Oxytocinergic Modulation of Stress-Associated Amygdala-Hippocampus Pathways in Humans Is Mediated by Serotonergic Mechanisms

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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 functional magnetic resonance imaging study aimed to determine 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 functional magnetic resonance imaging resting-state experiment with 4 treatment groups in n = 112 healthy male participants. Participants underwent a transient decrease in 5-HT signaling via acute tryptophan depletion (ATD) or a corresponding placebo-control protocol before the administration of intranasal OXT (24 IU) or placebo intranasal spray.

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, whereas this effect of OXT was significantly attenuated during transiently decreased central serotonergic signaling induced via acute tryptophan depletion. 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 the first evidence, to our knowledge, that the effects of OXT on stress-associated amygdala-hippocampal-midbrain pathways are critically mediated by the 5-HT system in humans.

Keywords: Oxytocin, serotonin, amygdala, stress, anxiety
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, for example, exert pro-social, anxiolytic, and stress-reducing effects (Heinrichs et al., 2005; 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; Dølen et al., 2013; Lefevre et al., 2017). In terms of the anxiety-protective 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 that 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 pretreatment 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 (see, 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; Varnás et al., 2004; Quintana et al., 2019) and thus may not influence only 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 (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) (Brodmann et al., 2017; Wu et al., 2020; Jiang et al., 2021; Xin et al., 2021).

Previous studies employing a placebo-control pharmaco-rsfMRI strategy to determine the effects of intranasal OXT on intrinsic amygdala networks reported an OXT-induced modulation of the intrinsic coupling of the amygdala with prefrontal regions, particularly the medial prefrontal cortex and orbitofrontal regions (Dodhia et al., 2014; Fan et al., 2014, Eckstein et al., 2017; Cheng et al., 2018; Jiang et al., 2021), posterior default mode network 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 default mode network (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 nonhuman 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; Vaisv aver et al., 2013) and amygdala-prefrontal circuits are sensitive to both long-term as well as acute stress exposure (Herrin ga et al., 2013; Fan et al., 2015, 2014; Park et al., 2018).

To summarize, accumulating evidence from animal and human models suggest an interaction of OXT and 5-HT in regulating anxiety- and stress-related behavior; however, although...
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-induced transient decrease in central 5-HT signaling or a matched ATD control (ATDc) protocol in a randomized controlled pharmaco-rsfMRI parallel group design in n = 121 healthy male participants (Liu et al., 2021a). Based on previous studies, we hypothesized that (1) 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.

MATERIALS AND METHODS

Participants

A total of 121 nonsmoking, right-handed, young, healthy male participants were enrolled (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 (Eckstein et al., 2017b), rsfMRI data were collected 45 minutes after OXT/PLC administration. Control variables were reassessed before and after fMRI acquisition (for schematic outline of the experimental protocols, see Figure 1). The rsfMRI acquisition was followed by a post-fMRI resting-state fMRI paradigm assessing amygdala threat reactivity and sensitization (Liu et al., 2021a). Briefly, a randomized, double-blind, placebo-controlled, between-participant pharmaco-rsfMRI design was employed during which 4 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 OXT signaling. To adhere to the pharmacodynamic profile of treatments, participants arrived between 7:30 and 10:00 am and underwent MRI acquisition between 1:30 and 4:00 pm. On arrival, participants received a standardized protein-poor diet for breakfast. Following the assessment of pretreatment control variables, participants underwent a previously validated tryptophan depletion protocol with ATD (for detailed components of mixtures, see supplementary Table 1), 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 reassessed and participants administered either OXT (24 IU, ingredients: OXT, glycerin, sodium chloride, and purified water) or PLC (identical ingredients except for OXT) intranasal spray. Both intranasal sprays were provided by Sichuan Meike Pharmaceutical Co. Ltd (Luzhou, Sichuan, China). In line with the pharmacokinetic profile of intranasal OXT (Spengler et al., 2017b), rsfMRI data were collected 45 minutes after OXT/PLC administration. Control variables were reassessed before and after fMRI acquisition (for 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).

Procedures

Experimental and treatment procedures were identical to our previous study that reported the results of a task-based fMRI paradigm in the same sample to assess interactive effects of OXT and 5-HT on amygdala threat reactivity and sensitization (Liu et al., 2021a). Briefly, a randomized, double-blind, placebo-controlled, between-participant pharmaco-rsfMRI design was employed during which 4 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 OXT signaling. To adhere to the pharmacodynamic profile of treatments, participants arrived between 7:30 and 10:00 am and underwent fMRI acquisition between 1:30 and 4:00 pm. On arrival, participants received a standardized protein-poor diet for breakfast. Following the assessment of pretreatment control variables, participants underwent a previously validated tryptophan depletion protocol with ATD (for detailed components of mixtures, see supplementary Table 1), 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 reassessed and participants administered either OXT (24 IU, ingredients: OXT, glycerin, sodium chloride, and purified water) or PLC (identical ingredients except for OXT) intranasal spray. Both intranasal sprays were provided by Sichuan Meike Pharmaceutical Co. Ltd (Luzhou, Sichuan, China). In line with the pharmacokinetic profile of intranasal OXT (Spengler et al., 2017b), rsfMRI data were collected 45 minutes after OXT/PLC administration. Control variables were reassessed before and after fMRI acquisition (for 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).
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 pretreatment differences between the groups. To this end, the State-Trait Anxiety Inventory (Spielberger et al., 1970) and Beck Depression Inventory (Beck et al., 1996) were applied. The Positive and Negative Affect Schedule (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 (for 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 (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, for example, 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 participants without active treatment.

MRI Data Acquisition

MRI data were 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 milliseconds; echo time, 1 millisecond; flip angle, 12°; field of view, 256×256 mm; resolution, 256×256; slice thickness, 1 mm; number of slices, 156. Resting-state fMRI data were acquired using an echo planar imaging sequence with the following acquisition parameters: repetition time, 2000 milliseconds; echo time, 30 milliseconds; 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 (approximately 7.5 minutes). During scanning, 2 head cushions were used to prevent excessive head motion while ensuring comfort. Participants were instructed to relax and think of nothing in particular while focusing on a fixation cross presented centrally via a rear mirror.

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 automated anatomic labeling served as a priori-defined seed regions. Individual functional connectivity maps were initially created for each participant using DPASFA 4.4 (advanced edition of i.e. Data Processing and Analysis for Brain Imaging, 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-participant factor and mean FD and age as covariates and the
grey matter mask template from Data Processing and Analysis for Brain Imaging 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 combined voxel-wise P < .001 with cluster-wise P < .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 false discovery rate (FDR) correction in R-Studio (Benjamini and Hochberg, 1995). To further examine associations with current stress levels, Spearman’s rank correlation was applied in SPSS (IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.) to explore associations between extracted parameter estimates in significant cluster and PSS scores with a focus on participants without active treatment (ATD-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-participant factors, and timepoint (T1–T4; pre-oral administration, pre-fMRI, post-fMRI) as within-participant factor. To this end, parameter estimates of atlas-based independent masks were extracted and subjected to post hoc comparisons with false discovery rate (FDR) correction in R-Studio (Benjamini and Hochberg, 1995). To further examine associations with current stress levels, Spearman’s rank correlation was applied in SPSS (IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.) to explore associations between extracted parameter estimates in significant cluster and PSS scores with a focus on participants without active treatment (ATD-PLC).

Interaction Between Reduced Serotonergic Signaling and OXT on Amygdala Networks

In the whole-brain analysis, a significant interaction effect between treatments was observed with respect to left amygdala intrinsic coupling with a cluster located in the left hippocampus extending into the adjacent midbrain (MNI coordinate, xyz = [−20, −28, −10], 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_{FDR} = .048), whereas pretreatment with ATD (ATD-OXT) significantly attenuated the OXT-induced (ATD-OXT) increase (P_{FDR} = .048) (see Figure 2B). Neither ATD (ATD-PLC) alone (P_{FDR} = .62) nor the combined application (ATD-OXT, P_{FDR} = .84) significantly increased amygdala connectivity compared with the PLC-group (ATDc-PLC). 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 supplementary Figure 2). No significant

![Interaction](image-url)
treatment interaction effects were observed for right amygdala intrinsic networks.

**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 ($r_{SPS} = -0.34, p = .08$; 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, $r_{SPS} = 0.25, p = .22$; ATD-OXT, $r_{SPS} = 0.10, p = .62$; ATD-PLC, $r_{SPS} = -0.07, p = .72$).

**Discussion**

In the present study, we aimed to determine the interactive effects of OXT and 5-HT system on the 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 ATD or the respective control conditions and resting-state fMRI. Examining treatment 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 subiculum of the hippocampus and extending to the left midbrain encompassing the superior colliculus. Post-hoc analyses convergently demonstrated that OXT increased intrinsic coupling in this pathway, whereas 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 was associated with higher levels of experienced stress. Although the association with current stress did not reach significance in the present sample and replication in larger samples is required, our findings in the placebo group may suggest 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 indicating that 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 who did not receive active treatment. Although the results in the present sample failed to reach statistical significance and require replication in larger samples, the findings may reflect 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 (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 primate studies have suggested that both the subiculum and the superior colliculus are brain regions with particularly dense OXT receptor expression (Elends et al., 1988; Shapiro and Insel, 1989; Freeman et al., 2014, 2014). The subiculum has been considered as a 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 gray matter of this region (Teicher et al., 2012) and the amygdala (Lim et al., 2014). Furthermore, previous studies...
reported strong functional connectivity between the amygdala and the hippocampus (Ki et al., 2013), and 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), whereas such reflexive defense reactions were attenuated when the activation of amygdala and superior colliculus were simultaneously inhibited in nonhuman 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 the amygdala and the superior colliculus toward both threatening and nonthreatening 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 (Straviga 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; Eber 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 (Liu et al., 2021a).

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 nonspecific negative affective states.

Findings of the present study need to be considered in the context of the following limitations. First, only male participants were enrolled due to previous studies that showed marked sex differences in the 5-HT synthesis (Nishizawa et al., 1997) and in the effects of OXT in rodents and humans (Dumais and Veenema, 2016; Dumais et al., 2017; Ma et al., 2018; Lieberz et al., 2020; Xu et al., 2020). 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 ATD or OXT treatment protocols to those 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 2 systems and should be included in future studies. Finally, although 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 although 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). Although 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). 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 the first evidence, to our knowledge, that effects of OXT on stress-associated amygdala-hippocampal-midbrain pathways are critically mediated by the 5-HT system in healthy men.

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**Conflict of Interest**

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

**References**

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, Berntsen 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 Neuroimaging 4:655–663.

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

Beck AT, Steer RA, Ball R, Ranieri WF (1996) Comparison of Beck Depression Inventories-I 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.

Bleuler 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.
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.

Fuwliler CE, King JA, Zhang N (2012) Amygdala-orbitofrontal resting-state functional connectivity is associated with trait anger. Neuroreport 23:606–10.

Gamer M, Zuroski 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, Schulz 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, 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.

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.

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 pieces 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 corticollimic 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 110:19119–19124.

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) Intrinsinc, 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. Neuropsychopharmacology 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.

Kirsch P (2005) Oxytocin modulates neural circuitry for social cognition and fear in humans. J Neurosci 25:11489–11493.

Koch SB, 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, Schulz 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, Richard N, Jazayeri M, Beurjat P-A, Fieux S, Zimmerman L, Duhamel J-R, Sirigu A (2017) Oxytocin and serotonin brain mechanisms in the nonhuman primate. J Neurosci 37:6741–6750.

Lefevre A, Mottolese R, Redouüt J, Kosten 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.

Lieberz J, Scheele D, Spengler FR, 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. Neuropsychopharmacology 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 Neuroimag. 6:1081–1089.

Liu C, Xu L, Li J, Zhou F, Yang X, Zhong 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, Veronez 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 111:8367–8374.

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 depression: a translational approach. Biol Psychiatry 79:213–221.

Nishizawa S, Benkelfat C, Young SN, Leyton M, Zangen 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.

Off 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, Cilibrasi J, Bradley C, Eden OB, MacKay 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, Apergis-Schoute AM, Clark L, Rowe JB, Calder AJ, Robbins TW (2012) Effects of acute tryptophan depletion on prefrontal-amygudala connectivity while viewing learned and future directions for clinical research. Mol Psychiatry 26:80–91.

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.

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 C (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.

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.

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.

Vainäns 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 POMS 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 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.

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.

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, 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.

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.

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 serotoninergic 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, 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) A distributed fMRI-based neuromarker for the subjective experience of fear. Nature Communications 12:6643.

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.