# Oxytocinergic modulation of stress-associated amygdala-hippocampus pathways in humans is mediated by serotonergic mechanisms

Chunmei Lan[a*] (MSc), Congcong Liu[a,b*] (PhD), Keshuang Li[a,c] (MSc), Zhiying Zhao[a,d] (PhD), Jiaxin Yang[e] (MSc), Yina Ma[e] (PhD), Dirk Scheele[f,g] (PhD), Yao Shuxia[a] (PhD), Keith M. Kendrick[a] (PhD), Benjamin Becker[a] (PhD)

*authors contributed equally

**Affiliations**

[a] The Clinical Hospital of the Chengdu Brain Science Institute, School of Life Science and Technology, University of Electronic Science and Technology of China, 611731 Chengdu, China

[b] Department of Psychology, Xinxiang Medical University, Henan 453003, China

[c] School of Psychology and Cognitive Science, East China Normal University, 200062 Shanghai, China

[d] Department of Radiology and Biomedical Imaging, Yale University School of Medicine, New Haven, Connecticut

[e] State Key Laboratory of Cognitive Neuroscience and Learning, IDG/McGovern Institute of Brain Research, Beijing Normal University, 100875 Beijing, China

[f] Division of Medical Psychology, Department of Psychiatry and Psychotherapy, University Hospital Bonn, 53105 Bonn, Germany

[g] Department of Psychiatry, School of Medicine & Health Sciences, University of Oldenburg, 26129 Oldenburg, Germany

**Address correspondence to**

Benjamin Becker

University of Electronic Science and Technology,

Xiyuan Avenue 2006, 611731 Chengdu, China

Phone: +86 2861 830 811

Email: ben_becker@gmx.de

## Abstract

**Background** The hypothalamic neuropeptide oxytocin (OXT) may exert anxiolytic and stress-reducing actions via modulatory effects on amygdala circuits. Animal models and initial findings in humans suggest that some of these effects are mediated by interactions with other neurotransmitter systems, in particular the serotonin (5-HT) system. Against this background, the present pharmacological resting state fMRI study aimed at determining whether effects of OXT on stress-associated amygdala intrinsic networks are mediated by 5-HT.

**Methods** We employed a randomized placebo-controlled double-blind parallel-group pharmacological fMRI resting state experiment during which n = 112 healthy male participants underwent a transient decrease in 5-HT signaling via acute tryptophan depletion (ATD) or the corresponding placebo-control protocols (ATDc) before the administration of intranasal OXT or placebo intranasal spray, respectively.

**Results** OXT and 5-HT modulation exerted interactive effects on the coupling of the left amygdala with the ipsilateral hippocampus and adjacent midbrain. OXT increased intrinsic coupling in this pathway, while this effect of OXT was significantly attenuated during transiently decreased central serotonergic signaling induced via ATD. In the absence of OXT or 5-HT modulation this pathway showed a trend for an association with self-reported stress perception in everyday life. No interactive effects were observed for the right amygdala.

**Conclusions** Together, the findings provide first evidence that effects of OXT on stress-associated amygdala-hippocampal-midbrain pathways are critically mediated by the 5-HT system in men.

**Keywords** oxytocin, serotonin, amygdala, stress, anxiety

## Introduction

The hypothalamic neuropeptide oxytocin (OXT) regulates socio-emotional behavior across species, with convergent evidence from experimental studies in humans suggesting that the intranasal administration of OXT can e.g. exert pro-social, anxiolytic and stress-reducing effects (Heinrichs et al., 2009; Meyer-Lindenberg et al., 2011; Yao et al., 2018; Xin et al., 2020; Grinevich and Neumann, 2021; Quintana et al., 2021). Accumulating evidence from sophisticated animal models suggests that some of the complex regulatory effects of OXT are critically mediated by interactions with other neurotransmitter systems, such that animal models demonstrated that dopamine partly mediated OXT's effects on pair bonding (Young and Wang, 2004) while the serotonin (5-HT) system critically mediated OXT's regulatory effect in the domains of social reward and anxiety (Yoshida et al., 2009; Dölen et al., 2013; Lefevre et al., 2017). In terms of the anxiolytic properties, a previous rodent study demonstrated a dense expression of OXT receptors in the raphe nucleus, the primary source of central 5-HT and reported that the administration of exogenous OXT facilitated 5-HT release in this region and subsequently reduced anxiety-like behavior (Yoshida et al., 2009). Based on these findings, we recently examined the interactive effects between OXT and 5-HT in humans on threat-related amygdala activity using a randomized parallel-group placebo-controlled pharmacological functional magnetic resonance imaging (fMRI) experiment which combined the intranasal administration of OXT with an acute tryptophan depletion (ATD) procedure (Liu et al., 2021a). The administration of an ATD procedure represents a robust means to induce a transient decrease in central serotonergic signaling (Veen et al., 2007; Crockett et al., 2008; Evers et al., 2010), and thus pre-treatment with ATD may attenuate some of the potential 5-HT-mediated effects of OXT. We observed that OXT

switched amygdala sensitization to social threat signals to desensitization and this potential anxiolytic action of OXT was attenuated following ATD-induced decreased central serotonergic signaling (Liu et al., 2021a).

The amygdala plays a crucial role in fear- and anxiety-related processes (e.g. (Phelps and LeDoux, 2005; Mihov et al., 2013); however, see also (LeDoux and Pine, 2016; Gothard, 2020; Zhou et al., 2021)) and both, OXT and 5-HT effects on the amygdala have been repeatedly documented (Cools et al., 2005; Fisher and Hariri, 2013; Kanat et al., 2015; Raab et al., 2016; Xin et al., 2020; Kreuder et al., 2020). OXT and 5-HT-sensitive receptors are widely distributed across limbic-striatal-frontal regions (Pasqualetti et al., 1998; Gimpl and Fahrenholz, 2001; Varnäs et al., 2004; Quintana et al., 2019) and thus may not only influence regional activity but also the functional cross-talk between nodes in these networks (Bethlehem et al., 2013). To examine modulatory effects of OXT on the network level while controlling for its valence- and context-specific effects (e.g. (Shamay-Tsoory and Abu-Akel, 2016; Yao et al., 2018; Chen et al., 2020)), an increasing number of studies employed pharmacological resting-state fMRI (pharmaco-rsfMRI) (see e.g. (Brodmann et al., 2017; Wu et al., 2020; Jiang et al., 2021; Xin et al., 2021)).

Previous studies employing placebo-control pharmaco-rsfMRI strategy to determine effects of the intranasal OXT on the intrinsic amygdala networks reported an OXT-induced modulation of the intrinsic coupling of the amygdala with prefrontal regions, particularly medial prefrontal (mPFC) and orbitofrontal regions (Fan et al., 2014; Dodhia et al., 2014; Eckstein et al., 2017; Cheng et al., 2018; Jiang et al., 2021), posterior default mode network (DMN) regions (Kumar et al., 2014) as well as with the hippocampal formation (Fan et al., 2015; Alaerts et al., 2019) with some studies reporting

associations between intranasal OXT effects and stress- and emotion processing-associated indices including subclinical depressive symptoms or stress exposure (Fan et al., 2014, 2015; Eckstein et al., 2017). Although fewer studies employed a pharmaco-rsfMRI or ATD-rsfMRI approach to examine modulatory effects of 5-HT on the intrinsic amygdala networks some evidence indicates a serotonergic modulation of pathways partly overlapping with those observed following oxytocinergic modulation, including amygdala-posterior DMN (Dutta et al., 2019; Zhang et al., 2019), amygdala-prefrontal (Eisner et al., 2017) and amygdala-hippocampal connectivity (Carhart-Harris et al., 2015).

Further support for a potential network-level modulatory role of the 5-HT-OXT interactions comes from molecular imaging studies which demonstrated that the administration of exogenous OXT increased 5-HT concentrations in key limbic regions including the amygdala and hippocampus in non-human primates (Lefevre et al., 2017) and that intranasal OXT influenced serotonergic signaling in a broad network encompassing limbic, insular and prefrontal regions in humans (Mottolese et al., 2014; Lefevre et al., 2018). Importantly the study confirmed the central role of the amygdala in regulating 5-HT signaling via OXT such that OXT-induced 5-HT1A receptor binding changes in the amygdala which correlated with changes in the hippocampus, insula and prefrontal cortex (Mottolese et al., 2014). The identified amygdala networks partly resemble pathways involved in stress reactivity and emotion regulation (Etkin et al., 2015). In particular pathways such as the amygdala-hippocampal (Admon et al., 2009; Vaisvaser et al., 2013) and amygdala-prefrontal circuits are sensitive to both, long-term as well as acute stress-exposure (Herringa et al., 2013; Fan et al., 2015, 2014; Park et al., 2018).

Summarizing, accumulating evidence from animal and human models suggest an interaction of OXT and 5-HT in regulating anxiety- and stress-related behavior, however, while both systems have been associated with modulating the amygdala-centered networks, interactive effects on the intrinsic amygdala networks in humans have not been systematically examined. To this end we combined the administration of OXT or placebo (PLC) intranasal spray with an ATD (Acute tryptophan depletion) - induced transient decrease in central 5-HT signaling or a matched acute tryptophan depletion control (ATDc) protocol in a randomized controlled pharmaco-rsfMRI parallel group design in n = 121 healthy male subjects (details see also (Liu et al., 2021a)). Based on previous studies, we hypothesized that OXT's effects on the amygdala intrinsic networks are (partly) mediated by interaction with the 5-HT system and that a transient reduction in 5-HT signaling following ATD would attenuate OXT's effect, and (2) given the role of OXT and 5-HT in stress processing and a high stress-sensitivity of the amygdala intrinsic networks (Sripada et al., 2012; Vaisvaser et al., 2013; Fan et al., 2015; Zhang et al., 2016; Feng et al., 2018) the identified pathways would be associated with levels of currently experienced stress exposure.

## Material and methods

### Participants

A total of 121 non-smoking, right-handed, young healthy male participants were enrolled (detailed study enrollment criteria see also (Liu et al., 2021a)). Given that previous studies reported sex-differences with respect to both, the effects of OXT on amygdala functional connectivity (Ma et al., 2018) and central 5-HT synthesis rates (Nishizawa et al., 1997) and to further control for potential

confounding effects of OXT administration with hormonal changes across the menstrual cycle the present study focused on male individuals (similar approach see also Eckstein et al., 2017; Zhao et al., 2019a; Xin et al., 2020). Participants were instructed to abstain from alcohol and caffeine for 24 hours and from food and drinks (except water) for 12 hours prior to the experiment. To examine interaction effects between the OXT and 5-HT systems, a randomized double-blind placebo-controlled between-group pharmacological resting state fMRI (pharmaco-rsfMRI) design was employed during which the subjects received ATD or an acute tryptophan depletion control (ATDc) drink which balanced for tryptophan before they were administered either OXT or a corresponding placebo (PLC) intranasal spray and subsequently underwent resting state fMRI. During initial quality assessments data from 9 subjects were excluded from the following analyses due to mania or depression history (n = 2), technical failure of the MRI system (n = 1), poor spatial registration quality (n = 1) and excessive head motion > 3mm (n = 5) (details see CONSORT flowchart Supplementary **Figure S1**) leading to a total number of n = 112 subjects for the final analysis (ATD-OXT, n = 29; ATD-PLC, n = 29; ATDc-OXT, n = 26; ATDc-PLC, n = 28, detailed group characteristics see **Table 1**).

All participants provided written informed consent after being fully informed about the experimental procedures. The study had full ethical approval from the Ethics Committee at the University of Electronic Science and Technology of China and adhered to the latest revision of the declaration of Helsinki. The study was preregistered on ClinicalTrials.gov (NCT03426176, https://clinicaltrials.gov/show/ NCT03426176).

**Procedure**

Experimental and treatment procedures were identical to our previous study which reports the results of a task-based fMRI paradigm in the same sample to assess interactive effects of oxytocin and serotonin on amygdala threat reactivity and sensitization (Liu et al., 2021a). Briefly, a randomized, double-blind, placebo-controlled between-subject pharmaco-rsfMRI design was employed during which four treatment groups received combinations of amino acid mixture drinks (ATD vs. ATDc) to induce a transient decrease in central serotonergic signaling and intranasal spray (OXT vs. PLC) to modulate central oxytocin signaling. To adhere to the pharmacodynamic profile of treatments participants arrived between 7:30 to 10:00 AM and underwent fMRI acquisition between 13:30 to 16:00 PM. Upon arrival, participants received a standardized protein-poor diet for breakfast. Following the assessment of pre-treatment control variables, participants underwent a previously validated tryptophan depletion protocol with ATD (detailed components of mixtures see Supplementary **Table S1**) which has been demonstrated to lead to a robust transient reduction in central 5HT signaling (Veen et al., 2007; Crockett et al., 2008; Passamonti et al., 2012) or ATDc. The ingestion of the amino acid mixtures was followed by a resting period of 5 hours to achieve a robust reduction in tryptophan levels. During the resting period participants were asked to relax and magazines were provided. Subsequently, control variables were re-assessed and participants administered either OXT (24IU, ingredients: oxytocin, glycerine, sodium chloride and purified water) or PLC (identical ingredients except for oxytocin) intranasal spray. Both intranasal sprays were provided by Sichuan Meike Pharmaceutical Co. Ltd in China. In line with the pharmacokinetic profile of intranasal OXT (Spengler et al., 2017b), rsfMRI data was collected 45 min after OXT/PLC administration. Control variables were re-assessed before and after fMRI acquisition (schematic

outline of the experimental protocols see **Figure 1**). The rsfMRI acquisition was followed by a task-based fMRI paradigm assessing amygdala threat reactivity and sensitization (Liu et al., 2021a).

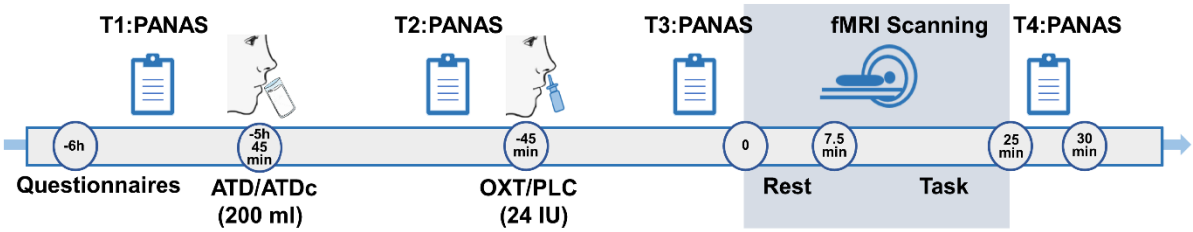


**Figure 1.** Experimental protocols and procedures of the pharmacological resting-state fMRI experiment.

**Behavioral assessments**

Anxiety and depression have been associated with amygdala functional networks (Spengler et al., 2017a; Zhao et al., 2019b; Xu et al., 2021; Liu et al., 2021b) and therefore corresponding indices were assessed to control for pre-treatment differences between the groups. To this end the State-Trait Anxiety Inventory (STAI) (Spielberger et al., 1970) and Beck Depression Inventory (BDI-II) (Beck et al., 1996) were applied. The Positive and Negative Affect Schedule (PANAS) (Watson et al., 1988) was repeatedly administered before administration of the amino acid drink (T1) and the intranasal spray (T2) as well as immediately before MRI acquisition (T3) and at the end of the experiment (T4) to control for unspecific effects of treatment on mood (procedure see **Figure 1**). No between-group differences in the potential confounders were observed (details see **Table 1**).

Both OXT and 5-HT have been strongly associated with stress processing and stress reactivity (Olff et al., 2013; Mahar et al., 2014) as well as intrinsic amygdala functional networks (e.g. Seeley et al., 2018; Zhang et al., 2019; Jiang et al., 2021). However, the intrinsic amygdala networks have been associated with numerous functional domains, including not only acute stress (Archer et al., 2018),

but also e.g. trait anger (Fulwiler et al., 2012), discrimination (Clark et al., 2018), social functioning (Johns et al., 2019), or early life stress exposure (Luo et al., 2022). We therefore included a measure of perceived stress during the previous month using the Perceived Stress Scale (PSS) (Cohen et al., 1983) to explore whether the identified pathways are associated with current stress. To control for treatment effects on the assessment and the neural pathway the questionnaire was applied before treatment and the analysis focused on the subjects without active treatment.

**Table 1 Group characteristics of subject age and questionnaire scores.**

| Measurements | ATD-OXT N = 29 | ATD-PLC N = 29 | ATDc-OXT N = 26 | ATDc-PLC N = 28 | $F_{(3,108)}$ | $p$ |
|---|---|---|---|---|---|---|
| Age(years) | 22.0 (2.4) | 21.9 (2.6) | 22.2 (2.3) | 22.1 (2.2) | 0.09 | 0.97 |
| BDI | 6.0 (6.3) | 7.0 (5.7) | 5.2 (6.6) | 5.1 (5.0) | 0.66 | 0.58 |
| STAI-SAI | 37.7 (8.9) | 34.8 (6.3) | 34.9 (8.2) | 35.9 (6.7) | 0.87 | 0.46 |
| STAI-TAI | 41.1 (8.3) | 40.5 (5.9) | 38.4 (7.5) | 41.2 (6.8) | 0.88 | 0.45 |
| PSS | 14.2 (5.5) | 14.9 (4.7) | 13.8 (4.9) | 15.5 (4.7) | 0.62 | 0.61 |
| PANAS-P(T1) | 24.9 (6.9) | 23.9 (8.3) | 24.3 (6.9) | 25.1 (8.2) | 0.15 | 0.93 |
| PANAS-N(T1) | 15.7 (9.0) | 13.1 (5.7) | 11.9 (5.2) | 13.3 (6.0) | 1.56 | 0.20 |

**Abbreviations:** OXT, Oxytocin; PLC, Placebo; ATD, Acute Tryptophan Depletion; ATDc, Acute Tryptophan Depletion control mixture; BDI, Beck's Depression Inventory; STAI-TAI, State-Trait Anxiety Inventory-Trait Anxiety Inventory; STAI-SAI, State-Trait Anxiety Inventory-State Anxiety Inventory; PSS, Perceived Stress Scale; PANAS-P, Positive and Negative Affect Schedule-Positive affect; PANAS-N, Positive and Negative Affect Schedule-Negative affect; T1, pre-oral administration.

**MRI data acquisition**

MRI data was obtained on a 3-T GE MR750 Discovery MRI system (General Electric Medical System, Milwaukee, WI, USA). High-resolution brain structural data were acquired with a T1-weighted sequence using the following parameters: repetition time, 6.0 ms; echo time, 1 ms; flip angle, 12°; field of view, 256×256 mm; resolution, 256×256; slice thickness, 1 mm; number of slices, 156. Resting-state functional MRI data were acquired using an echo planar imaging sequence with the

following acquisition parameters: repetition time, 2000 ms; echo time, 30 ms; field of view, 220 × 220 mm; flip angle, 90°; resolution, 64×64; slice thickness, 3.2 mm; number of slices, 43. A total number of 225 whole-brain volumes were collected (about 7.5 min). During scanning, two head cushions were used to prevent excessive head motion while ensuring comfort. Participants were instructed to relax and think of nothing in particular while fixating a fixation cross presented centrally via a rear mirror.

**Functional MRI data preprocessing**

The first 10 volumes of the resting-state time-series were discarded for each subject and subsequent fMRI data preprocessing was carried out using FSL FEAT 6.0 (https://www.fmrib.ox.ac.uk/fsl). The following preprocessing steps were applied: brain extraction with BET (Brain Extraction Tool, v2.1), motion correction, slice-timing correction, spatial smoothing with a Gaussian kernel of 5 mm full width at half maximum, intensity normalization and 0.01-0.1 Hz band-pass filtering after ICA-AROMA was employed (Pruim et al., 2015). Mean signals from white matter and cerebrospinal fluid (CSF) were removed by means of linear regression. Registration of functional data to high resolution structural images was carried out using FLIRT with boundary-based registration (Jenkinson and Smith, 2001; Jenkinson et al., 2002; Greve and Fischl, 2009). Registration from high resolution structural to standard space was then further improved using FNIRT nonlinear registration with 12 degrees of freedom. To control for the critical impact of head motion on functional connectivity analyses (Van Dijk et al., 2012), ICA-AROMA was applied to remove motion-related artifacts before filtering, subjects with head motion > 3 mm or high mean frame-wise displacement (mean FD, > 0.3

mm) were excluded, and for the group-level analysis (Power et al., 2012) mean FD was included as a covariate. Kruskal-Wallis one way-ANOVA indicated that there was no significant difference between the four treatment groups with respect to mean FD ($FD_{ATD\text{-}OXT}$=0.1, *SD*=0.05, $FD_{ATD\text{-}PLC}$ = *SD*= 0.03, $FD_{ATDc\text{-}OXT}$=0.08, *SD* =0.02, $FD_{ATDc\text{-}PLC}$=0.07, *SD* = 0.03, $F_{(3,108)}$=6.65, $p > 0.05$).

**Functional connectivity analyses**

In line with our research question, the main and interaction effects of treatments (ATD and OXT vs. the respective control treatment conditions) were examined on the amygdala intrinsic networks by means of a seed-to-whole brain resting state connectivity analysis. Anatomical masks of the left amygdala and right amygdala from the Automated Anatomic Labeling (AAL) served as a priori defined seed regions. Individual functional connectivity (FC) maps were initially created for each participant using DPASFA 4.4 (advanced edition of DPABI, http://rfmri.org/dpabi) and each seed region by calculating Pearson correlations between the mean time-course extracted from the amygdala masks and all other voxels in the brain and subsequently transformed to z-maps using Fisher r-to-z transformation.

Effects of treatment were examined separately for the left and right amygdala intrinsic connectivity networks by means of a 2 × 2 ANCOVA in SPM12 (Friston et al., 1994) with amino acid mixture (ATD/ATDc) and intranasal spray (OXT/PLC) as between-subjects factor and mean FD and age as covariates and the grey matter mask template from DPABI as explicit mask. Our main hypothesis in terms of interactive effects between the OXT and 5-HT system was evaluated by means of applying a whole brain analysis with a stringent initial cluster-forming threshold which combine

voxel wise $p < 0.001$ with cluster wise $p < 0.05$ family-wise error (FWE) corrected (Woo et al., 2014; Eklund et al., 2016; Daniel Kessler, 2017). The probabilistic maps from the Anatomy toolbox (version 2.2) (Eickhoff et al., 2005) were used to pinpoint regions exhibiting significant interaction effects. Post-hoc group comparisons were conducted to further disentangle significant interaction effects. To this end, parameter estimates of atlas-based independent masks were extracted and subjected to post-hoc comparisons with FDR-correction in R-Studio (Benjamini and Hochberg, 1995). To further examine associations with current stress levels Spearman's rank correlation were applied in SPSS to explore associations between extracted parameter estimates in significant cluster and PSS scores with a focus on participants without active treatment (ATDc-PLC).

## Results

### Mood state

Effects of treatment on mood were assessed using a mixed-design ANOVA model including mixture (ATD vs. ATDc) and intranasal spray (OXT vs. PLC) as between-subject factors, and timepoint (T1-T4; pre-oral administration, pre-intranasal administration, pre-fMRI, post-fMRI) as within-subject factor. In line with our previous study (Liu et al., 2021a), we observed significant main effects of timepoint on positive ($F_{(3,327)} = 16.68$, $p < 0.001$, $\eta^2_p = 0.13$) and negative ($F_{(3,327)} = 14.10$, $p < 0.001$, $\eta^2_p = 0.12$) affect, suggesting a general decline of positive and negative mood during the experiment (details see Supplementary **Table S2**). We also observed a trend for an interaction effect between of ATD and OXT on negative affect ($F_{(1,108)} = 3.891$, $p = 0.051$, $\eta^2_p = 0.035$). Post-hoc comparisons revealed that participants who received ATD-OXT treatment reported a higher negative affect than who received ATD-PLC treatment ($p = 0.043$) or received ATDc-OXT treatment ($p = 0.057$, trend),

while there was no significant difference between participants who received ATDc-PLC treatment and those received the ATD-PLC condition ($p$ = 0.40) or the ATDc-OXT condition ($p$ = 0.45), suggesting that the combination ATD and OXT treatment produced stronger increases on negative mood, while neither ATD nor OXT treatment did affect mood changes over the experiment in healthy individuals. No significant main effect of mixture or intranasal spray on both two types of mood was observed.

**Interaction between reduced serotonergic signaling and OXT on amygdala networks**

In the whole-brain analysis, a significant interaction effect between treatments were observed with respect to left amygdala intrinsic coupling with a cluster located in the left hippocampus extending into the adjacent midbrain (MNI, *xyz* = [-20 -28 -10], $t_{106}$ = 4.69, $p_{\text{FWE-cluster}}$ = 0.046, $k$ = 118 voxels, see **Figure 2A**). Probabilistic mapping by means of the Anatomy (version 2.2b) toolbox indicated that the interaction effect primarily encompassed the subiculum subregion of the left hippocampus. To disentangle the significant interaction effect, parameter estimates were extracted from the left subiculum using independently defined anatomical mask (Anatomy toolbox) for post-hoc comparisons which revealed that OXT (ATDc-OXT) significantly increased the connectivity relative to the PLC-reference (ATDc-PLC) group ($p_{\text{FDR}}$ = 0.048), whereas pre-treatment with ATD (ATD-OXT) significantly attenuated the OXT-induced (ATDc-OXT) increase ($p_{\text{FDR}}$ = 0.048) (see **Figure 2B**). Neither ATD (ATD-PLC) alone ($p_{\text{FDR}}$ = 0.62) nor the combined application (ATD-OXT, $p_{\text{FDR}}$ = 0.84) significantly increased amygdala connectivity in comparison to the PLC-treated reference (ATDc-PLC) group. To further determine which midbrain regions were included in the cluster we

employed an atlas encompassing the corresponding regions (https://github.com/canlab/Neuroimaging_Pattern_Masks/tree/master/Atlases_and_parcellations/2018_Wager_combined_atlas) and observed that the cluster extended into the superior colliculus and dorsal part of the midbrain (see also **Figure S2**). No significant treatment interaction effects were observed for right amygdala intrinsic networks.

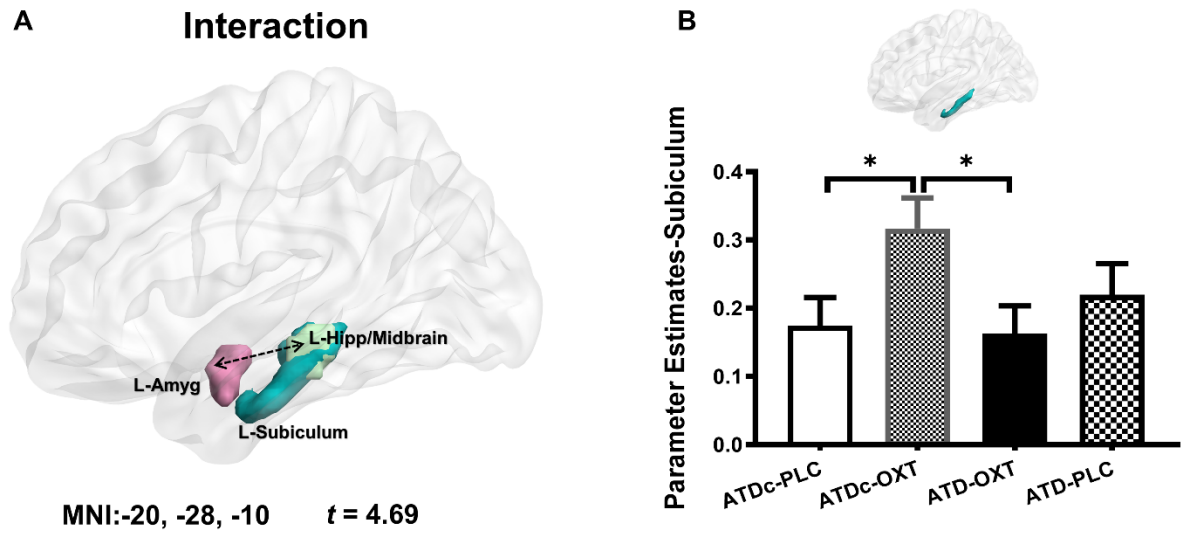


**Figure 2. Interaction effects between oxytocin and serotonin on the intrinsic connectivity of the left amygdala.** (A) The whole brain voxel-wise analysis revealed a significant negative interaction effect of treatments on the functional connectivity between the left amygdala and a left hippocampus/midbrain cluster (in light green, whole brain corrected at $p_{FWE\text{-}cluster} < 0.05$). The mask in light blue represents the left subiculum from the SPM Anatomy toolbox. (B) Post hoc comparisons between the treatment groups using extracted parameter estimates from the left subiculum anatomical mask. Group difference between ATDc-PLC and ATDc-OXT indicates that OXT significantly increased connectivity in this pathway in relative to the PLC-reference group, whereas the group difference between ATDc-OXT and ATD-OXT indicates that pre-treatment with acute tryptophan depletion significantly attenuated the OXT-induced increase. Bars indicate M ± SEM, * $p_{FDR} < 0.05$. Abbreviations: L, left; Amyg, amygdala; Hipp, hippocampus; OXT, Oxytocin; PLC, Placebo; ATD, Acute Tryptophan Depletion; ATDc, Acute Tryptophan Depletion control mixture.

**Associations with current stress**

Associations between current stress and neural indices were examined by means of examining correlations between PSS scores and neural indices of significant clusters. Examining general associations of the identified pathway with current stress in the group without OXT or 5-HT

modulation (ATDc-PLC) revealed a trend for a significant negative correlation between PSS scores and the left amygdala-left hippocampus/midbrain connectivity ($r_{S(26)}$ = -0.34, p = 0.08, see **Figure 3**), suggesting that this pathway may be associated with current stress experience. Further control analyses in the groups receiving treatment did not reveal significant associations between this pathway and perceived stress (ATDc-OXT, $r_{S(24)}$ = 0.25, $p$ = 0.22; ATD-OXT, $r_{S(27)}$= 0.10, $p$ = 0.62; ATD-PLC, $r_{S(27)}$ = -0.07, $p$ = 0.72).

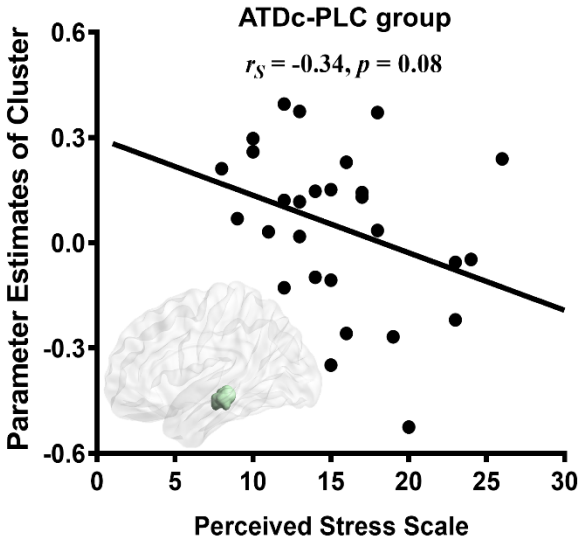


**Figure 3**. **Associations of with currently experienced stress in ATDc-PLC group.** Associations between Perceived Stress Scale scores and parameter estimates extracted from the amygdala-hippocampal pathway displaying correlation. Dots represent individual data. Abbreviations: PLC, Placebo; ATDc, Acute Tryptophan Depletion control mixture.

## Discussion

The present study aimed at determining interactive effects of the OXT and 5-HT system on intrinsic organization of the amygdala-centered networks. To this end, we employed a pharmaco-rsfMRI design that combined the administration of intranasal OXT with a transient decrease in central 5-HT signaling via acute tryptophan depletion or the respective control conditions and resting state fMRI. Examining treatments effects on the intrinsic amygdala networks revealed an interactive effect of

OXT and 5-HT on the coupling of the left amygdala and a cluster encompassing the left subiculum of the hippocampus and extend to the left midbrain encompassing the superior colliculus. Post-hoc analyses convergently demonstrated that OXT increased intrinsic coupling in this pathway while this effect of OXT was significantly attenuated during transiently decreased central serotonergic signaling induced via ATD. In the placebo (ATDc-PLC) group lower connectivity in this pathway correlated with higher levels of experienced stress, suggesting a sensitivity of this pathway to current stress exposure. Together the present findings indicate that the effects of OXT on stress-associated amygdala intrinsic pathways with the hippocampus are critically mediated by 5-HT.

Previous pharmaco-rsfMRI studies have demonstrated that intranasal OXT modulates the connectivity of the amygdala with prefrontal regions (Dodhia et al., 2014; Koch et al., 2016; Eckstein et al., 2017; Jiang et al., 2021) and the hippocampus (Fan et al., 2015; Kirkby et al., 2018; Alaerts et al., 2019), while animal models and an initial human study suggest that some effects of OXT in the domains of anxiety and amygdala threat-reactivity are critically mediated by the 5-HT system (Yoshida et al., 2009; Mottolese et al., 2014). Within this context the present findings further extend previous findings that specifically the oxytocinergic modulation of the intrinsic coupling between the amygdala and the hippocampus/midbrain is critically mediated by interactive effects with the 5-HT system.

Across species, the amygdala-hippocampus-midbrain circuitry plays a role in the reactivity to potential threatening stimuli and stress reactivity (Belujon and Grace, 2011). In humans, intrinsic coupling in the amygdala-hippocampus pathway has been associated with acute and prolonged effects of stress exposure (Sripada et al., 2012; Vaisvaser et al., 2013; Fan et al., 2015; Zhang et al., 2016)

and a recent intracranial electroencephalography study suggests that this pathway accurately tracks mood variations in humans (Kirkby et al., 2018). Whereas the functional role of the amygdala-midbrain pathways has not been extensively examined by means of rsfMRI in humans, animal models have documented its critical role in the selection of threat-induced behavioral responses and associated aversive learning processes (Steinberg et al., 2020). Previous intranasal OXT studies in humans reported an oxytocinergic modulation of amygdala-midbrain coupling during exposure to threatening social stimuli (Kirsch, 2005) as well as on oxytocinergic regulation of stress-induced changes in intrinsic amygdala-hippocampal coupling (Fan et al., 2015), suggesting a potential role of this pathway in the stress modulating effects of OXT. We found some evidence that the intrinsic communication in the amygdala-midbrain pathway is associated with current stress experience in the participants that did not receive active treatment, suggesting a role of the identified pathway in current stress experience. This may link the observed interactive effects to overarching theories on the regulatory role of OXT on stress- and anxiety-related processes (e.g. (Neumann and Slattery, 2016; Kendrick et al., 2018; Matsushita et al., 2019)).

The cluster exhibiting interactive effects of 5-HT and OXT encompassed the subiculum of hippocampus and the left superior colliculus and further dorsal parts of the midbrain. Previous rodent and nonhuman primates studies have suggested that both the subiculum and the superior colliculus are brain regions with particular dense OXT receptor expression (Elands et al., 1988; Shapiro and Insel, 1989; Freeman et al., 2014, 2014). The subiculum has been considered as stress-sensitive brain region in both animal and human studies (Mueller et al., 2004; Belujon and Grace, 2011; Teicher et al., 2012), and several studies reported an association between long term stress exposure and decreased

the gray matter of this region (Teicher et al., 2012) and the amygdala (Lim et al., 2014). While previous studies reported strong functional connectivity between the amygdala and the hippocampus (Kiem et al., 2013), previous studies also reported that OXT can modulate this stress-related pathway (Fan et al., 2014, 2015; Hernández et al., 2015; Alaerts et al., 2019). The superior colliculus has been associated with defense reactions toward threat in rodent models (Coimbra et al., 2006), while such reflexive defense reactions were attenuated when the activation of amygdala and superior colliculus were inhibited simultaneously in non-human primates (Forcelli et al., 2016), suggesting a role of this pathway in defensive responses. In line with the functional role of this pathway previous OXT studies in humans reported that OXT induced a modulation effect on the functional connectivity between amygdala and the superior colliculus toward both threatening and non-threatening social stimuli (Kirsch, 2005; Gamer et al., 2010). Together, the current findings suggest that the effects of OXT on the intrinsic coupling between the amygdala with the subiculum and superior colliculus are critically modulated by interactive effects with the 5-HT system.

The interactive effects between the OXT and 5-HT system specifically affected the left amygdala intrinsic networks. Previous studies revealed inconsistent lateralization effects of OXT on the intrinsic amygdala networks in studies that examined the left and right amygdala separately (Sripada et al., 2013; Fan et al., 2014; Grace et al., 2019). In the previous literature such lateralization effects of OXT have been related to age, gender, stimuli type, or early life stress exposure (Striepens et al., 2012; Fan et al., 2015; Ebner et al., 2016; Grace et al., 2018, 2019; Alaerts et al., 2019). Left lateralized OXT effects have been repeatedly observed on cerebral blood flow activity and intrinsic connectivity of the left amygdala in young males (Paloyelis et al., 2016; Grace et al., 2019). Although the functional

lateralization of the amygdala remains debated some early studies hypothesized that the left amygdala is stronger related to the conscious perception and regulation of emotional responses (Morris et al., 1998). Within this context the increased left amygdala-hippocampus/midbrain functional connectivity after OXT may reflect effects on threat perception and regulation (see also Liu et al., 2021).

We additionally observed an interaction effect between ATD and OXT on negative mood. In line with a number of previous studies ATD (Benkelfat et al., 1994; Evers et al., 2006; Talbot and Cooper, 2006) or OXT (Xu et al., 2019; Chen et al., 2020; Zhuang et al., 2021) per se did not affect mood. The interactive effect on negative mood was not predicted and may reflect complex interaction effects of 5-HT and OXT on rather non-specific negative affective states.

Findings of the present study need to be considered in the context of the following limitations. First, only male subjects were enrolled due to previous studies showing marked sex-differences in the 5-HT synthesis (Nishizawa et al., 1997) and sex-differences in the effects of OXT in rodents and humans (Dumais and Veenema, 2016; Dumais et al., 2017; Xu et al., 2020; Lieberz et al., 2020; Ma et al., 2018). Future studies need to determine whether the observed effects generalize to women. Second, tryptophan blood and OXT blood levels were not assessed. Although previous studies reported robust and selective decreases in 5-HT signaling (Crockett et al., 2008; Passamonti et al., 2012) and increases in (peripheral) OXT levels (Burri et al., 2008; Gossen et al., 2012) following similar the ATD or OXT treatment protocols as used in the present study, the additional examination of blood-level measures particularly in the combined treatment group may reveal important additional information on the interaction of the two systems and should be included in future studies. Finally, while the study involved a total of 112 participants in a complex pharmaco-fMRI design we did not include an a priori sample size calculation. The priori calculation for sample size and power in complex pharmaco-fMRI design is still limited and while our results are in line with our task-based findings in this sample demonstrating interactive OXT and 5-HT effects on threat-related amygdala processing (Liu et al., 2021a) the comparably low sample size in our study requires larger validation

and replication studies ( for details see also Cremers et al., 2017). While the present study focused on the connectivity of the amygdala as a single region, previous studies revealed subregional and nuclei specific effects of OXT on subcortical systems including the amygdala and basal ganglia (Eckstein et al., 2017; Zhao et al., 2019a; Martins et al., 2022) and future studies with larger samples may open the opportunity to examine OXT and 5-HT interaction effects on the subregional level. Overall, the present findings provide first evidence that effects of OXT on stress-associated amygdala-hippocampal-midbrain pathways are critically mediated by the 5-HT system in healthy men.

**Funding**

This work was supported by the National Key Research and Development Program of China (Grant No. 2018YFA0701400).

**Acknowledgments**

We thank all subjects who participated in this study.

**Conflict of Interest**

The authors report no biomedical financial interests or potential conflicts of interest.

## Reference


Admon R, Lubin G, Stern O, Rosenberg K, Sela L, Ben-Ami H, Hendler T (2009) Human vulnerability to stress depends on amygdala's predisposition and hippocampal plasticity. Proc Natl Acad Sci U S A 106:14120–14125.

Alaerts K, Bernaerts S, Vanaudenaerde B, Daniels N, Wenderoth N (2019) Amygdala-Hippocampal Connectivity Is Associated with Endogenous Levels of Oxytocin and Can Be Altered by Exogenously Administered Oxytocin in Adults with Autism. Biol Psychiatry Cogn Neurosci Neuroimaging 4:655–663.

Archer JA, Lee A, Qiu A, Annabel Chen S-H (2018) Functional connectivity of resting-state, working memory and inhibition networks in perceived stress. Neurobiol Stress 8:186–201.

Beck AT, Steer RA, Ball R, Ranieri WF (1996) Comparison of Beck Depression Inventories-IA and-II in Psychiatric Outpatients. J Pers Assess 67:588–597.

Belujon P, Grace AA (2011) Hippocampus, amygdala, and stress: interacting systems that affect susceptibility to addiction. Ann N Y Acad Sci 1216:114–121.

Benjamini Y, Hochberg Y (1995) Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. J R Stat Soc Ser B Methodol 57:289–300.

Benkelfat C, Ellenbogen MA, Dean P, Palmour RM, Young SN (1994) Mood-lowering effect of tryptophan depletion. Enhanced susceptibility in young men at genetic risk for major affective disorders. Arch Gen Psychiatry 51:687–697.

Bethlehem RAI, van Honk J, Auyeung B, Baron-Cohen S (2013) Oxytocin, brain physiology, and functional connectivity: A review of intranasal oxytocin fMRI studies. Psychoneuroendocrinology 38:962–974.

Brodmann K, Gruber O, Goya-Maldonado R (2017) Intranasal Oxytocin Selectively Modulates Large-Scale Brain Networks in Humans. Brain Connect:brain.2017.0528.

Burri A, Heinrichs M, Schedlowski M, Kruger THC (2008) The acute effects of intranasal oxytocin administration on endocrine and sexual function in males. Psychoneuroendocrinology 33:591–600.

Carhart-Harris RL et al. (2015) The Effects of Acutely Administered 3,4-Methylenedioxymethamphetamine on Spontaneous Brain Function in Healthy Volunteers Measured with Arterial Spin Labeling and Blood Oxygen Level-Dependent Resting State Functional Connectivity. Biol Psychiatry 78:554–562.

Chen Y, Becker B, Zhang Y, Cui H, Du J, Wernicke J, Montag C, Kendrick KM, Yao S (2020) Oxytocin increases the pleasantness of affective touch and orbitofrontal cortex activity

independent of valence. Eur Neuropsychopharmacol J Eur Coll Neuropsychopharmacol 39:99–110.

Cheng W, Rolls ET, Qiu J, Xie X, Lyu W, Li Y, Huang C-C, Yang AC, Tsai S-J, Lyu F, Zhuang K, Lin C-P, Xie P, Feng J (2018) Functional connectivity of the human amygdala in health and in depression. Soc Cogn Affect Neurosci 13:557–568.

Clark US, Miller ER, Hegde RR (2018) Experiences of Discrimination Are Associated With Greater Resting Amygdala Activity and Functional Connectivity. Biol Psychiatry Cogn Neurosci Neuroimaging 3:367–378.

Cohen S, Kamarck T, Mermelstein R (1983) A global measure of perceived stress. J Health Soc Behav 24:385–396.

Coimbra NC, De Oliveira R, Freitas RL, Ribeiro SJ, Borelli KG, Pacagnella RC, Moreira JE, da Silva LA, Melo LL, Lunardi LO, Brandão ML (2006) Neuroanatomical approaches of the tectum-reticular pathways and immunohistochemical evidence for serotonin-positive perikarya on neuronal substrates of the superior colliculus and periaqueductal gray matter involved in the elaboration of the defensive behavior and fear-induced analgesia. Exp Neurol 197:93–112.

Cools R, Calder AJ, Lawrence AD, Clark L, Bullmore E, Robbins TW (2005) Individual differences in threat sensitivity predict serotonergic modulation of amygdala response to fearful faces. Psychopharmacology (Berl) 180:670–679.

Cremers HR, Wager TD, Yarkoni T (2017) The relation between statistical power and inference in fMRI. PLOS ONE 12:e0184923.

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

Daniel Kessler MA (2017) Reevaluating “cluster failure” in fMRI using nonparametric control of the false discovery rate. Proc Natl Acad Sci U S A 114:E3372.

Dodhia S, Hosanagar A, Fitzgerald DA, Labuschagne I, Wood AG, Nathan PJ, Phan KL (2014) Modulation of Resting-State Amygdala-Frontal Functional Connectivity by Oxytocin in Generalized Social Anxiety Disorder. Neuropsychopharmacology 39:2061–2069.

Dölen G, Darvishzadeh A, Huang KW, Malenka RC (2013) Social reward requires coordinated activity of nucleus accumbens oxytocin and serotonin. Nature 501:179–184.

Dumais KM, Kulkarni PP, Ferris CF, Veenema AH (2017) Sex differences in neural activation following different routes of oxytocin administration in awake adult rats. Psychoneuroendocrinology 81:52–62.

Dumais KM, Veenema AH (2016) Vasopressin and oxytocin receptor systems in the brain: Sex differences and sex-specific regulation of social behavior. Front Neuroendocrinol 40:1–23.

Dutta A, McKie S, Downey D, Thomas E, Juhasz G, Arnone D, Elliott R, Williams S, Deakin JFW, Anderson IM (2019) Regional default mode network connectivity in major depressive disorder: modulation by acute intravenous citalopram. Transl Psychiatry 9:116.

Ebner NC, Chen H, Porges E, Lin T, Fischer H, Feifel D, Cohen RA (2016) Oxytocin's effect on resting-state functional connectivity varies by age and sex. Psychoneuroendocrinology 69:50–59.

Eckstein M, Markett S, Kendrick KM, Ditzen B, Liu F, Hurlemann R, Becker B (2017) Oxytocin differentially alters resting state functional connectivity between amygdala subregions and emotional control networks: Inverse correlation with depressive traits. NeuroImage 149:458–467.

Eickhoff SB, Stephan KE, Mohlberg H, Grefkes C, Fink GR, Amunts K, Zilles K (2005) A new SPM toolbox for combining probabilistic cytoarchitectonic maps and functional imaging data. NeuroImage 25:1325–1335.

Eisner P, Klasen M, Wolf D, Zerres K, Eggermann T, Eisert A, Zvyagintsev M, Sarkheil P, Mathiak KA, Zepf F, Mathiak K (2017) Cortico-limbic connectivity in MAOA-L carriers is vulnerable to acute tryptophan depletion. Hum Brain Mapp 38:1622–1635.

Eklund A, Nichols TE, Knutsson H (2016) Cluster failure: Why fMRI inferences for spatial extent have inflated false-positive rates. Proc Natl Acad Sci U S A 113:7900–7905.

Elands J, Beetsma A, Barberis C, de KE (1988) Topography of the oxytocin receptor system in rat brain: an autoradiographical study with a selective radioiodinated oxytocin antagonist. J Chem Neuroanat 1:293–302.

Etkin A, Büchel C, Gross JJ (2015) The neural bases of emotion regulation. Nat Rev Neurosci 16:693–700.

Evers EAT, Sambeth A, Ramaekers JG, Riedel WJ, van der Veen FM (2010) The Effects of Acute Tryptophan Depletion on Brain Activation During Cognition and Emotional Processing in Healthy Volunteers. Curr Pharm Des 16:1998–2011.

Evers EAT, van der Veen FM, van Deursen JA, Schmitt JAJ, Deutz NEP, Jolles J (2006) The effect of acute tryptophan depletion on the BOLD response during performance monitoring and response inhibition in healthy male volunteers. Psychopharmacology (Berl) 187:200–208.

Fan Y, Herrera-Melendez AL, Pestke K, Feeser M, Aust S, Otte C, Pruessner JC, Böker H, Bajbouj M, Grimm S (2014) Early life stress modulates amygdala-prefrontal functional connectivity: Implications for oxytocin effects. Hum Brain Mapp 35:5328–5339.

Fan Y, Pestke K, Feeser M, Aust S, Pruessner JC, Böker H, Bajbouj M, Grimm S (2015) Amygdala–Hippocampal Connectivity Changes During Acute Psychosocial Stress: Joint Effect of Early Life Stress and Oxytocin. Neuropsychopharmacology 40:2736–2744.

Feng P, Becker B, Zheng Y, Feng T (2018) Sleep deprivation affects fear memory consolidation: bi-stable amygdala connectivity with insula and ventromedial prefrontal cortex. Soc Cogn Affect Neurosci 13:145–155.

Fisher PM, Hariri AR (2013) Identifying serotonergic mechanisms underlying the corticolimbic response to threat in humans. Philos Trans R Soc Lond B Biol Sci 368:20120192.

Forcelli PA, DesJardin JT, West EA, Holmes AL, Elorette C, Wellman LL, Malkova L (2016) Amygdala selectively modulates defensive responses evoked from the superior colliculus in non-human primates. Soc Cogn Affect Neurosci 11:2009–2019.

Freeman SM, Inoue K, Smith AL, Goodman MM, Young LJ (2014) The neuroanatomical distribution of oxytocin receptor binding and mRNA in the male rhesus macaque (Macaca mulatta). Psychoneuroendocrinology 45:128–141.

Friston KJ, Holmes AP, Worsley KJ, Poline J-P, Frith CD, Frackowiak RSJ (1994) Statistical parametric maps in functional imaging: A general linear approach. Hum Brain Mapp 2:189–210.

Fulwiler CE, King JA, Zhang N (2012) Amygdala-orbitofrontal resting-state functional connectivity is associated with trait anger. Neuroreport 23:606–610.

Gamer M, Zurowski B, Büchel C (2010) Different amygdala subregions mediate valence-related and attentional effects of oxytocin in humans. Proc Natl Acad Sci U S A 107:9400–9405.

Gimpl G, Fahrenholz F (2001) The oxytocin receptor system: structure, function, and regulation. Physiol Rev 81:629–683.

Gossen A, Hahn A, Westphal L, Prinz S, Schultz RT, Gründer G, Spreckelmeyer KN (2012) Oxytocin plasma concentrations after single intranasal oxytocin administration – A study in healthy men. Neuropeptides 46:211–215.

Gothard KM (2020) Multidimensional processing in the amygdala. Nat Rev Neurosci 21:565–575.

Grace SA, Labuschagne I, Castle DJ, Rossell SL (2019) Intranasal oxytocin alters amygdala-temporal resting-state functional connectivity in body dysmorphic disorder: A double-blind placebo-controlled randomized trial. Psychoneuroendocrinology 107:179–186.

Grace SA, Rossell SL, Heinrichs M, Kordsachia C, Labuschagne I (2018) Oxytocin and brain activity in humans: A systematic review and coordinate-based meta-analysis of functional MRI studies. Psychoneuroendocrinology 96:6–24.

Greve DN, Fischl B (2009) Accurate and robust brain image alignment using boundary-based registration. NeuroImage 48:63–72.

Grinevich V, Neumann ID (2021) Brain oxytocin: how puzzle stones from animal studies translate into psychiatry. Mol Psychiatry 26:265–279.

Heinrichs M, von Dawans B, Domes G (2009) Oxytocin, vasopressin, and human social behavior. Front Neuroendocrinol 30:548–557.

Hernández J, Prieto I, Segarra AB, de Gasparo M, Wangensteen R, Villarejo AB, Banegas I, Vives F, Cobo J, Ramírez-Sánchez M (2015) Interaction of neuropeptidase activities in cortico-limbic regions after acute restraint stress. Behav Brain Res 287:42–48.

Herringa R, Birn R, Ruttle P, Burghy C, Stodola D, Davidson R, Essex M (2013) Childhood maltreatment is associated with altered fear circuitry and increased internalizing symptoms by late adolescence. Proc Natl Acad Sci U S A 110.

Jenkinson M, Bannister P, Brady M, Smith S (2002) Improved optimization for the robust and accurate linear registration and motion correction of brain images. NeuroImage 17:825–841.

Jenkinson M, Smith S (2001) A global optimisation method for robust affine registration of brain images. Med Image Anal 5:143–156.

Jiang X, Ma X, Geng Y, Zhao Z, Zhou F, Zhao W, Yao S, Yang S, Zhao Z, Becker B, Kendrick KM (2021) Intrinsic, dynamic and effective connectivity among large-scale brain networks modulated by oxytocin. NeuroImage 227:117668.

Johns CB, Lacadie C, Vohr B, Ment LR, Scheinost D (2019) Amygdala functional connectivity is associated with social impairments in preterm born young adults. NeuroImage Clin 21:101626.

Kanat M, Heinrichs M, Mader I, van Elst LT, Domes G (2015) Oxytocin Modulates Amygdala Reactivity to Masked Fearful Eyes. Neuropsychopharmacol Off Publ Am Coll Neuropsychopharmacol 40:2632–2638.

Kendrick KM, Guastella AJ, Becker B (2018) Overview of Human Oxytocin Research. Curr Top Behav Neurosci 35:321–348.

Kiem SA, Andrade KC, Spoormaker VI, Holsboer F, Czisch M, Sämann PG (2013) Resting state functional MRI connectivity predicts hypothalamus-pituitary-axis status in healthy males. Psychoneuroendocrinology 38:1338–1348.

Kirkby LA, Luongo FJ, Lee MB, Nahum M, Van Vleet TM, Rao VR, Dawes HE, Chang EF, Sohal VS (2018) An Amygdala-Hippocampus Subnetwork that Encodes Variation in Human Mood. Cell 175:1688-1700.e14.

Kirsch P (2005) Oxytocin Modulates Neural Circuitry for Social Cognition and Fear in Humans. J Neurosci 25:11489–11493.

Koch SBJ, van Zuiden M, Nawijn L, Frijling JL, Veltman DJ, Olff M (2016) Intranasal Oxytocin Normalizes Amygdala Functional Connectivity in Posttraumatic Stress Disorder. Neuropsychopharmacology 41:2041–2051.

Kreuder A-K, Scheele D, Schultz J, Hennig J, Marsh N, Dellert T, Ettinger U, Philipsen A, Babasiz M, Herscheid A, Remmersmann L, Stirnberg R, Stöcker T, Hurlemann R (2020) Common and dissociable effects of oxytocin and lorazepam on the neurocircuitry of fear. Proc Natl Acad Sci U S A 117:11781–11787.

Kumar J, Völlm B, Palaniyappan L (2014) Oxytocin affects the connectivity of the precuneus and the amygdala: a randomized, double-blinded, placebo-controlled neuroimaging trial. Int J Neuropsychopharmacol 18:pyu051.

LeDoux JE, Pine DS (2016) Using Neuroscience to Help Understand Fear and Anxiety: A Two-System Framework. Am J Psychiatry 173:1083–1093.

Lefevre A, Mottolese R, Redouté J, Costes N, Le Bars D, Geoffray M-M, Leboyer M, Sirigu A (2018) Oxytocin Fails to Recruit Serotonergic Neurotransmission in the Autistic Brain. Cereb Cortex 28:4169–4178.

Lefevre A, Richard N, Jazayeri M, Beuriat P-A, Fieux S, Zimmer L, Duhamel J-R, Sirigu A (2017) Oxytocin and Serotonin Brain Mechanisms in the Nonhuman Primate. J Neurosci 37:6741–6750.

Lieberz J, Scheele D, Spengler FB, Matheisen T, Schneider L, Stoffel-Wagner B, Kinfe TM, Hurlemann R (2020) Kinetics of oxytocin effects on amygdala and striatal reactivity vary between women and men. Neuropsychopharmacol Off Publ Am Coll Neuropsychopharmacol 45:1134–1140.

Lim L, Radua J, Rubia K (2014) Gray Matter Abnormalities in Childhood Maltreatment: A Voxel-Wise Meta-Analysis. Am J Psychiatry 171:854–863.

Liu C, Lan C, Li K, Zhou F, Yao S, Xu L, Yang N, Zhou X, Yang J, Yong X, Ma Y, Scheele D, Kendrick KM, Becker B (2021a) Oxytocinergic modulation of threat-specific amygdala sensitization in humans is critically mediated by serotonergic mechanisms. Biol Psychiatry Cogn Neurosci Neuroimaging.

Liu C, Xu L, Li J, Zhou F, Yang X, Zheng X, Fu M, Li K, Sindermann C, Montag C, Ma Y, Scheele D, Ebstein RP, Yao S, Kendrick KM, Becker B (2021b) Serotonin and early life stress interact to shape brain architecture and anxious avoidant behavior - a TPH2 imaging genetics approach. Psychol Med 51:2476–2484.

Luo L, Yang T, Zheng X, Zhang X, Gao S, Li Y, Stamatakis EA, Sahakian B, Becker B, Lin Q, Kendrick KM (2022) Altered centromedial amygdala functional connectivity in adults is associated with childhood emotional abuse and predicts levels of depression and anxiety. J Affect Disord 303:148–154.

Ma X, Zhao W, Luo R, Zhou F, Geng Y, Xu L, Gao Z, Zheng X, Becker B, Kendrick KM (2018) Sex- and context-dependent effects of oxytocin on social sharing. NeuroImage 183:62–72.

Mahar I, Bambico FR, Mechawar N, Nobrega JN (2014) Stress, serotonin, and hippocampal neurogenesis in relation to depression and antidepressant effects. Neurosci Biobehav Rev 38:173–192.

Martins D, Brodmann K, Veronese M, Dipasquale O, Mazibuko N, Schuschnig U, Zelaya F, Fotopoulou A, Paloyelis Y (2022) “Less is more”: A dose-response account of intranasal oxytocin pharmacodynamics in the human brain. Prog Neurobiol 211:102239.

Matsushita H, Latt HM, Koga Y, Nishiki T, Matsui H (2019) Oxytocin and Stress: Neural Mechanisms, Stress-Related Disorders, and Therapeutic Approaches. Neuroscience 417:1–10.

Meyer-Lindenberg A, Domes G, Kirsch P, Heinrichs M (2011) Oxytocin and vasopressin in the human brain: social neuropeptides for translational medicine. Nat Rev Neurosci 12:524–538.

Mihov Y, Kendrick KM, Becker B, Zschernack J, Reich H, Maier W, Keysers C, Hurlemann R (2013) Mirroring fear in the absence of a functional amygdala. Biol Psychiatry 73:e9-11.

Morris JS, Ohman A, Dolan RJ (1998) Conscious and unconscious emotional learning in the human amygdala. Nature 393:467–470.

Mottolese R, Redoute J, Costes N, Le Bars D, Sirigu A (2014) Switching brain serotonin with oxytocin. Proc Natl Acad Sci U S A 111:8637–8642.

Mueller NK, Dolgas CM, Herman JP (2004) Stressor-selective role of the ventral subiculum in regulation of neuroendocrine stress responses. Endocrinology 145:3763–3768.

Neumann ID, Slattery DA (2016) Oxytocin in General Anxiety and Social Fear: A Translational Approach. Biol Psychiatry 79:213–221.

Nishizawa S, Benkelfat C, Young SN, Leyton M, Mzengeza S, de Montigny C, Blier P, Diksic M (1997) Differences between males and females in rates of serotonin synthesis in human brain. Proc Natl Acad Sci U S A 94:5308–5313.

Olff M, Frijling JL, Kubzansky LD, Bradley B, Ellenbogen MA, Cardoso C, Bartz JA, Yee JR, van Zuiden M (2013) The role of oxytocin in social bonding, stress regulation and mental health: An update on the moderating effects of context and interindividual differences. Psychoneuroendocrinology 38:1883–1894.

Paloyelis Y, Doyle OM, Zelaya FO, Maltezos S, Williams SC, Fotopoulou A, Howard MA (2016) A Spatiotemporal Profile of In Vivo Cerebral Blood Flow Changes Following Intranasal Oxytocin in Humans. Biol Psychiatry 79:693–705.

Park AT, Leonard JA, Saxler PK, Cyr AB, Gabrieli JDE, Mackey AP (2018) Amygdala–medial prefrontal cortex connectivity relates to stress and mental health in early childhood. Soc Cogn Affect Neurosci 13:430–439.

Pasqualetti M, Ori M, Nardi I, Castagna M, Cassano GB, Marazziti D (1998) Distribution of the 5-HT5A serotonin receptor mRNA in the human brain. Brain Res Mol Brain Res 56:1–8.

Passamonti L, Crockett MJ, Apergis-Schoute AM, Clark L, Rowe JB, Calder AJ, Robbins TW (2012) Effects of Acute Tryptophan Depletion on Prefrontal-Amygdala Connectivity While Viewing Facial Signals of Aggression. Biol Psychiatry 71:36–43.

Phelps EA, LeDoux JE (2005) Contributions of the amygdala to emotion processing: from animal models to human behavior. Neuron 48:175–187.

Power JD, Barnes KA, Snyder AZ, Schlaggar BL, Petersen SE (2012) Spurious but systematic correlations in functional connectivity MRI networks arise from subject motion. NeuroImage 59:2142–2154.

Quintana DS, Lischke A, Grace S, Scheele D, Ma Y, Becker B (2021) Advances in the field of intranasal oxytocin research: lessons learned and future directions for clinical research. Mol Psychiatry 26:80–91.

Quintana DS, Rokicki J, van der Meer D, Alnæs D, Kaufmann T, Córdova-Palomera A, Dieset I, Andreassen OA, Westlye LT (2019) Oxytocin pathway gene networks in the human brain. Nat Commun 10:668.

Raab K, Kirsch P, Mier D (2016) Understanding the impact of 5-HTTLPR, antidepressants, and acute tryptophan depletion on brain activation during facial emotion processing: A review of the imaging literature. Neurosci Biobehav Rev 71:176–197.

Seeley SH, Chou Y, O'Connor M-F (2018) Intranasal oxytocin and OXTR genotype effects on resting state functional connectivity: A systematic review. Neurosci Biobehav Rev 95:17–32.

Shamay-Tsoory SG, Abu-Akel A (2016) The Social Salience Hypothesis of Oxytocin. Biol Psychiatry 79:194–202.

Shapiro LE, Insel TR (1989) Ontogeny of oxytocin receptors in rat forebrain: a quantitative study. Synap N Y N 4:259–266.

Spengler FB, Becker B, Kendrick KM, Conrad R, Hurlemann R, Schade G (2017a) Emotional Dysregulation in Psychogenic Voice Loss. Psychother Psychosom 86:121–123.

Spengler FB, Schultz J, Scheele D, Essel M, Maier W, Heinrichs M, Hurlemann R (2017b) Kinetics and Dose Dependency of Intranasal Oxytocin Effects on Amygdala Reactivity. Biol Psychiatry 82:885–894.

Spielberger CD, Gorsuch RL, Lushene RE (1970) Manual for the State-Trait Anxiety Inventory. Palo Alto, CA: Consulting Psychologists Press. Available at: http://ubir.buffalo.edu/xmlui/handle/10477/2895 [Accessed May 19, 2020].

Sripada CS, Phan KL, Labuschagne I, Welsh R, Nathan PJ, Wood AG (2013) Oxytocin enhances resting-state connectivity between amygdala and medial frontal cortex. Int J Neuropsychopharmacol 16:255–260.

Sripada RK, King AP, Garfinkel SN, Wang X, Sripada CS, Welsh RC, Liberzon I (2012) Altered resting-state amygdala functional connectivity in men with posttraumatic stress disorder. J Psychiatry Neurosci JPN 37:241–249.

Steinberg EE, Gore F, Heifets BD, Taylor MD, Norville ZC, Beier KT, Földy C, Lerner TN, Luo L, Deisseroth K, Malenka RC (2020) Amygdala-Midbrain Connections Modulate Appetitive and Aversive Learning. Neuron 106:1026-1043.e9.

Striepens N, Scheele D, Kendrick KM, Becker B, Schafer L, Schwalba K, Reul J, Maier W, Hurlemann R (2012) Oxytocin facilitates protective responses to aversive social stimuli in males. Proc Natl Acad Sci U S A 109:18144–18149.

Talbot PS, Cooper SJ (2006) Anterior Cingulate and Subgenual Prefrontal Blood Flow Changes Following Tryptophan Depletion in Healthy Males. Neuropsychopharmacology 31:1757–1767.

Teicher MH, Anderson CM, Polcari A (2012) Childhood maltreatment is associated with reduced volume in the hippocampal subfields CA3, dentate gyrus, and subiculum. Proc Natl Acad Sci U S A 109:E563–E572.

Vaisvaser S, Lin T, Admon R, Podlipsky I, Greenman Y, Stern N, Fruchter E, Wald I, Pine DS, Tarrasch R, Bar-Haim Y, Hendler T (2013) Neural traces of stress: cortisol related sustained enhancement of amygdala-hippocampal functional connectivity. Front Hum Neurosci 7:313.

Van Dijk KRA, Sabuncu MR, Buckner RL (2012) The influence of head motion on intrinsic functional connectivity MRI. NeuroImage 59:431–438.

Varnäs K, Halldin C, Hall H (2004) Autoradiographic distribution of serotonin transporters and receptor subtypes in human brain. Hum Brain Mapp 22:246–260.

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.

Watson D, Clark LA, Tellegen A (1988) Development and validation of brief measures of positive and negative affect: The PANAS scales. J Pers Soc Psychol 54:1063–1070.

Woo C-W, Krishnan A, Wager TD (2014) Cluster-extent based thresholding in fMRI analyses: Pitfalls and recommendations. NeuroImage 91:412–419.

Wu H, Feng C, Lu X, Liu X, Liu Q (2020) Oxytocin effects on the resting-state mentalizing brain network. Brain Imaging Behav 14:2530–2541.

Xin F, Zhou F, Zhou X, Ma X, Geng Y, Zhao W, Yao S, Dong D, Biswal BB, Kendrick KM, Becker B (2021) Oxytocin Modulates the Intrinsic Dynamics Between Attention-Related Large-Scale Networks. Cereb Cortex 31:1848–1860.

Xin F, Zhou X, Dong D, Zhao Z, Yang X, Wang Q, Gu Y, Kendrick KM, Chen A, Becker B (2020) Oxytocin Differentially Modulates Amygdala Responses during Top-Down and Bottom-Up Aversive Anticipation. Adv Sci 7:2001077.

Xu L, Becker B, Luo R, Zheng X, Zhao W, Zhang Q, Kendrick KM (2020) Oxytocin amplifies sex differences in human mate choice. Psychoneuroendocrinology 112:104483.

Xu X, Dai J, Chen Y, Liu C, Xin F, Zhou X, Zhou F, Stamatakis EA, Yao S, Luo L, Huang Y, Wang J, Zou Z, Vatansever D, Kendrick KM, Zhou B, Becker B (2021) Intrinsic connectivity of the prefrontal cortex and striato-limbic system respectively differentiate major depressive from generalized anxiety disorder. Neuropsychopharmacology 46:791–798.

Xu X, Liu C, Zhou X, Chen Y, Gao Z, Zhou F, Kou J, Becker B, Kendrick KM (2019) Oxytocin Facilitates Self-Serving Rather Than Altruistic Tendencies in Competitive Social Interactions Via Orbitofrontal Cortex. Int J Neuropsychopharmacol 22:501–512.

Yao S, Zhao W, Geng Y, Chen Y, Zhao Z, Ma X, Xu L, Becker B, Kendrick KM (2018) Oxytocin Facilitates Approach Behavior to Positive Social Stimuli via Decreasing Anterior Insula Activity. Int J Neuropsychopharmacol 21:918–925.

Yoshida M, Takayanagi Y, Inoue K, Kimura T, Young LJ, Onaka T, Nishimori K (2009) Evidence That Oxytocin Exerts Anxiolytic Effects via Oxytocin Receptor Expressed in Serotonergic Neurons in Mice. J Neurosci 29:2259–2271.

Young LJ, Wang Z (2004) The neurobiology of pair bonding. Nat Neurosci 7:1048–1054.

Zhang X, Huettel SA, Mullette-Gillman OA, Guo H, Wang L (2019) Exploring common changes after acute mental stress and acute tryptophan depletion: Resting-state fMRI studies. J Psychiatr Res 113:172–180.

Zhang X, Zhang J, Wang L, Li R, Zhang W (2016) Altered resting-state functional connectivity of the amygdala in Chinese earthquake survivors. Prog Neuropsychopharmacol Biol Psychiatry 65:208–214.

Zhao Z, Ma X, Geng Y, Zhao W, Zhou F, Wang J, Markett S, Biswal BB, Ma Y, Kendrick KM, Becker B (2019a) Oxytocin differentially modulates specific dorsal and ventral striatal functional connections with frontal and cerebellar regions. NeuroImage 184:781–789.

Zhao Z, Yao S, Li K, Sindermann C, Zhou F, Zhao W, Li J, Lührs M, Goebel R, Kendrick KM, Becker B (2019b) Real-Time Functional Connectivity-Informed Neurofeedback of Amygdala-Frontal Pathways Reduces Anxiety. Psychother Psychosom 88:5–15.

Zhou F, Zhao W, Qi Z, Geng Y, Yao S, Kendrick KM, Wager TD, Becker B (2021) Beyond fear centers: a distributed fMRI-based neuromarker for the subjective experience of fear. Available at: https://www.biorxiv.org/content/10.1101/2020.11.23.394973v2 [Accessed August 16, 2021].

Zhuang Q, Zhu S, Yang X, Zhou X, Xu X, Chen Z, Lan C, Zhao W, Becker B, Yao S, Kendrick KM (2021) Oxytocin-induced facilitation of learning in a probabilistic task is associated with reduced feedback- and error-related negativity potentials. J Psychopharmacol (Oxf) 35:40–49.

**Legends to figures**

**Figure 1** Experimental protocols and procedures of the pharmacological resting-state fMRI experiment.

**Figure 2. Interaction effects between oxytocin and serotonin on the intrinsic connectivity of the left amygdala.** (A) The whole brain voxel-wise analysis revealed a significant negative interaction effect of treatments on the functional connectivity between the left amygdala and a left hippocampus/midbrain cluster (in light green, whole brain corrected at $p_{\text{FWE-cluster}} < 0.05$). The mask in light blue represents the left subiculum from the SPM Anatomy toolbox. (B) Post hoc comparisons between the treatment groups using extracted parameter estimates from the left subiculum anatomical mask. Group difference between ATDc-PLC and ATDc-OXT indicates that OXT significantly increased connectivity in this pathway in relative to the PLC-reference group, whereas the group difference between ATDc-OXT and ATD-OXT indicates that pre-treatment with acute tryptophan depletion significantly attenuated the OXT-induced increase. Bars indicate M ± SEM, * $p_{\text{FDR}} < 0.05$. Abbreviations: L, left; Amyg, amygdala; Hipp, hippocampus; OXT, Oxytocin; PLC, Placebo; ATD, Acute Tryptophan Depletion; ATDc, Acute Tryptophan Depletion control mixture.

**Figure 3**. **Associations of with currently experienced stress in ATDc-PLC group.** Associations between Perceived Stress Scale scores and parameter estimates extracted from the amygdala-hippocampal pathway displaying correlation. Dots represent individual data. Abbreviations: PLC, Placebo; ATDc, Acute Tryptophan Depletion control mixture.

**Legends to tables**

**Table 1 Group characteristics of subject age and questionnaire scores.**

**Abbreviations:** OXT, Oxytocin; PLC, Placebo; ATD, Acute Tryptophan Depletion; ATDc, Acute Tryptophan Depletion control mixture; BDI, Beck's Depression Inventory; STAI-TAI, State-Trait Anxiety Inventory-Trait Anxiety Inventory; STAI-SAI, State-Trait Anxiety Inventory-State Anxiety Inventory; PSS, Perceived Stress Scale; PANAS-P, Positive and Negative Affect Schedule-Positive affect; PANAS-N, Positive and Negative Affect Schedule-Negative affect; T1, pre-oral administration.