48	-43	25
R	Precuneus	55	4.76*	15	-76	37
R	Thalamus	116	4.70*	9	-19	4
R	Caudate			9	5	10
R	Caudate			12	5	-2
L	Thalamus	132	4.34*	-15	-19	4
L	Thalamus			-6	-7	4
L	Caudate			-15	-4	19
R	Insula	42	4.24*	27	44	19
R	Anterior Cingulate[a]	181	4.99**	9	23	28
L	Anterior Cingulate			-6	20	31
L	Anterior Cingulate			-6	29	19
R	Middle Frontal Gyrus[a]	108	5.36**	42	-4	58
R	Middle Frontal Gyrus			51	-10	52
R	Middle Frontal Gyrus[a]	34	4.14*	30	44	22
L	Middle Frontal Gyrus[a]	32	4.72*	-30	-1	55
L	Middle Frontal Gyrus			-24	-4	49
L	Middle Frontal Gyrus			-27	8	55

*P < .05 FWE and **P < .01 FWE corrected.
[#]Height threshold 0.001.
[a]Analysis based on predefined anatomical ROIs.

SUPPLEMENTAL REFERENCES

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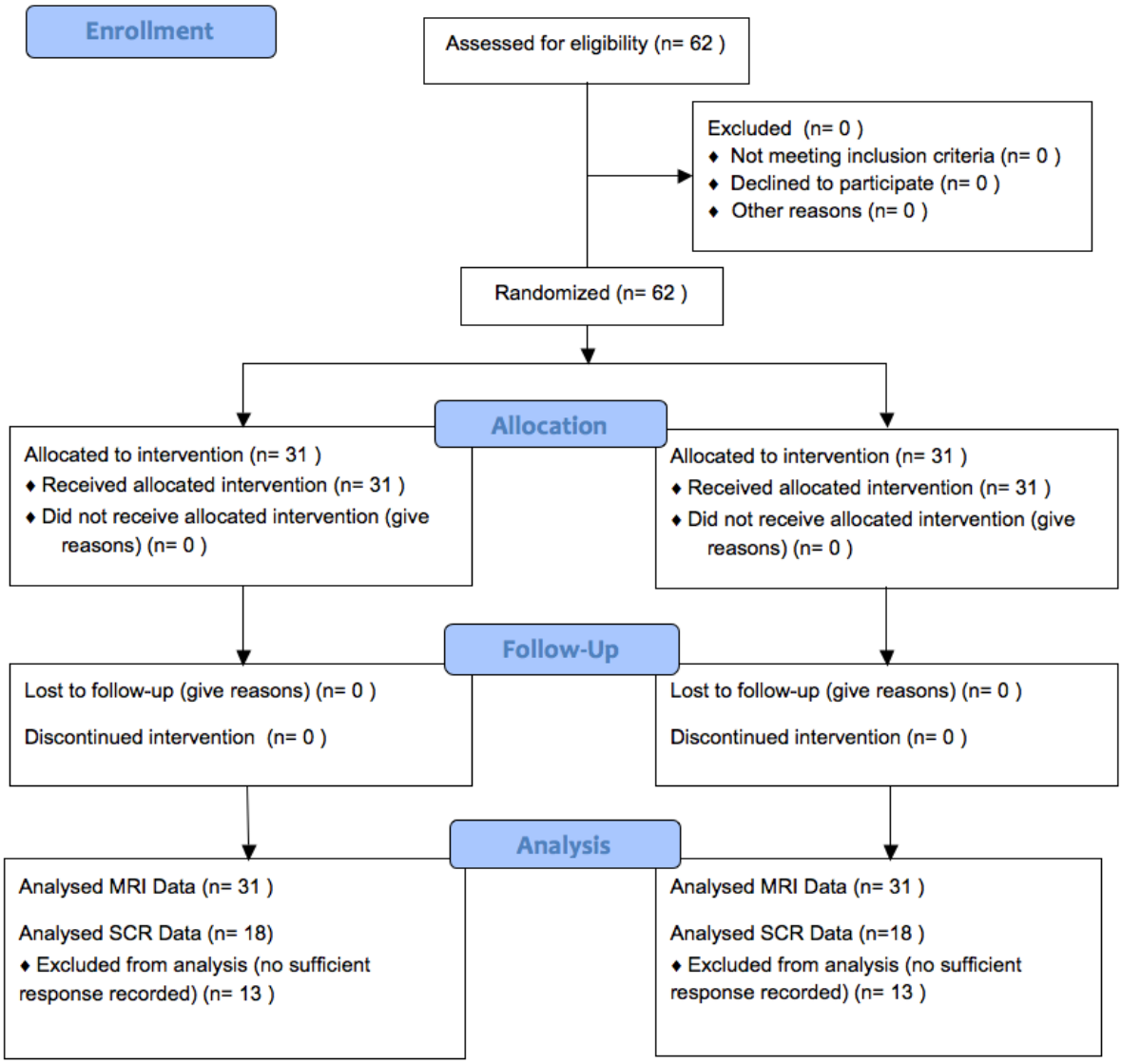
CONSORT
TRANSPARENT REPORTING of TRIALS

CONSORT 2010 Flow Diagram



CONSORT 2010 checklist of information to include when reporting a randomised trial*

Section/Topic	Item No	Checklist item	Reported on page No
Title and abstract			
	1a	Identification as a randomised trial in the title	2
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)	2
Introduction			
Background and objectives	2a	Scientific background and explanation of rationale	3
	2b	Specific objectives or hypotheses	4
Methods			
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	5
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	5
Participants	4a	Eligibility criteria for participants	5
	4b	Settings and locations where the data were collected	5
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	5
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	5/6
	6b	Any changes to trial outcomes after the trial commenced, with reasons	6
Sample size	7a	How sample size was determined	5
	7b	When applicable, explanation of any interim analyses and stopping guidelines	-
Randomisation:			
Sequence generation	8a	Method used to generate the random allocation sequence	5
	8b	Type of randomisation; details of any restriction (such as blocking and block size)	5
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	5
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	22
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those	5

		assessing outcomes) and how	
	11b	If relevant, description of the similarity of interventions	5
Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes	6/7
	12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses	6/7
Results			
Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analysed for the primary outcome	5
	13b	For each group, losses and exclusions after randomisation, together with reasons	7/8
Recruitment	14a	Dates defining the periods of recruitment and follow-up	5
	14b	Why the trial ended or was stopped	-
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	19
Numbers analysed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	7/8
Outcomes and estimation	17a	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval)	9/10/11
	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory	9/10/11
Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	-
Discussion			
Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	13
Generalisability	21	Generalisability (external validity, applicability) of the trial findings	13
Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	12
Other information			
Registration	23	Registration number and name of trial registry	3
Protocol	24	Where the full trial protocol can be accessed, if available	
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	15

*We strongly recommend reading this statement in conjunction with the CONSORT 2010 Explanation and Elaboration for important clarifications on all the items. If relevant, we also recommend reading CONSORT extensions for cluster randomised trials, non-inferiority and equivalence trials, non-pharmacological treatments, herbal interventions, and pragmatic trials. Additional extensions are forthcoming: for those and for up to date references relevant to this checklist, see www.consort-statement.org.