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

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ABSTRACT

BACKGROUND: Overarching conceptualizations propose that the complex social-emotional effects of oxytocin (OXT) in humans are partly mediated by interactions with other neurotransmitter systems. Recent animal models suggest that the anxiolytic effects of OXT are critically mediated by the serotonin (5-HT) system, yet direct evidence in humans is lacking.

METHODS: To determine the role of 5-HT in OXT-induced attenuation of amygdala threat reactivity and sensitization/desensitization, we conducted a parallel-group, randomized, placebo-controlled, double-blind experiment during which 121 healthy subjects underwent a transient decrease in 5-HT signaling via acute tryptophan depletion or the corresponding placebo-control protocol before the administration of intranasal OXT or placebo intranasal spray, respectively. Mean and repetition-dependent changes in threat-specific amygdala reactivity toward threatening stimuli (angry faces) as assessed by functional magnetic resonance imaging served as the primary outcome.

RESULTS: No main or interaction effects of treatment on amygdala threat reactivity were observed, yet OXT switched bilateral amygdala threat sensitization to desensitization, and this effect was significantly attenuated during decreased central 5-HT signaling via pretreatment with acute tryptophan depletion.

CONCLUSIONS: The present findings provide the first evidence for a role of OXT in threat-specific amygdala desensitization in humans and suggest that these effects are critically mediated by the 5-HT system. OXT may have a therapeutic potential to facilitate amygdala desensitization, and adjunct upregulation of 5-HT neurotransmission may facilitate OXT's anxiolytic potential.

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The hypothalamic peptide oxytocin (OXT) regulates a broad range of peripheral and central functions (1). Across species, OXT plays an important role in complex social behavior and basal emotion processes, particularly salience and threat processing (2). Overarching conceptualizations of the role of OXT in human social-emotional behavior have proposed that the complex behavioral effects of OXT are partly mediated by interactions with other neurotransmitter systems (3). Such interactions have been evidenced by initial animal models demonstrating that OXT's effects in the domains of pair bonding are partly mediated by dopamine (4), whereas social reward and anxiolytic effects involve interactions with the serotonin (5-HT) system (5–7).

Accumulating evidence further suggests that the anxiolytic properties of OXT are (partly) mediated by the 5-HT system. Direct evidence for a role of 5-HT in OXT's anxiolytic effects has been demonstrated in a seminal rodent model combining genetic editing with OXT infusion (7). This study demonstrated that OXT receptors are expressed in one third of the 5-HT releasing neurons in the raphe nucleus, which represents the principal source of central 5-HT as well as afferent serotonergic projections to the amygdala (8). More recently, initial studies combined intranasal or intracerebroventricular OXT administration with concomitant molecular imaging of 5-HT neurotransmission in nonhuman primates and humans and reported OXT-induced modulations of serotonergic signaling in regions strongly engaged in salience and threat processing, particularly the amygdala and insula, with further analyses suggesting a central role of the amygdala in the oxytocinergic regulation of 5-HT release (6,9).

To directly examine whether the anxiolytic effects of OXT on threat-related amygdala reactivity in humans are mediated by the 5-HT system, we conducted a parallel-group, randomized, placebo-controlled, double-blind functional magnetic resonance imaging (fMRI) experiment during which 121 healthy male participants underwent either transient decreases in 5-HT signaling (via acute tryptophan depletion [ATD^+]) or a matched control protocol (ATD^-) before the administration of intranasal OXT or placebo (PLC). Based on previous animal models, we specifically hypothesized that 1) OXT would dampen amygdala

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threat reactivity to angry faces relative to placebo and 2) pretreatment with 5-HT depletion would attenuate OXT-induced dampening of amygdala responses relative to OXT treatment alone.

Amygdala responses show rapid adaptations with repeated stimulus presentations, with both reduced (desensitization) (10–12) and increased (sensitization) reactivity (13–15) being reported depending on the respective amygdala subregions involved and emotional content. Furthermore, a higher retest reliability of repetition-dependent amygdala signal changes was found when compared with mean amplitude measurements, suggesting a particular stable marker for pharmacological imaging [e.g., (16)]. Although a number of previous studies suggest that amygdala sensitization/desensitization is modulated by serotonergic signaling (17) and that OXT may modulate arousal and amygdala habituation in trust and cooperation contexts (18,19), the role of OXT for threat-related habitation is still unclear. Therefore, we additionally explored the interactive effects of OXT and 5-HT on sensitization/desensitization, employing a comparable analysis strategy as for amygdala mean amplitudes. Given that previous rodent models demonstrated that OXT regulates amygdala threat responses via direct hypothalamic-amygdala neuronal projections (20) as well as indirect pathways via OXT receptors expressed on serotonergic raphe neurons (7), we hypothesized that downregulation of serotonergic signaling would decrease but not fully abolish the effects of intranasal OXT on amygdala threat reactivity. Finally, although amygdala desensitization as assessed by the mean of a block difference approach represents a robust and comparably reliable within-subject fMRI index (21), we additionally included an independent dataset to determine the robustness of threat-specific amygdala sensitization/desensitization across samples.

METHODS AND MATERIALS

Participants

A total of 121 right-handed, healthy male participants were enrolled. To reduce variance related to sex differences in the effects of oxytocin on amygdala reactivity (22), only male participants were included. Given the complexity of the design, a pragmatic approach for sample size determination was employed based on a recent fMRI study (23) comparing effects of different OXT dosages on threat-related amygdala activity [for a similar approach, see a recent study comparing OXT with another anxiolytic agent (24)]. For the behavioral and mood analysis, based on initial quality assessments, data from 9 subjects were excluded owing to technical problems during data acquisition, poor performance (accuracy >3 SD from mean accuracy), or exclusion criteria. During subsequent quality assessment of the MRI data, 1 subject was further excluded from the fMRI analyses owing to poor normalization quality (for details see CONSORT flowchart, Figure S1). We used the compromise option in G*power (version 3.1.9.4; https://www.softpedia.com/get/Science-CAD/G-Power.shtml) to estimate power using the number of subjects in the main analyses of treatments (two-way analysis of variance [ANOVA]: amino acid mixture × intranasal spray) with an expected medium effect size (η_p^2 = 0.06), which revealed 87% power.

Study protocols were approved by the ethics committee (University of Electronic Science and Technology of China) and adhered to the latest revision of the Declaration of Helsinki. Written informed consent was obtained, and the study was preregistered on clinicaltrials.gov (https://clinicaltrials.gov/ct2/show/NCT03426176, NCT03426176).

Procedure

The present study employed a between-subject, randomized, double-blind pharmacological fMRI design incorporating 4 treatment groups that received combinations of ATD^+ (ATD^+ vs. ATD^- drink) and intranasal OXT (OXT vs. placebo nasal spray). Participants were instructed to abstain from alcohol and caffeine for 24 hours and from food and drinks (except water) for 12 hours before the experiment. To adhere to the pharmacodynamic profile of the treatments, participants arrived between 7:30 AM and 10:00 AM and underwent fMRI acquisition between 1:30 PM and 4:00 PM. Upon arrival, participants received a standardized protein-poor food for breakfast. After the assessment of pretreatment control variables, participants were administered either a tryptophan-depleted amino acid mixture to induce ATD^+ or a control drink, which was balanced in tryptophan and thus did not induce acute tryptophan depletion (ATD^-), followed by a 5-hour resting period 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 assessed, and 5 hours after the amino acid drink, participants self-administered either OXT (24 IU) or PLC nasal spray (standardized according to previous OXT administration studies) (25). In line with the pharmacokinetic profile of intranasal OXT (23), the fMRI paradigm was scheduled 50 minutes after OXT administration. Control variables were assessed before and after fMRI acquisition (for schematic outline of the experimental protocols, see Figure 1).

Control Variables

To control for between-group differences in depressive symptom load, anxiety, and current stress, the Beck Depression Inventory (26), State-Trait Anxiety Inventory (27), and Perceived Stress Scale (28) were administered before treatment. To assess the effects of treatment on mood during the entire experimental procedure, the Positive and Negative Affect Schedule (29) was repeatedly administered before administration of the amino acid drink (T1) and the nasal spray (T2) as well as immediately before MRI acquisition (T3) and at the end of the experiment (T4).

Serotonin Dietary Manipulation (Oral Administration)

A previously validated dietary drink inducing ATD^+ and a control protocol (ATD^-) (30,31) was used to temporarily lower central 5-HT level (ATD^+ group) in a randomized, double-blind, placebo-controlled, between-subject design (Supplement).

OXT (Intranasal Administration)

OXT nasal spray comprised OXT, glycerin, sodium chloride, and purified water, whereas the PLC nasal spray included identical ingredients except for OXT (both provided in identical

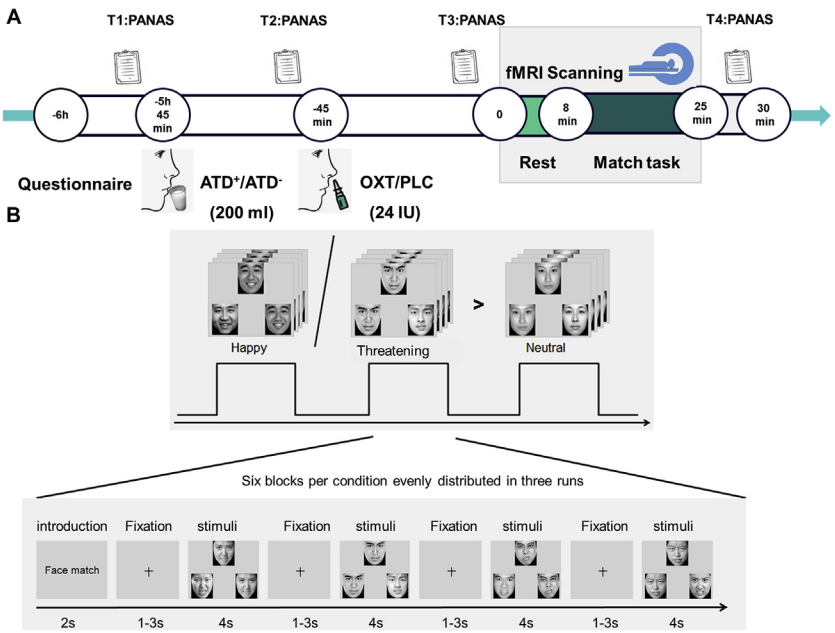

Figure 1. Experimental design and treatment protocols. ATD$^+$, acute tryptophan depletion; ATD$^-$, no acute tryptophan depletion (control treatment for ATD); fMRI, functional magnetic resonance imaging; OXT, oxytocin; PANAS, Positive and Negative Affect Schedule; PLC, placebo (for oxytocin); T, time.

spray bottles by Sichuan Meike Pharmaceutical Co Ltd). In line with previous intranasal OXT administration studies (32), a single dose of 24 international units was administered with 3 puffs per nostril.

Experimental Paradigm

The blocked-design fMRI paradigm has been previously validated and demonstrated to produce robust amygdala responses in response to threatening (angry) faces (21,33). The paradigm consisted of 3 runs, and every run comprised 6 blocks of facial stimuli as well as 2 blocks of nonfacial stimuli serving as nonsocial control stimuli. During the face-processing blocks, a trio of condition-specific (neutral, angry, or happy expressions) facial stimuli was presented, and subjects were required to select one of the two faces (bottom) that was identical to a target face (top). Each block comprised 4 condition-specific trials, balanced for gender. Asian facial stimuli were selected from a standardized Asian facial expression database (34). During the nonsocial control blocks, a trio of simple geometric shapes (circles and ellipses) was presented, and subjects were required to select one of the two shapes (bottom) that matched the target shape presented on the top (Figure 1B). Each control block comprised 4 different shape trios. All blocks were preceded by a brief instruction (face match or shapes match) that lasted 2 seconds. Within each block, each trial was presented for 4 seconds with a variable interstimulus interval of 1–3 seconds (mean, 2 s). The total block length was 24 seconds, and the total paradigm lasted 16 minutes 48 seconds.

MRI Data Acquisition and Processing

MRI data was acquired on a 3T MRI system and preprocessed using routines in SPM 12 (Wellcome Department of Cognitive Neurology, Institute of Neurology, London, United Kingdom; see the Supplement). On the first level, a general linear model was employed and included condition-specific regressors modeling the experimental conditions, the cue-phase, and the 6 head motion parameters. To examine (de)sensitization effects, a separate first-level model was designed, which additionally modeled the blocks separately. The corresponding design matrices were convolved with the default SPM hemodynamic response function. The design matrices additionally included a high-pass filter to control for low-frequency components and a first-order autoregressive model to account for autocorrelation in the time series. To evaluate our a priori hypotheses, analyses focused on threat-specific brain activity using (angry > neutral faces) as the primary contrast of interest.

Statistical Analysis

Effects on Mean Amygdala Threat Reactivity. Effects on threat-related amygdala reactivity were examined using a standard general linear model approach employing the mean contrast of all angry facial expression blocks relative to neutral faces ($\text{angry}_{\text{all-block}} > \text{neutral}_{\text{all-block}}$). On the second level, effects of treatment were examined by means of mixed ANOVA models including treatments (amino acid mixture, ATD$^+$/ATD$^-$, and intranasal spray OXT/PLC) as between-subject factors.

Effects on Amygdala Threat Sensitization/Desensitization. Effects on amygdala threat sensitization/desensitization were analyzed using the mean of a block difference model including the first and last block (last block minus first block), which is more sensitive than the means of the regression approach with respect to complex nonlinear dependencies during habituation (35). To this end, amplitude

differences between the first block in the first run and the corresponding last block in the last run were calculated. To separate threat-specific amygdala habituation from nonspecific habituation to facial stimuli [e.g., (36)], the primary outcome employed a subtraction of the neutral facial stimuli ([$angry_{last\text{-}block}$ − $neutral_{last\text{-}block}$] > [$angry_{first\text{-}block}$ − $neutral_{first\text{-}block}$]). These contrast images were subjected to second-level mixed ANOVA models including amino acid mixture (ATD^+ vs. ATD^-) and intranasal spray (OXT vs. PLC) as between-subject factors.

A Priori Region of Interest and Statistical Thresholding. In line with our regional a priori hypotheses, previous evidence that the anxiolytic effects of OXT in rodents and humans are mediated by the amygdala, and a central role of the amygdala in OXT-induced 5-HT release in humans (9), analyses focused on the amygdala as a priori region of interest. To this end, a bilateral mask for the entire left and right amygdalae were defined using the Automatic Anatomical Labelling template (37) and employed for familywise error (FWE) correction using a small-volume correction ($p_{FWE} < .05$). An additional exploratory whole-brain analysis was computed to explore treatment interaction effects in regions outside of the a priori defined region of interest using a whole-brain threshold of $p_{FWE} < .05$. For post hoc comparisons, individual parameter estimates were extracted from the amygdala mask. To evaluate our hypotheses, post hoc comparisons focused on comparing the treatment groups with the respective PLC-treated reference groups.

Robustness of Threat-Specific Amygdala Sensitization/Desensitization. To further determine the robustness of threat-specific amygdala sensitization/desensitization, we included data from an independent sample of 25 healthy males who underwent a control protocol (ATD^-) administration before fMRI acquisition with a similar block design paradigm (for details see the Supplement). In line with the analysis in the original sample, the second-level analysis focused on the sensitization/desensitization contrast ([$angry_{last\text{-}block}$ − $neutral_{last\text{-}block}$] > [$angry_{first\text{-}block}$ − $neutral_{first\text{-}block}$]).

RESULTS

Sample Characteristics, Confounders, and Mood

There were no pretreatment group differences in age, depressive symptoms, anxiety, current stress levels, and mood (all $p > .16$; for details see Table 1). Examining effects of treatment on mood during the course of the experiment by means of mixed ANOVA models with amino acid mixture (ATD^+ vs. ATD^-) and intranasal spray (OXT vs. PLC) as between-subject factors and time point (T1–T4: pre-oral administration, pre-intranasal administration, pre-fMRI, post-fMRI) as within-subject factor revealed a significant main effect of time on both positive ($F_{3,306} = 20.03$, $p < .001$, $\eta^2_p = 0.164$) and negative ($F_{3,306} = 14.73$, $p < .001$, $\eta^2_p = 0.126$) affect, suggesting a general decrease of mood over the experiment. Moreover, a significant interaction effect of ATD and OXT on negative affect ($F_{1,102} = 7.99$, $p = .006$, $\eta^2_p = 0.073$) was observed, with post hoc analyses suggesting that when the participants received OXT treatment after ATD^+, they reported higher negative affect as compared with the ATD^- condition ($p < .01$), while when participants received PLC treatment after ATD^+, they reported no significant difference as compared with the ATD^- condition ($p = .33$). This suggests that in line with some previous studies using the same acute tryptophan depletion protocols (31), ATD^+ treatment alone did not affect mood in healthy individuals.

Behavioral Results

Examining accuracy and response times by means of mixed ANOVAs with condition (angry face vs. happy face vs. neutral face vs. geometric shape) as within-subject factor and amino acid mixture (ATD^+ vs. ATD^-) and intranasal spray (OXT vs. PLC) as between-subject factors revealed no significant effects of treatment on accuracy and reaction time, arguing against potential confounding effects of treatment on basal attention and vigilance (for details see the Supplement).

Effects on Mean Threat-Related Amygdala Amplitude

We conducted a one-sample t test with the contrast ($angry_{all\text{-}block}$ > $neutral_{all\text{-}block}$) to validate the paradigm and found that threatening stimuli induced increased bilateral amygdala activation (for details see the Supplement and Figure S2). Contrary to our hypothesis, no significant main or interaction effects of ATD and OXT on amygdala threat reactivity were observed (contrast [$angry_{all\text{-}block}$ > $neutral_{all\text{-}block}$]).

Effects on Amygdala Threat Sensitization/Desensitization

Examination of treatment effects on threat-specific amygdala sensitization/desensitization by means of voxelwise ANOVA models including the between-subject factors treatment (amino acid mixture [ATD^+ vs. ATD^-] and intranasal spray [OXT vs. PLC]) revealed a significant time (first block vs. last block) × treatment interaction effect in the bilateral amygdala (left: MNI [−21, −3, −15], $p_{FWE} = .008$, k = 20, $t_{107} = 3.64$; right: MNI [18, −3, −15], $p_{FWE} = .019$, k = 1, $t_{107} = 3.25$; small volume corrected for the bilateral amygdala mask) (Figure 2). Post hoc comparisons on the extracted parameter estimates from the amygdala mask (contrast of interest, $angry_{last\text{-}block}$ − $neutral_{last\text{-}block}$ > $angry_{first\text{-}block}$ − $neutral_{first\text{-}block}$) revealed that the PLC-treated reference group (ATD^--PLC) demonstrated increased amygdala responses, suggesting threat-specific sensitization rather than habituation of the amygdala. Compared with the reference group, OXT (ATD^--OXT) switched amygdala sensitization to desensitization as reflected by significantly decreased threat-specific amygdala responses (false discovery rate-corrected p (p_{FDR}) < .001; Cohen's d = 0.99), while decreased serotonin signaling by ATD^+ pretreatment before OXT administration significantly attenuated this effect of OXT (ATD^--OXT vs. ATD^+-OXT, $p_{FDR} = .038$; Cohen's d = 0.52). In addition, there was no significant difference between ATD^+-PLC group and ATD^--PLC group ($t_{53} = 1.05$, p = .30). An additional post hoc analysis on the condition-specific parameter estimates ($neutral_{last\text{-}block}$ > $neutral_{first\text{-}block}$ and $angry_{last\text{-}block}$ > $angry_{first\text{-}block}$, respectively) employing a

Table 1. Sample Characteristics (n = 111)

Characteristics	ATD^+-OXT (n = 28)	ATD^+-PLC (n = 26)	ATD^--OXT (n = 28)	ATD^--PLC (n = 29)	p Value
Age, Years	21.89 ± 2.39	21.73 ± 2.66	22.24 ± 2.29	22.10 ± 2.13	.86
STAI-Trait	41.79 ± 8.00	41.00 ± 5.85	38.72 ± 7.97	40.83 ± 7.00	.43
STAI-State	38.77 ± 8.81	35.16 ± 6.36	34.71 ± 8.50	35.43 ± 6.64	.21
BDI	6.07 ± 6.44	8.08 ± 6.05	4.90 ± 6.40	5.21 ± 4.97	.21
PSS	14.50 ± 5.45	15.42 ± 5.25	13.59 ± 4.69	15.41 ± 4.58	.46
PANAS-P (T1)	24.19 ± 7.17	24.08 ± 8.38	24.46 ± 6.95	26.04 ± 8.07	.76
PANAS-N (T1)	15.88 ± 9.18	13.04 ± 6.09	11.75 ± 5.02	13.43 ± 5.95	.16

Values are presented as mean ± SD.

ATD^+, acute tryptophan depletion; ATD^-, no acute tryptophan depletion (control treatment for ATD); BDI, Beck Depression Inventory; OXT, oxytocin; PANAS-N, Positive and Negative Affect Schedule–Negative affect; PANAS-P, Positive and Negative Affect Schedule–Positive affect; PLC, placebo (for oxytocin); PSS, Perceived Stress Scale; STAI-TAI, State-Trait Anxiety Inventory; T1, time point 1 (pretreatment assessment).

two-way ANOVA with treatments as between-subject factors aimed at further exploring whether the observed treatment effects were specifically driven by the angry face condition. A lack of significant effects on neutral faces in the context of a significant difference between the ATD^--PLC group and ATD^--OXT (p_{FDR} = .003; Cohen's d = 0.83) and between the ATD^--OXT and ATD^+-OXT (p_{FDR} = .037; Cohen's d = 0.55) for the angry condition ($angry_{last\text{-}block} > angry_{first\text{-}block}$) (Figure 3) further confirmed effects on threat-specific sensitization/desensitization.

Additional control analysis examining effects of treatment on sensitization/desensitization to positive stimuli ($happy_{last\text{-}block} - neutral_{last\text{-}block} > happy_{first\text{-}block} - neutral_{first\text{-}block}$) and nonsocial stimuli ($shapes_{last\text{-}block} > shapes_{first\text{-}block}$) did not reveal significant differences, further suggesting threat-specific effects.

Exploratory Whole-Brain Analysis

In line with our hypothesis, the primary analysis focused on the amygdala. In addition, an unrestricted exploratory whole-brain analysis was conducted on the voxel level. Results from this analysis revealed a significant time × treatment effect in cortical midline regions, insula, and the bilateral superior temporal gyrus (for details see the Supplement and Figure S3).

Robustness and Replicability of Threat-Specific Amygdala Desensitization

Data from an independent validation sample of 25 healthy male subjects was employed to determine the robustness of the threat-specific amygdala sensitization observed in the control group (ATD^--PLC). A voxelwise second-level analysis of the sensitization/desensitization contrast ($angry_{last\text{-}block} - neutral_{last\text{-}block} > angry_{first\text{-}block} - neutral_{first\text{-}block}$) confirmed threat-specific bilateral amygdala sensitization ($p_{FWE} < .001$) in the validation sample (see the Supplement and Figure S4).

DISCUSSION

Overarching conceptualization suggests a modulatory influence of OXT on 5-HT signaling, and animal models demonstrated a functional relevance of this interaction for the anxiolytic potential of OXT. Building on these previous findings, the present pharmacological fMRI study employed an experimental protocol to reduce central 5-HT signaling before the administration of intranasal OXT to determine the role of 5-HT in mediating OXT-induced attenuation of amygdala threat reactivity. In contrast to our hypothesis, no effects on the mean amplitude of amygdala threat reactivity were observed; however, further analyses on repetition-dependent threat-related amygdala reactivity revealed a sensitization of

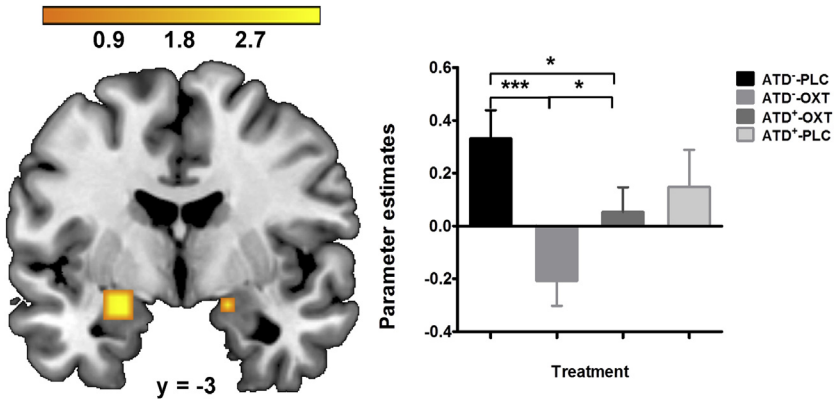

Figure 2. Effect of treatment on threat-specific amygdala sensitization/desensitization. The threat-specific effect in the bilateral amygdala is displayed at $p_{FWE\text{-}SVC} < .05$ thresholded for the entire bilateral amygdala. The color bar codes the t value. Bars on the right correspond to the extracted estimates for threat-specific sensitization/desensitization ($[angry_{last\text{-}block} - neutral_{last\text{-}block}] > [angry_{first\text{-}block} - neutral_{first\text{-}block}]$) for each treatment group. Results indicate that after placebo treatment (ATD^--PLC), the bilateral amygdala exhibited threat sensitization, which was switched to desensitization after administration of oxytocin (ATD^--OXT), and that this effect of oxytocin was significantly attenuated yet not fully abolished after pretreatment with acute tryptophan depletion (ATD^+-OXT). *Significant post hoc differences at $p_{FDR} < .05$; ***significant post hoc differences at $p_{FDR} < .001$. ATD^+, acute tryptophan depletion; ATD^-, no acute tryptophan depletion (control treatment for ATD); FDR, false discovery rate; FWE, familywise error; OXT, oxytocin; PLC, placebo (for oxytocin); SVC, small volume correction.

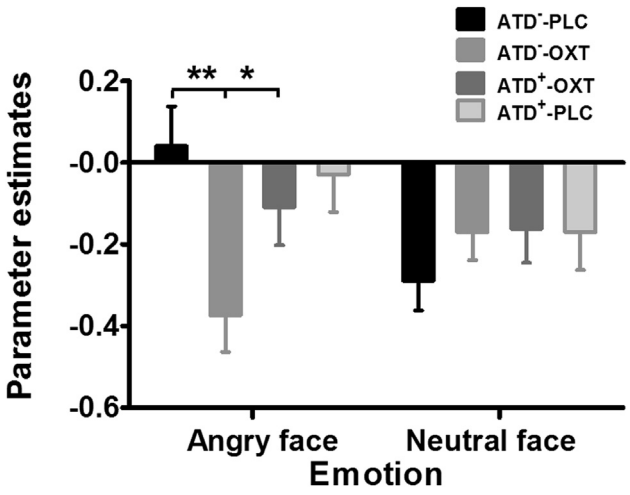

Figure 3. Condition-specific amygdala sensitization/desensitization. Condition-specific parameter estimates for amygdala sensitization/desensitization for angry and neutral faces. Bars correspond to the extracted estimates for the identified amygdala region and suggest threat-specific differences between the treatment groups. *Significant post hoc differences at $p_{FDR} < .01$; **significant post hoc differences at $p_{FDR} < .05$. ATD^+, acute tryptophan depletion; ATD^-, no acute tryptophan depletion (control treatment for ATD); FDR, false discovery rate; OXT, oxytocin; PLC, placebo (for oxytocin).

the bilateral amygdala with repeated presentation of threatening faces after control treatment (ATD^--PLC), which was switched to desensitization after OXT administration (ATD^--OXT), and showed that this effect of OXT was attenuated after decreased central serotonin signaling via pretreatment with ATD^+ (ATD^+-OXT). Together, these findings provide the first evidence that OXT facilitates amygdala threat desensitization and that this effect is (partly) mediated via a 5-HT–dependent mechanism.

In contrast to our hypothesis, no effects of OXT on the mean amplitude of amygdala threat reactivity were observed, which might be related to the specific threat stimuli chosen. We chose angry faces as direct threat stimuli, and while previous studies demonstrated convergent evidence for a serotonergic modulation of amygdala reactivity to angry as well as fearful faces (38), the effects of intranasal OXT on amygdala threat processing appear to depend strongly on the specific emotion of the faces displayed. Whereas previous intranasal OXT studies reported enhanced recognition of fearful faces and attenuated amygdala reactivity toward fearful faces, OXT did not consistently modulate recognition of or amygdala reactivity toward angry facial expressions (39–41). The differences may be explained in the different motivational tendencies inherent to the facial expressions, such that the dominant response to angry expressions is threat avoidance, whereas the dominant response to fearful expressions is approach (42). Likewise, the observation that OXT reduces amygdala habituation to unreciprocated cooperation in men (19) indicates that the effects of OXT on habituation may be domain specific.

The amygdala exhibits rapid adaptations to repeated presentation of salient stimuli, including facial expressions (10), and these changes might be a more reliable marker of amygdala function as assessed by fMRI activation (16,21). Desensitization (habituation) of amygdala responses has been most consistently reported, but amygdala sensitization may also occur with repeated presentation of particularly salient or aversive stimuli (14). The currently prevailing dual-process framework proposes that the incremental (sensitization) and decremental (habituation) adaptations on the physiological and affective level are based on independent yet interacting processes (43). Sensitization has most consistently been observed in response to repeated presentations of reward- and threat-related stimuli (43), and sustained attention and less habituation to threat, including angry facial stimuli, have been demonstrated on the behavioral level (15,44). Consistently, increased neural responses with repeated presentation of angry emotional stimuli have been reported in limbic regions (15), including the amygdala (14). Partly resembling these previous observations, we found threat-specific amygdala sensitization in the control group (ATD^--PLC), while OXT switched the direction of the repetition-dependent adaptation, leading to a threat-specific habituation in this region.

The amygdala is particularly sensitive to social information (45) and exhibits widespread functional interactions with limbic and prefrontal systems [e.g., (46)]. Previous studies in animals have pinpointed anxiolytic effects of OXT to the amygdala (20,47), and in humans, intranasal OXT enhanced communication between the amygdala and prefrontal regulatory regions (48). By modulating anger-related habituation of amygdala reactivity, OXT may facilitate rapid and flexible adaptation to social threat signals (3,48).

In line with a previous rodent model demonstrating that the anxiolytic effects of OXT are critically mediated by the 5-HT system (7), we found that ATD^+-induced reduction in serotonergic signaling attenuated, but did not fully abolish, the effects of OXT on amygdala threat reactivity. Animal models suggest that the anxiolytic action of OXT is mediated via hypothalamic-amygdala projection neurons (20) as well as OXT-sensitive receptors expressed on serotonergic raphe neurons (7). Whereas initial data suggest that ATD^+ may reduce peripheral OXT levels in the absence of behavioral effects (49), the combination of ATD^+ with PLC nasal spray did not produce effects on amygdala reactivity in the present study, arguing against an ATD^+-induced nonspecific decrease in OXT signaling.

In contrast, molecular imaging studies have demonstrated that intranasal OXT induces central serotonin release (6,9), and ATD^+ leads to stable and selective reductions in central 5-HT signaling (50), including attenuation of stimulated serotonin release (50,51) and availability of serotonin in presynaptic neurons (52). This suggests that pretreatment with ATD^+ diminished OXT-induced serotonin release via OXT-sensitive receptors on serotonergic raphe neurons, which in turn attenuated anxiolytic effects mediated by serotonergic raphe-amygdala pathways.

Given the increasing interest in the clinical application of OXT to attenuate anxiety and exaggerated amygdala responses (2,39), the present results have important clinical implications. First, deficient amygdala threat desensitization has been reported in several psychiatric disorders, including anxiety disorders and autism, and may represent a core

pathological mechanism for the development and maintenance of exaggerated anxious arousal (17,53), with the present results indicating that OXT may facilitate amygdala threat habituation. Second, serotonin dysfunction is a core biomarker of anxiety and autism spectrum disorder (54,55). We found that the strength of the effect of OXT on amygdala threat desensitization was mediated by endogenous 5-HT levels, suggesting that individuals with low endogenous serotonergic levels may not fully capitalize on the anxiolytic effects of OXT, and thus combined upregulation of 5-HT and OXT transmission may be needed for optimal facilitation of anxiolytic effects.

Findings of the present study need to be interpreted in the context of the following limitations. First, we employed a between-subject design, which may add heterogeneity to the sample yet control for repeated assessment effects. Future studies should employ crossover designs to validate the robustness of the findings. Second, only male subjects were investigated because of sex differences in 5-HT synthesis rate (56) and the effects of OXT on amygdala reactivity (19,22), and future studies need to determine whether the observed effects generalize to women. Third, tryptophan levels were not assessed in the present study; however, the study adhered to previously validated ATD^{+} protocols, which have been shown to induce robust and selective decreases in 5-HT signaling (30,57). Fourth, blood levels of OXT were not assessed to validate the increase in (peripheral) OXT levels; however, previous studies using identical intranasal administration protocols reported increased blood levels of OXT (58,59). Nevertheless, the examination of blood-level measures, particularly in the combined treatment group, may have revealed important additional information on the interaction of the two systems. Finally, the present study did not include fearful facial expression stimuli. Based on previous studies reporting that intranasal OXT may have pronounced effects on the recognition of and amygdala reactivity toward fearful faces [(39–41; however, see (60–64)] and that neural responses to angry and fearful facial expressions are separable, future studies should include fearful and angry faces to directly compare effects on the divergent approach and avoidance motivation induced by these facial expression (42).

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