Supplemental Material

Oxytocin facilitates Pavlovian fear learning in males

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SUPPLEMENTAL METHODS

FMRI conditioning paradigm

After arrival at the MRI scanner, subjects were first habituated to all four stimuli by presenting them outside the scanner. The face stimuli were selected from the Karolinska face data base (Lundqvist *et al*, 1998). The same face and house stimuli were used for all subjects, yet CS+ and CS- assignment within the two stimuli pairs was counterbalanced across treatment groups. To ensure attentive processing, 50 % of the subjects were instructed to press the right response button for a face and the left button for a house and the other 50 % of subjects vice versa. All subjects were naive with respect to the aim of the study.

Psychophysiological measurement and electrical stimulation

The UüS consisted of brief electrical shocks of 2 ms. The electrical shocks were delivered 3750 ms after onset of the CS+ via a Biopac stimulator module STM100C and a STIMSOC adapter (Biopac Systems, Inc., Goleta CA, USA) coupled with a notebook computer presenting the fMRI paradigm. A 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." Skin conductance responses (SCRs) were acquired at a sampling rate of 1000 Hz from Ag/AgCl electrodes filled with isotonic electrolyte gel on the thenar and hypothenar of the left (non-dominant) hand via Biopac

Module EDA100C-MRI and acquisition module MP150 (Biopac Systems Inc., Goleta CA, USA). The SCRs were acquired and analyzed with the *AcqKnowlege 4.3* software package.

SUPPLEMENTAL RESULTS

Behavioral Results

We repeated all behavioral and imaging analyses for the reduced sample used for the analysis of electrodermal responses. Mean reaction times were submitted to a repeated measures ANOVA with the within-subject factors 'time' (early: trials 1-15, late: trials 16-30), 'type' (CS+, CS-), and 'sociality' (face, house), and 'treatment' (OXT, PLC) as between-subject factor.

The analysis yielded a significant main effect of type ($F_{(1,42)}$ = 6.28, P = 0.02, η^2 = 0.13), but no further main effects. Across all conditions, participants showed faster responses to the CS+ than the CS- indicating successful conditioning. Furthermore, we detected interaction effects of 'type' and 'sociality' ($F_{(1,42)}$ = 4.04, P = 0.02, η^2 = 0.13) as well as 'sociality' and 'phase' ($F_{(1,42)}$ = 7.384, P = 0.01, η^2 = 0.15), indicating stronger differential responses in the social condition and generally accelerated responses to faces towards the late phase of conditioning. Importantly, we also observed an interaction effect of 'type', 'sociality', and 'treatment' ($F_{(1,42)}$ = 4.04, P = 0.025 one tailed, η^2 = 0.09). Post hoc paired t-tests showed significantly faster responses to all CS+ ($M \pm SD$= 597.87 ± 103.6 ms) than to all CS- ($M \pm SD$ = 615.7 ± 110.7 ms) under OXT ($t_{(15)}$ = -2.44, P = 0.02). For the PLC group, the reaction time difference did not reach statistical significance in the reduced sample (P = 0.16). Therefore, enhanced conditioning under OXT was also evident in this subsample.

fMRI Results

The region of interest analysis for the amygdala did not yield significant OXT-specific effects. A main effect of conditioning [CS+ > CS-] was evident only at an uncorrected P level (for the left amygdala: peak MNI coordinates x, y, z = -24, -7, -11, $t_{(94)}$ = 1.74, $P_{\text{uncorrected}}$ = 0.04; for the right amygdala: peak MNI coordinates x, y, z = 30, -7, -11, $t_{(94)}$ =2.15, $P_{\text{uncorrected}}$ = 0.02).

The fMRI analysis with the reduced subsample used for the psychophysiological analysis revealed a pattern of results very similar to the one that we obtained for the whole sample. We found a main effect of OXT^{IN} on conditioning [CS+ > CS-] such that OXT-treated subjects compared to the PLC group exhibited a significantly higher activation in the right sACC (peak MNI x, y, z = 3, 29, -2, $t_{(45)}$ = 3.18, $P_{uncorrected}$ = 0.001). Analysis for social fear acquisition [face CS+ > face CS-] revealed that OXT^{IN} enhanced activity in the left pMCC (peak MNI x, y, z = -12, -40, 37, $t_{(45)}$ = 2.75, $P_{uncorrected}$ = 0.004).

A correlational analysis of the oxytocin (OXT) effect on reaction times and electrodermal responses did not yield significant associations (all *P*s > 0.05). Likewise, there was no significant correlation between parameter estimates and OXT-induced differences in reaction times or electrodermal responses.

SUPPLEMENTAL TABLES

Supplemental Table 1. Activation table for the GLM analysis **Treatment Effects**

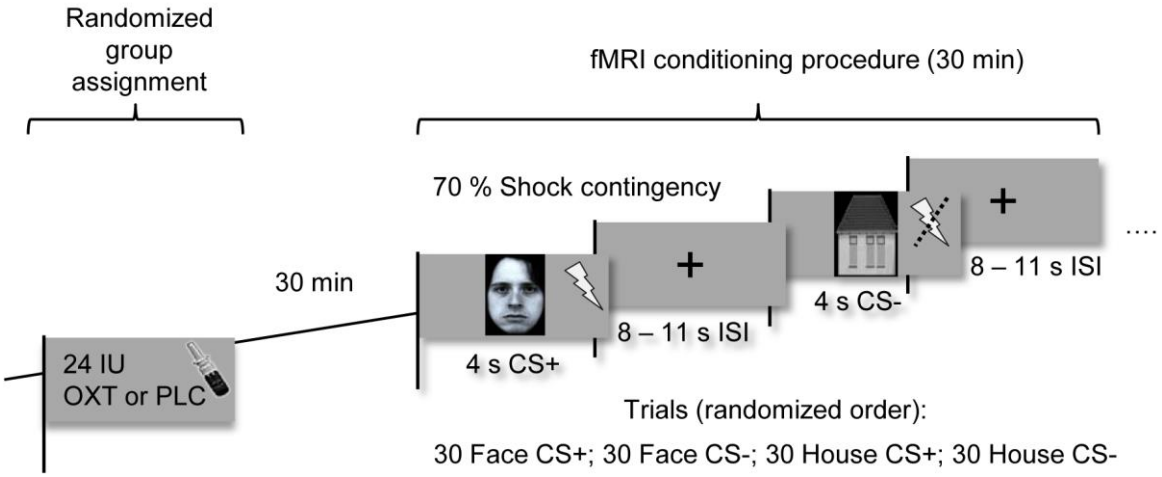
Region		Cluster size	Peak Z	MNI coordinates		
Conditioning				**x**	**y**	**z**
OXT$^{[CS+> CS-]}$ > PLC$^{[CS+> CS-]}$						
R	Anterior Cingulate[a]	2	3.45*	3	29	-2
OXT$^{[CS+> CS-]}$ > PLC$^{[CS+> CS-]}$						
L	Middle Cingulate[a]		5.59*	-15	-43	37
PLC$^{[Shock > Baseline]}$ > OXT$^{[Shock > Baseline-]}$						
L	Anterior Cingulate[a]	56	3.92*	-3	44	4

*P< 0.05 FWE-corrected. [a]Analysis based on predefined anatomical regions of interest (ROIs) with a height threshold = 0.001

SUPPLEMENTAL FIGURES

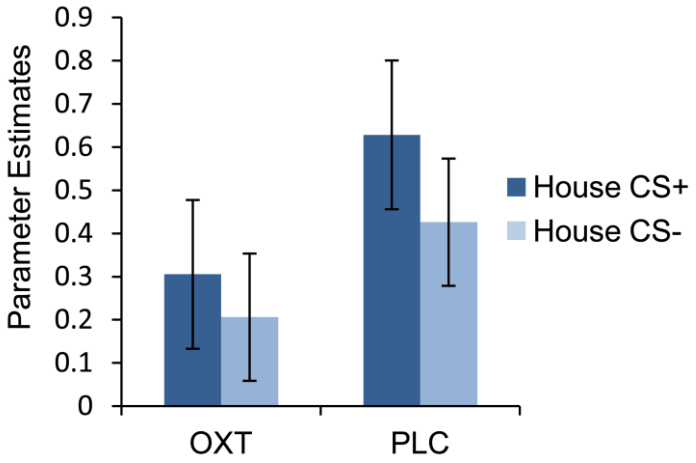
Supplemental Figure S1. Schematic representation of the fear conditioning experiment.

The fMRI conditioning procedure started 30 min after inhalation of the nasal spray. All CS+ and CS- stimuli were presented 30 times in total for 4000 ms each in a randomized order throughout the conditioning procedure (restriction: there were no more than two consecutive presentations of any one type of CS). CSs were separated by a variable interstimulus interval (ISI) ranging from 8 to 11 s, during which subjects viewed a central fixation cross (low-level baseline). To ensure attentive processing, 50 % of the subjects were instructed to press the right response button for a face and the left button for a house and the other 50 % of subjects vice versa. Abbreviations: CS+, fear associated stimulus; CS-, safety associated stimulus; Flash denotes electric shock; fMRI, functional magnetic resonance imaging; OXT, oxytocin; PLC, placebo; ISI, interstimulus interval.



Supplemental Figure S2. Neural effects of oxytocin on fear conditioning with non-social stimuli

Intranasal oxytocin increased neural responses in the posterior midcingulate cortex selectively in the social stimuli condition, but it had no effect with non-social stimuli *Abbreviations:* OXT, oxytocin; PLC, placebo; Error bars indicate standard error of the mean (SEM).



SUPPLEMENTAL REFERENCES

Lundqvist D, Flykt A, Öhman A (1998). The Karolinska directed emotional faces (KDEF). *CD ROM from Department of Clinical Neuroscience, Psychology section, Karolinska Institute*.