Oxytocinergic Modulation of Threat-Specific Amygdala Sensitization in Humans Is Critically Mediated by Serotonergic Mechanisms

Supplementary Information

Supplementary Methods

Participants

The primary aim of the study was to determine interactions between the OXT and 5-HT systems on threat-related amygdala reactivity and amygdala sensitization/desensitization in humans. To this end a total of *N*=121 right-handed healthy male participants were enrolled in the present randomized placebo-controlled double-blind between-subject pharmacological fMRI study. Exclusion criteria included: (1) current/history of physical or psychiatric disorders, (2) current/regular use of licit or illicit psychotropic substances, (3) weight >85 kilograms, (4) MRI contraindications, (5) cardiovascular disorders including high blood pressure, (6) contraindications for either oxytocin administration (OXT) or the acute tryptophan depletion (ATD) protocol. Subjects were explicitly asked about their subjective state (e.g. exhaustion, emotional state, somatic state including nausea or stomach problems) and whether their current state could interfere with proceeding with the assessments (e.g. treatment administration, MRI, questionnaires). None of the participants reported negative side effects. For the behavioral and mood analysis, based on initial quality assessments data from n=9 subjects were excluded from the analysis (n=5, technical problems during data acquisition; n=2, performance >3 SDs from mean accuracy suggesting a lack of attention or adherence to the experimental protocols; n=2, history of mania or depression). During subsequent quality assessment of the MRI data one subject was further excluded from the fMRI analyses due to poor normalization quality. (details see CONSORT flowchart, **Figure S1**).

Serotonin manipulation procedure

Previous studies have demonstrated that after administration of ATD^+ tryptophan levels continuously decrease until it reaches a plateau after about 5 hours and that the robust decrease lasts around two hours (1-3). Given that tryptophan is the amino acid precursor of serotonin, the ATD^+ procedure induces a transient selective reduction in central serotonergic neurotransmission (4). The contents of the amino-acid mixtures were based on previously validated proportions (3, 5, 6). The amino acid mixture (ATD^+) consisted of 4.1g L-alanine, 3.7 g L-arginine, 2 g L-cystine, 2.4 g glycine, 2.4g L-histidine, 6g L-isoleucine, 10.1 g L-leucine, 6.7g L-lysine, 2.3 g L-methionine, 9.2g L-proline, 4.3 g L-phenylalanine, 5.2 g L-serine, 4.9 g L-threonine, 5.2 g L-tyrosine, 6.7 g L-valine, (total: 75.2g). The control drink (ATD^-) contained identical

ingredients plus 3.0g of L-tryptophan (total: 78.2g). The drinks were prepared by stirring the mixture into 200-ml water and lemon-lime flavor was added to mask the taste of mixture.

MRI data acquisition and pre-processing

MRI data were acquired on a 3.0 Tesla GE MR750 system (General Electric Medical System, Milwaukee, WI, USA). T1-weighted high-resolution anatomical images were acquired with a spoiled gradient echo pulse sequence, repetition time (TR) = 5.9 ms, echo time (TE) = minimum, flip angle = 9°, field of view (FOV) = 256 × 256 mm, acquisition matrix = 256 × 256, thickness = 1 mm, number of slice = 156. For the functional MRI timeseries a total of 504 functional volumes were acquired using a T2*-weighted Echo Planar Imaging (EPI) sequence (TR = 2000 ms, TE = 30 ms, FOV = 220 × 220 mm, flip angle = 90°, image matrix = 64 × 64, thickness/gap = 3.2/0mm, 43 axial slices with an interleaved ascending order). Functional time-series were pre-processed using statistical parametric mapping (SPM12; Wellcome Department of Cognitive Neurology, Institute of Neurology, London, United Kingdom). For each subject and run the first seven volumes were discarded to allow for T1 equilibration. The remaining functional images were slice-time corrected and realigned to the first image to correct for head motion. The EPI images were next co-registered to the T1-weighted structural images and normalized to Montreal Neurological Institute (MNI) standard space using the segmentation parameters obtained from segmenting the structural images and interpolated at 3×3×3mm voxel size. Finally, the normalized images were spatially smoothed with an 8-mm full-width at half maximum (FWHM) Gaussian filter.

Robustness of threat-specific amygdala sensitization / desensitization in an independent sample

Using an identical fMRI paradigm a previous study demonstrated the robustness and within-subject replicability of amygdala desensitization as assessed by the mean of the block difference approach (7). However, the previous study employed a within-subject validation approach and examined amygdala habituation irrespective of emotional content of the faces whereas the present study examined threat-specific amygdala adaptations in a between-subject treatment design. To further test the replicability of the threat-specific amygdala sensitization / desensitization effects, we therefore employed a dataset from an independent study from our lab (details of this study see also pre-registration https://clinicaltrials.gov/ct2/show/NCT03549182, ID NCT03549182), henceforward referred to a 'validation study' in the context of the present manuscript). Briefly, in the validation study we employed the same paradigm, except that additionally a fearful face condition was included. Subjects were administered either an ATD^{+} or an ATD-placebo drink before fMRI acquisition, for the validation analysis the data from the n = 25 ATD-placebo treated healthy male subjects (mean age 21.44 ± 2.50) was included. The paradigm consisted of 6 runs and every run comprised 4 blocks of facial stimuli as well as 1 block of

non-facial stimuli serving as non-social control stimuli. During the face-processing blocks, a trio of condition-specific (neutral, angry, fear or happy expressions) facial stimuli was presented and subjects required to select one of the two faces (bottom) that was identical to a target face (top). Each block comprised four condition-specific trials, balanced for gender. Asian facial stimuli were selected from a standardized Asian facial expression database (8). During the non-social control blocks a trio of simple geometric shapes (circles and ellipses) was presented and subjects required to select one of two shapes (bottom) that was identical to a target shape (top). Each control block comprised four different shape trios. All blocks were preceded by a brief instruction ('Face match' or 'Shape match') that lasted 2s. Within each block, each trial was presented for 4s with a variable interstimulus interval (ISI) of 1-3 s (mean, 2s). The total block length was 26s and the total paradigm lasted 21min 36s. Preprocessing and first-level modelling were conducted in SPM 12 and were identical to the procedures in the main study. In line with the original study, the main contrast of interest to determine the robustness of the amygdala sensitization / desensitization in the validation sample was [($angry_{last\text{-}block}$-$neutral_{last\text{-}block}$) > ($angry_{first\text{-}block}$-$neutral_{first\text{-}block}$)].

Supplementary Results

Behavioral results

Significant main effects of condition were observed on both behavioral indices (accuracy: $F(3,324) = 5.69$, $p = 0.001$, $\eta^2_p = 0.051$; RT: $F(3,324) = 188.70$, $p < 0.001$, $\eta^2_p = 0.638$) with post-hoc analyses suggesting that accuracy for angry faces was significantly higher compared to all other conditions (ps < 0.001, all accuracies higher than 95%). Response times for geometric shapes (1045.34 ± 193.63) were faster compared to angry faces (1205.03 ± 230.79), angry faces compared happy faces (1279.80 ± 274.64) and slower response times for neutral faces (1351.16 ± 287.85) compared to all other conditions (all ps < 0.001).

Effects on mean threat-related amygdala amplitude

We conducted a one sample t-test with the contrast [$angry_{all\text{-}block}$ > $neutral_{all\text{-}block}$] to validate whether the threatening stimuli generally induced activation in our target region, specifically the amygdala. Results revealed activation in the bilateral amygdala (left, MNI [-27 -3 -21], $p_{FWE} = 0.012$, k = 7, $t_{110} = 3.34$; right, MNI [33 0 -21], $p_{FWE} = 0.01$, k = 9, $t_{110} = 3.40$; small volume corrected for the bilateral amygdala mask, **Figure S2**).

Exploratory whole-brain analysis

The exploratory voxel-wise whole-brain analysis additionally revealed a significant sensitization ×treatment interaction effect in cortical midline regions (CMR), including right paracentral lobule, middle cingulate gyrus and precuneus (MNI [0 -12 51], $p_{FWE\text{-}cluster} = 0.014$, k = 185, $t = 4.38$), right superior temporal gyrus (STG, MNI [51 -18 6], $p_{FWE\text{-}cluster} = 0.002$, k = 283, $t = 4.77$), left superior temporal gyrus (MNI [-42 -24 3], $p_{FWE\text{-}cluster} = 0.006$, k = 229, $t = 4.87$) and right insula/superior temporal gyrus (MNI [39 -9 -9], $p_{FWE\text{-}cluster} = 0.032$, k = 147, $t = 4.35$, **Figure S3**).

Validation dataset – behavioral results

Mixed ANOVAs with condition (angry face vs. happy face vs. neutral face vs. geometric shape) as within-subject factor and group (ATD^--PLC group from the current study vs. ATD^- group from validation study) as between-subject factor revealed no significant main effect of group and condition on accuracy and no significant main effect of group on reaction time (RT) except for a significant main effect of condition on RT (RT: $F(3,153) = 90.10$, $p < 0.001$, $\eta^2_p = 0.639$). Post-hoc analysis suggested that the response times for geometric shapes (1012.41 ± 199.75) were faster compared to angry faces (1164.12 ± 219.50), angry faces compared happy faces (1231.80 ± 276.55) and slower response times for neutral faces (1320.77 ± 290.00) compared to all other conditions (all ps < 0.001).

Validation dataset – threat-specific amygdala sensitization / desensitization

A voxel-wise one sample *t*-test on the validation dataset revealed a significant threat-specific sensitization effect in the right amygdala (MNI [27 3 -27], $p_{FWE} < 0.001$, k = 22, $t = 14.69$, small volume corrected for the entire bilateral amygdala) and left amygdala (MNI [-24 -9 -12], $p_{FWE} < 0.001$, k = 54, $t = 15.40$, small volume corrected for the entire bilateral amygdala) (**Figure S4**).

Supplementary References

1. Carpenter LL, Anderson GM, Pelton GH, Gudin JA, Kirwin PD, Price LH, Heninger GR, McDougle CJ (1998) Tryptophan depletion during continuous CSF sampling in healthy human subjects. Neuropsychopharmacology 19:26–35.

2. Williams WA, Shoaf SE, Hommer D, Rawlings R, Linnoila M (1999) Effects of Acute Tryptophan Depletion on Plasma and Cerebrospinal Fluid Tryptophan and 5‐Hydroxyindoleacetic Acid in Normal Volunteers. J Neurochem 72:1641–1647.

3. Young SN, Smith SE, Pihl RO, Ervin FR (1985) Tryptophan depletion causes a rapid lowering of mood in normal males. Psychopharmacology 87:173–177.

4. Crockett MJ, Clark L, Roiser JP, Robinson OJ, Cools R, Chase HW, den Ouden H, Apergis-Schoute A, Campbell-Meikeljohn D, Seymour B, Sahakian BJ, Rogers RD, Robbins TW (2012) Converging evidence for central 5-HT effects in acute tryptophan depletion. Mol Psychiatry 17:121–123.

5. Crockett MJ, Clark L, Tabibnia G, Lieberman MD, Robbins TW (2008) Serotonin Modulates Behavioral Reactions to Unfairness. Science 320:1739–1739.

6. Veen FM van der, Evers EAT, Deutz NEP, Schmitt JAJ (2007) Effects of Acute Tryptophan Depletion on Mood and Facial Emotion Perception Related Brain Activation and Performance in Healthy Women with and without a Family History of Depression. Neuropsychopharmacology 32:216–224.

7. Plichta MM, Grimm O, Morgen K, Mier D, Sauer C, Haddad L, Tost H, Esslinger C, Kirsch P, Schwarz AJ, Meyer-Lindenberg A (2014) Amygdala habituation: A reliable fMRI phenotype. Neuroimage 103:383–390.

8. Gong X, Huang YX, Wang Y, Luo YJ (2011) Revision of the Chinese facial affective picture system. Chinese Mental Health Journal 25:40–46.

Supplementary Figures

Figure S1 CONSORT flowchart

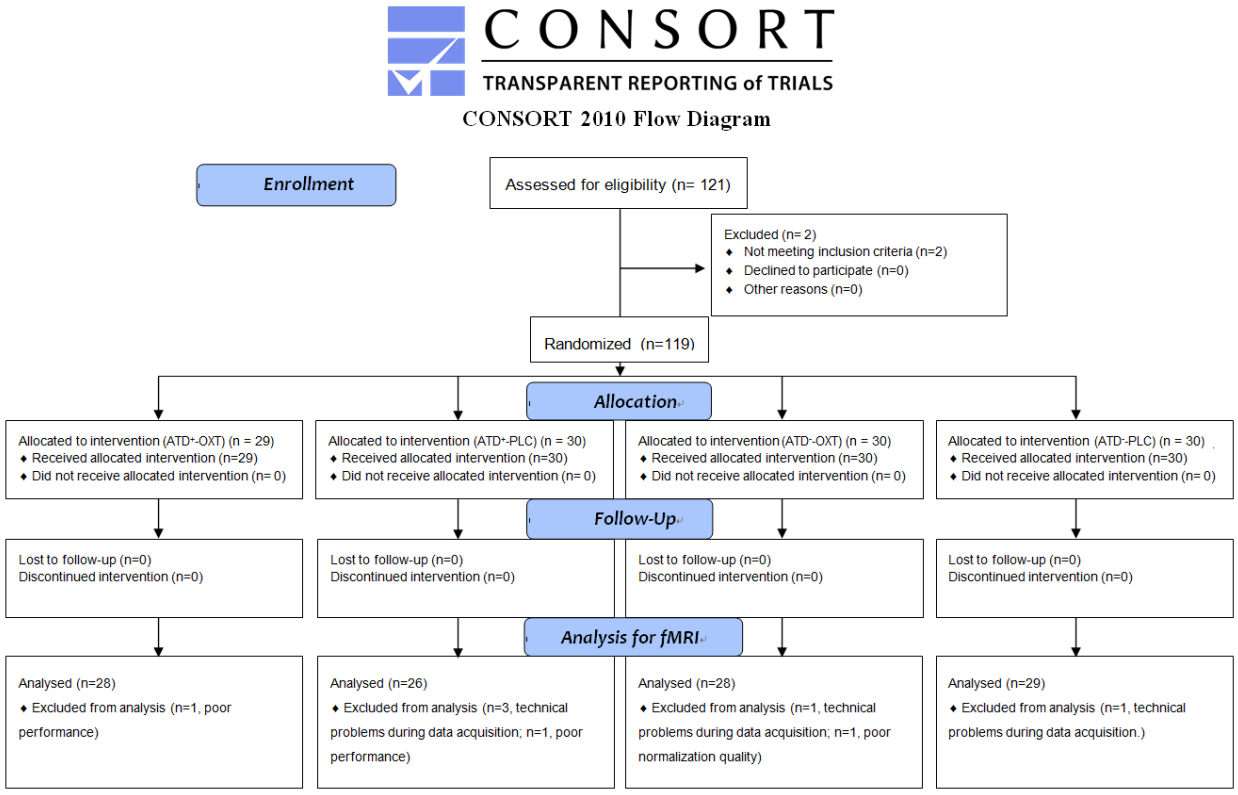


Figure S2 Results from a one sample *t*-test with the contrast [$angry_{all\text{-}block}$ > $neutral_{all\text{-}block}$]. The threat-specific effect in the bilateral amygdala is displayed at $p_{FWE\text{-}SVC} < 0.05$ thresholded for the entire bilateral amygdala. The color bar codes the *t* value.

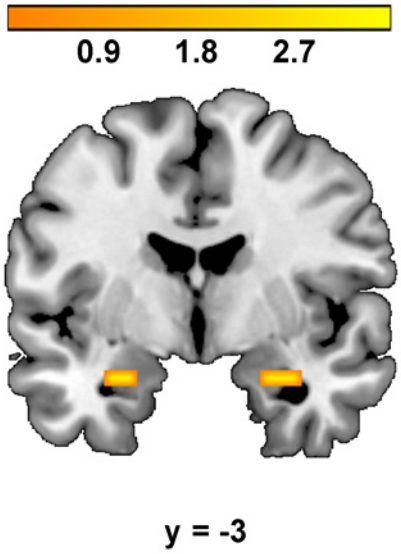
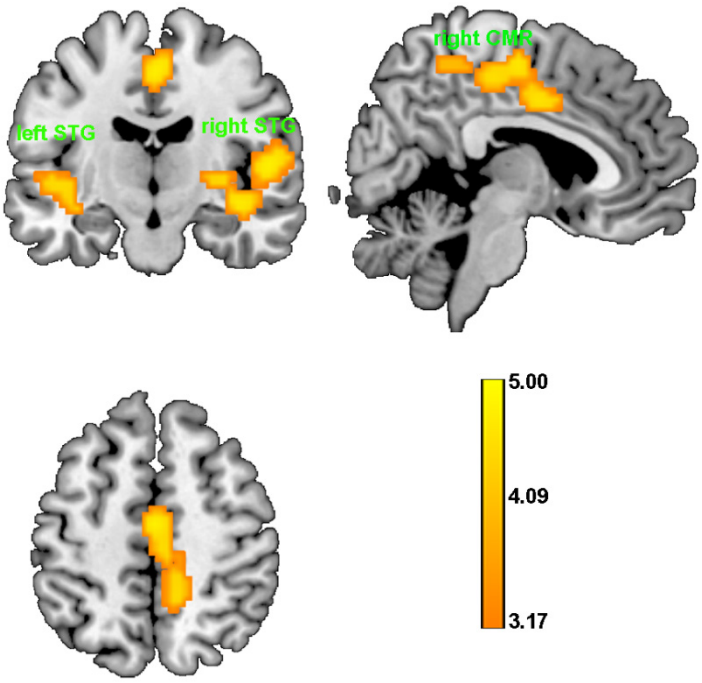


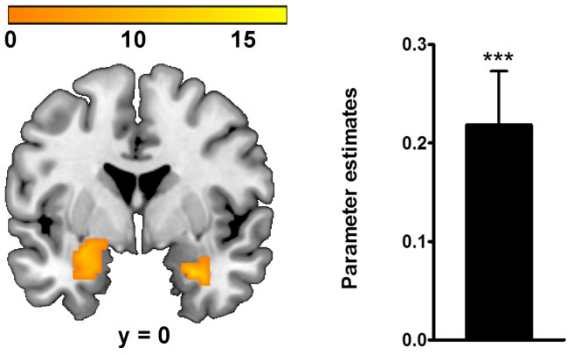
Figure S3 Results from the exploratory whole-brain analysis of sensitization / desensitization differences between the treatment groups.



The exploratory whole brain analysis revealed significant time × treatment interaction effects on the whole brain level ($p_{FWE\text{ - cluster}} < 0.05$).

Abbreviations: STG, superior temporal gyrus; CMR, cortical middle region, including right paracentral lobule/middle cingulum gyrus/precuneus.

Figure S4 Results from the validation dataset



Examination of threat-specific amygdala sensitization / desensitization in the independent validation sample confirmed the robustness of increased amygdala reactivity over the course of the experiment (small volume corrceted for the entire amygdala, $p_{FWE} < 0.001$). Extraction of parameter estimates further confirmed the threat-specific sensitization effects [$(angry_{last\text{-}block}>neutral_{last\text{-}block}) > (angry_{first\text{-}block}>neutral_{first\text{-}block})$. *** $p < 0.001$