Psychoneuroendocrinology 79 (2017) 160–166

Contents lists available at ScienceDirect

Psychoneuroendocrinology

journal homepage: www.elsevier.com/locate/psyneuen

Oxytocin reduces amygdala responses during threat approach

Sina Radke[a,d,*], Inge Volman[a,b,e], Idil Kokal[a], Karin Roelofs[a,b,1], Ellen R.A. de Bruijn[c,1], Ivan Toni[a,1]

[a] Radboud University Nijmegen, Donders Institute for Brain, Cognition and Behaviour, P.O. Box 9101, 6500 HB Nijmegen, The Netherlands
[b] Radboud University Nijmegen, Behavioural Science Institute, P.O. Box 9104, 6500 HE Nijmegen, The Netherlands
[c] Leiden University, Department of Clinical Psychology and Leiden Institute for Brain and Cognition, Wassenaarseweg 52, 2333 AK, Leiden, The Netherlands
[d] RWTH Aachen University, Medical Faculty, Department of Psychiatry, Psychotherapy and Psychosomatics & Jülich Aachen Research Alliance (JARA) – BRAIN Institute Brain Structure-Function Relationships: Decoding the Human Brain at Systemic Levels, Forschungszentrum Jülich GmbH and RWTH Aachen University, Pauwelsstr. 30, 52074 Aachen, Germany
[e] University College London, Sobell Department for Motor Neuroscience and Movement Disorders, UCL Institute of Neurology, Queen Square, London WC1E 6 BT, United Kingdom

ARTICLE INFO

Article history:
Received 10 October 2016
Received in revised form 30 January 2017
Accepted 27 February 2017

Keywords:
Oxytocin
Amygdala
Social approach
Motivation
Emotion
fMRI

ABSTRACT

Oxytocin reduces amygdala responses to threatening social stimuli in males and has been suggested to facilitate approach-related processing by either decreasing anxiety or intensifying salience. The current administration study tested whether oxytocin either reduces or enhances amygdala responses during threat approach in a placebo-controlled randomized, double-blind, between-subjects design with 52 healthy males undergoing fMRI during a social approach-avoidance task. Oxytocin decreased amygdala activation during threat approach and not during threat avoidance. This neural effect supports oxytocin's social anxiolytic effects and provides a neuroendocrine mechanism promoting social approach. The findings may yield clinical implications for individuals suffering from dysregulations of social approach such as patients with anxiety disorders.

1. Introduction

Oxytocin is a neuromodulatory hormone involved in social approach behaviors across a number of species (Heinrichs and Domes, 2008; Insel and Young, 2000). Various mechanisms underlying the effects of oxytocin have been postulated, e.g., a reduction of social anxiety (Bale et al., 2001; Heinrichs and Domes, 2008; Labuschagne et al., 2010), an increased sensitivity to social cues, i.e., increased salience of social information (e.g., Bethlehem et al., 2014; Shamay-Tsoory and Abu-Akel, 2016), an up-regulation of social approach (e.g., Kemp and Guastella, 2010), and a modulation of approach and avoidance motivation in general (Harari-Dahan and Bernstein, 2014). Particularly for human oxytocin research, most functional accounts are based on assessing emotional face perception (for a meta-analysis, see Shahrestani et al., 2013). Processing advantages for positively or negatively valenced faces have been interpreted in favor of either hypothesis. However, it remains unknown how oxytocin influences neural processing of emotional faces that require motivational actions, i.e., approach or avoidance.

Behaviorally, we have recently shown that oxytocin administration leads healthy males to increase their approach tendencies toward faces signaling social threat (Radke et al., 2013). This observation was obtained in the context of a social approach-avoidance task, where participants respond (approach, avoid) to affective faces (angry, happy) by pulling or pushing a joystick, respectively (Radke et al., 2015; Volman et al., 2011a; Volman et al., 2011b; Volman et al., 2013; Volman et al., 2016). In this task, the interpretation of arm flexion (pulling) as approach and arm extension (pushing) as avoidance, respectively, is enforced by explicit instructions, i.e., evaluating the faces based on valence, and a self-related reference frame (e.g., "If you see a happy face, move the joystick TOWARDS yourself")(see Phaf et al., 2014 for further discussion). While participants are usually slower when providing affect-incongruent responses (approach-angry, avoid-happy), males with low levels of social anxiety showed enhanced oxytocin-induced approach tendencies toward social threat (Radke et al., 2013). This aligns well with the anxiolytic account of oxytocin function (Heinrichs and Domes, 2008), and may also fit with facilitated (social) approach-related processing (Harari-Dahan and Bernstein,

* Corresponding author at: Uniklinik RWTH Aachen, Department of Psychiatry Psychotherapy and Psychosomatics, Pauwelsstr. 30, 52074 Aachen, Germany.
E-mail address: sradke@ukaachen.de (S. Radke).
[1] These authors contributed equally to this study.

http://dx.doi.org/10.1016/j.psyneuen.2017.02.028
0306-4530/

2014; Kemp and Guastella, 2010), but the corresponding neurobiological implementation remains to be investigated.

Building on the cerebral distribution of oxytocin receptors observed in rodents (Mitre et al., 2016; Veinante and Freund-Mercier, 1997), the amygdala has been established as a crucial region for oxytocin research, centering around its role in social cognition, face processing and threat detection (Adolphs, 2010; Davis and Whalen, 2001). While the effects of oxytocin likely involve other brain regions and hormone systems, most human studies on the neuromodulatory effects of oxytocin on face processing have focused on the amygdala, showing reduced activation to threatening scenes and faces in males (Domes et al., 2007; Gamer et al., 2010; Kirsch et al., 2005; Petrovic et al., 2008), along with increased activation to happy faces in one report (Gamer et al., 2010).

Here, following a previous behavioral study (Radke et al., 2013), we investigate how oxytocin modulates amygdala activation during threat approach-avoidance in a double-blind, randomized, placebo-controlled oxytocin administration study. Fifty-two healthy males performed a well-established fMRI-compatible social approach-avoidance task 45 min after self-administration of 24 IU of oxytocin (n = 28) or matched placebo (n = 24). A body of neuroimaging research with this task has consistently demonstrated the involvement of the amygdala during motivational actions to emotional faces (Radke et al., 2015; Volman et al., 2013; Volman et al., 2016). Moreover, this task is able to capture subtle between-groups differences in cerebral effects, unconfounded by behavioral differences in emotional processing (Radke et al., 2015; Volman et al., 2013; Volman et al., 2016). The implementation of affect-incongruent behavioral responses is subserved by the anterior prefrontal cortex (aPFC), showing increased activation when approach of angry and avoidance of happy faces is required (Radke et al., 2015; Volman et al., 2011a; Volman et al., 2011b; Volman et al., 2013; Volman et al., 2016).

Focusing on the causal role of oxytocin on amygdala reactivity during threat approach, we distinguish between the following two hypotheses: If anxiolytic effects of oxytocin (Heinrichs and Domes, 2008) underlie our previous behavioral findings of increased approach tendencies towards social threat (Radke et al., 2013), then amygdala activation would be attenuated during the execution of approach behaviors in response to social threat. This oxytocin-driven down-regulation of the amygdala should be confined to threat approach, and not extend to threat avoidance, given the automatic response tendencies to avoid social threat. Alternatively, oxytocin could promote social approach by enhancing the sensitivity to social cues in a context-dependent manner (Prehn et al., 2013; Shamay-Tsoory and Abu-Akel, 2016). If oxytocin increases the salience of threat signals in aggressive contexts (Shamay-Tsoory and Abu-Akel, 2016), one might expect increased neural processing when angry faces with direct gaze have to be approached. Given the role of the amygdala in assigning salience (Davis and Whalen, 2001), this would be evident in increased amygdala activation during threat approach.

Given the modulatory impact of steroid hormones and social anxiety on social motivational behavior and amygdala activation (e.g., Enter et al., 2014, 2016; Radke et al., 2013; Radke et al., 2015; van Peer et al., 2007), we control for endogenous hormone levels (testosterone, cortisol) and social anxiety in our analyses.

2. Methods and materials

2.1. Participants

Fifty-seven male volunteers were recruited through advertisements placed across the university's campus and gave written informed consent in accordance with the study procedures approved by the Medical Ethics Committee of the Radboud University Nijmegen Medical Center (Commissie Mensengebonden Onderzoek Region Arnhem-Nijmegen). Participants were financially compensated.

All participants reported neither current nor a history of neurological or endocrine disease, medication, and drug or alcohol abuse. Exclusion criteria included age of <18 or >35, smoking more than 5 cigarettes per day, participation in another pharmacological study within the last two months, and presence of metal objects in the body. Participants were asked to abstain from caffeine, alcohol and nicotine for 24 h as well as from eating and drinking (except water) 2 h prior to substance administration.

Five participants (n = 4 oxytocin, n = 1 placebo) were excluded due to technical problems or poor task compliance, i.e., less than 50% of usable trials for analyses, resulting in 52 participants (*M* age = 22.4, *SD* = 3) for the final analyses.

2.2. Pharmacological procedure

A randomized, placebo-controlled, double-blind between-subjects design was used in this study. Participants self-administered either oxytocin (Syntocinon; Novartis, Basel, Switzerland; n = 24) or a saline solution (i.e., placebo n = 28) via a nasal spray with three puffs per nostril (each with 4 IU, i.e., a dose of 24 IU). All sprays were manufactured according to the guidelines on Good Manufacturing Practice (GMP) and Good Clinical Practice (GCP). All sessions were scheduled for the afternoon on weekdays and consisted of two testing blocks, each preceded by substance administration and a waiting period. In order to preclude any experimenter's biases due to potential differences between the sprays, the experimenter was not present during substance administration. An independent assistant who was blind to the experimental hypotheses supervised substance administration. Participants were not able to identify the substance they had received (chi-square $\chi^2(2) = 416$, $P = 0.812$). In the first block, the effect of oxytocin on semantic integration was investigated using magnetoencephalography (Ye et al., 2016). The MR block always took place after the second substance administration, which immediately followed completion of the first block. In more details, after a waiting period of 30 min, participants were positioned in the MR scanner and completed a short training session (5 min) before the experiment began (45 min after substance administration).

2.3. Endocrine and psychometric control measurements

Three saliva samples (prior to any substance administration, 15 min after first substance administration and 100 min after second substance administration) were obtained with salicaps (Immuno-Biological Laboratories GmbH, Hamburg, Germany) and stored at −25 °C. Samples were analyzed in duplicate for testosterone and cortisol and the average was used in subsequent analyses. Hormone concentrations were measured using Luminescence Immunoassays (Immuno-Biological Laboratories GmbH, Hamburg, Germany). For the cortisol assay, the intra-assay and inter-assay coefficients were less than 4% and for the testosterone analyses, these were 0–2% and 5–9%, respectively.

2.4. Liebowitz Social Anxiety Scale

The Liebowitz Social Anxiety Scale (LSAS; Liebowitz, 1987) assesses fear and avoidance in 24 social situations via self-report. After the MR scans, participants rated on a 4-point scale how anxious they would feel during a particular social situation (anxiety) and how often they would avoid this situation (avoidance).

2.5. Approach-avoidance (AA) task

During this fMRI-adapted reaction time task (e.g., Volman et al., 2011b; Volman et al., 2013), participants had to respond to visually presented emotional facial expressions by pulling a joystick either towards their body (approach movement) or pushing it away from their body (avoidance movement). As stimuli, happy and angry facial expressions (of 18 female and 18 male models) from several databases were used (e.g., Ekman and Friesen, 1976; Lundqvist et al., 1998). Pictures were trimmed to exclude influences from hair and non-facial contours and matched for brightness and contrast values.

In 16 blocks with 12 trials each, participants had to categorize the affective expression. In other words, they had to respond with an approach movement (pull joystick) to one emotion and with avoidance (push joystick) to the other. Written instructions on the stimulus-response mapping were presented at the beginning of each block (e.g., "If you see a happy face, move the joystick TOWARDS yourself as fast as possible. If you see an angry face, move the joystick AWAY from yourself as fast as possible"). This operationalization is in line with a recent meta-analysis (Phaf et al., 2014) demonstrating strongest effects when requesting explicit evaluation of the affective value of stimuli (happy/angry). The combination of the two factors Emotion and Movement entailed two different stimulus-response mappings, one congruent (approach-happy, avoid-angry) and one incongruent (approach-angry, avoid-happy). The mapping changed after each block, with the order being counterbalanced across participants. Blocks were separated by an inter-block-interval of 21–24 s.

Each trial started with a blank screen (300 ms) followed by a fixation cross (100 ms) and a black screen (300 ms), after which the stimulus was presented in grayscale against a black background (100 ms). During the response period, another blank screen was presented. Following previous studies using the AA task during fMRI (Volman et al., 2011a; Volman et al., 2011b; Volman et al., 2013), images were presented without a 'zooming' feature to avoid neural confounds related to an image moving towards or away from the participants as well as mere exposure effects. Valid responses were defined as joystick displacements of at least 80% along the sagittal plane within 2 s after stimulus presentation. Subsequent to their response, participants had to move the joystick back to the starting position (the central area of 20% on the sagittal plane) before the end of the inter-trial interval (blank screen; 2–4 s). Written feedback was given only for invalid responses ("you did not move the joystick far enough"; "please return the joystick to the starting position"); in this case, the inter-trial interval was repeated after the joystick reached the starting position. Participants completed a training session of 4 blocks (each 8 trials) inside the MR scanner, for which different stimuli were used.

Stimuli were projected (visual angle $4^\circ \times 6^\circ$) at the center of a screen that was viewed via a mirror above participants' head. An MR-compatible joystick (Fiber Optic Joystick, Current Designs; sampling rate 550 Hz) was placed on the abdomen of the participants. Stimulus presentation and response acquisition were run by Presentation software version 16.

2.6. Behavioral analyses

The time from stimulus presentation until movement onset reflects the time of movement initiation (MI). The reaction time (RT) and movement time (MT) was defined as the time from stimulus presentation and movement onset, respectively until attainment of the target position of the joystick.

Trials with missed (3.0%), incorrect (7.5%) and extreme responses (3.1%; MTs <400 ms; MI <100 or >1500 ms and >3 SDs of the subject-specific mean) were excluded. Blocks in which the error rate exceeded chance level were regarded as a misapprehension of the instructions and therefore also excluded (1.9%; cf. Volman et al., 2011a; Volman et al., 2011b; Volman et al., 2013). After log-transformation to correct for a skewed distribution (cf. Volman et al., 2011a; Von Borries et al., 2012), mean RTs were calculated for each level of the two experimental factors (Emotion, Movement) and subjected to a repeated measures (rm) ANCOVA, with the within-subject factors Emotion (happy, angry) and Movement (approach, avoid) and the between-subject factor Substance (oxytocin, placebo). The standardized testosterone and cortisol levels from the first saliva measurement were included as covariates as previously done (cf. Volman et al., 2011b; Volman et al., 2013). Moreover, following earlier research (Radke et al., 2013), the LSAS total score was included as a covariate as well.

In addition, the amount of trials showing either no response or a joystick movement in the wrong direction was summed per level of the two experimental factors per participant. These error rates were subjected to a rm ANCOVA (Substance x Emotion x Movement) with standardized baseline cortisol, testosterone and LSAS as covariates.

An independent *t*–test was used to compare the LSAS total score. Two rm ANCOVAs with the within-subject factor Time (three time points) and the between-subject factor Substance (oxytocin, placebo) were performed for cortisol and testosterone, respectively, with standardized baseline levels of the other hormone and LSAS as covariates. The α-level was set at $P < 0.05$. For the ANCOVAs, within-subject effects with Greenhouse Geisser correction are reported with partial eta squared as an indication of effect size. Statistical testing was performed with the Statistical Package for the Social Sciences (IBM SPSS 20).

2.7. Image acquisition

Functional images were acquired on a 1.5 T MRI scanner (Avanto, Siemens Medical Systems, Erlangen, Germany) equipped with a 32-channel head coil using a multi-echo GRAPPA sequence (Poser et al., 2006) (repetition time [TR]: 2.14s, echo times [TEs, 5]: 9.4/21/33/44/56 ms, 34 transversal slices, ascending acquisition, distance factor: 17%, voxel size $3.3 \times 3.3 \times 3.0$ mm, field of view [FoV]: 212 mm, flip angle 90°). Subsequently, an anatomical scan (TR: 2730 ms, TE: 2.95 ms, 176 sagittal slices, voxel size $1.0 \times 1.0 \times 1.0$ mm, FoV: 256 mm, flip angle 7°) was performed.

2.8. Imaging data

Preprocessing and analyses of the imaging data were performed with statistical parametric mapping (SPM8, Wellcome Department of Imaging Neuroscience, London). For each data set, the first four volumes were excluded to allow for magnetic saturation. Head motion parameters were derived from the MR images of the first echo (TE 9.4s; Volman et al., 2011b) and estimated by using a least-squares approach with 6 rigid body transformation parameters (translations, rotations). They were then applied to all five echo images. Subsequently, the five echo images were combined into single volumes based on an optimized echo weighting method (Poser et al., 2006). After slice-time correction, the anatomical scan was coregistered with the mean of the functional images, and images were normalized into Montreal Neurological Institute (MNI) space on the basis of a segmentation algorithm (Ashburner and Friston, 2005). Images were resampled to 2 mm^3 and smoothed with an 8 mm^3 full-width-at-half-maximum Gaussian kernel.

First-level analyses were based on an event-related approach in the general linear model with four task-relevant regressors: approach-happy, avoid-happy, approach-angry, avoid-angry. Furthermore, trials without responses were modeled as misses, and presentation of task instructions were modeled as info, resulting in two additional regressors of no interest to the experimental ques-

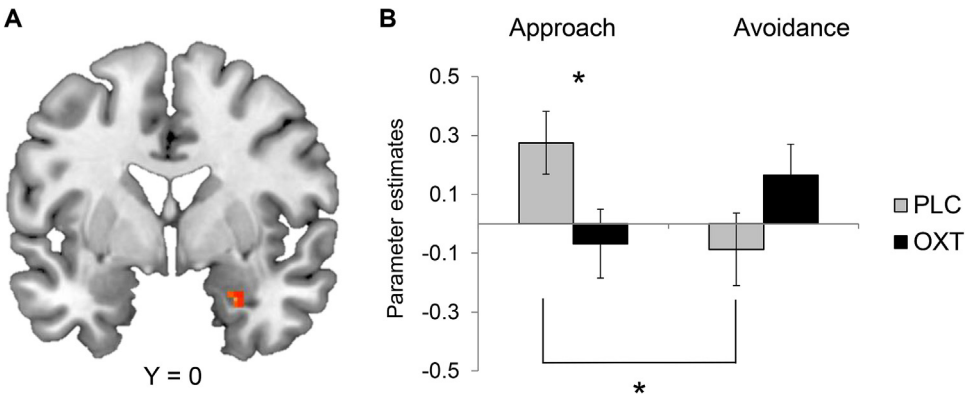

Fig. 1. Oxytocinergic modulation of amygdala responses during threat approach-avoidance. (A) Amygdala region showing a significant Substance × Emotion × Movement interaction, i.e., reduced amygdala activation during threat approach after oxytocin administration, compared to placebo. Coronal slice from a representative template brain with SPM T map overlaid at $P < 0.05$ uncorrected for visualization purposes. (B) Contrast estimates for the amygdala cluster during approach and avoidance of angry faces in the oxytocin (OXT) and placebo (PLC) groups. Error bars represent SEM. * $P < 0.05$.

tion. Regressors were constructed for the times of stimulus onset with RT as duration and convolved with a canonical hemodynamic response function to model brain activity. To minimize residual head movement effects, 57 additional regressors were derived from incorporating the original, squared, cubic, first-order, and second-order derivatives of the movement parameters (Lund et al., 2005) as well as signal intensities of white matter, cerebrospinal fluid, and the portion of the MR image outside the skull. Lastly, a high pass filter (128s) and an autoregressive AR(1) model were applied to the images to account for serial correlations in the functional series.

2.9. Multiple regression analyses

On the group level, a random effects multiple regression analysis was performed based on participants' task-relevant effects (i.e., four contrast images: approach-happy, avoid-happy, approach-angry, avoid-angry). This entailed 8 conditions based on the combination of three factors Substance (between-subject factor, with two levels: oxytocin, placebo), Emotion (within-subject factor, with two levels: angry, happy), and Movement (within-subject factor, with two levels: approach, avoid). Consistent with previous reports (cf. e.g., Volman et al., 2011b), we also considered how those effects were modulated by standardized endogenous testosterone and cortisol levels (obtained from the first saliva sample) as well as the standardized total score on the LSAS, resulting in another 24 regressors. The main experimental question of this study was formalized as a three-way interaction between the factors Substance, Emotion, and Movement. This question was focused on the amygdala (bilaterally) as derived from the aal atlas (Tzourio-Mazoyer et al., 2002) using the Wake Forest University (WFU) PickAtlas tool (Maldjian et al., 2003), as in Volman et al. (2013).We additionally tested for two-way interactions between the factors Substance and Movement, and Substance and Emotion, respectively. The reported activations are corrected for multiple comparisons using family-wise error (FWE) correction ($P_{FWE} < 0.05$) (Friston, 1997). Inferences were made at the voxel-level, applying a small volume correction. Parameter estimates for each factor/level were extracted for follow-up analyses in SPSS with α set at $P < 0.05$.

In addition, this study considered a second issue, namely whether the anterior prefrontal cortex (aPFC) is involved in task performance, as previously reported (Radke et al., 2015; Volman et al., 2011a; Volman et al., 2011b; Volman et al., 2013; Volman et al., 2016). Accordingly, activation evoked during affect-incongruent trials (approach-angry, avoid-happy) was compared to activation evoked during affect-congruent trials (approach-happy, avoid-angry) across both oxytocin and placebo groups. Those effects were compared within two spheres (8 mm radius) centered on 32 54 8 and −30 58 2, respectively, following prior findings using this task (Radke et al., 2015; Volman et al., 2011a; Volman et al., 2011b; Volman et al., 2013; Volman et al., 2016). Inferences were made at the voxel-level, based on small volume FWE corrections ($P_{FWE} < 0.05$) (Friston, 1997), whole brain effects are presented in Supplementary Table S1.

2.10. Effective connectivity analyses

The psychophysiological interactions (PPIs) method (Friston et al., 1997) was used to test for substance-induced differences in the coupling of the right aPFC and the amygdala during affect-incongruent versus affect-congruent trials. As a seed region, voxel selection for the volume of interest (VOI) was based on a sphere of 8 mm radius around the peak voxel corresponding to the activated aPFC cluster of the cerebral congruency effect (incongruent > congruent; coordinates 26 58 8; see Results). Participant-specific contrast images describing the PPI between the time course of the aPFC VOI and the time course of incongruent vs. congruent trials were subjected to a multiple regression analysis as described above for activation. We tested for substance-induced differences both across the whole brain ($P_{FWE} < 0.05$) and within the amygdala as defined with the WFU PickAtlas tool (Maldjian et al., 2003), using small volume family-wise error correction ($P_{FWE} < 0.05$) (Friston, 1997).

3. Results

3.1. Amygdala

Oxytocin modulated task-related activity associated with emotional control in the right amygdala (Substance [placebo > oxytocin] x Emotion [angry > happy] × Movement [approach > avoid] interaction [coordinates: 32 −8 −12; z-value = 3.65; $P_{FWE} = 0.02$]; see Fig. 1). This effect was due to an oxytocinergic modulation during approach-avoidance of angry faces ($F_{1,50} = 13.25$, $P < 0.001$, partial $\eta 2 = 0.21$), and not significant for happy faces ($F_{1,50} = 3.04$, $P = 0.087$, partial $\eta 2 = 0.06$). Post-hoc tests revealed that, compared to placebo, oxytocin decreased amygdala activation during approach of angry faces ($F_{1,50} = 4.69$, $P = 0.035$, partial $\eta 2 = 0.09$), but not during avoidance of angry faces ($F_{1,50} = 2.36$, $P = 0.13$, partial $\eta 2 = 0.05$). Moreover, amygdala activation differed significantly between threat approach and avoidance after placebo ($F_{1,50} = 7.0$, $P = 0.011$, partial $\eta 2 = 0.12$), but not after oxytocin administration ($F_{1,50} = 2.50$, $P = 0.12$, partial $\eta 2 = 0.05$). Analyses

Table 1
Means (SE) for endocrine and behavioral characteristics of study participants (n= 28 Placebo, n = 24 Oxytocin, otherwise indicated). LSAS = Liebowitz Social Anxiety Scale.

	Placebo		Oxytocin	
Testosterone (pg/mL)				
T1 (baseline)	79.4 (9.6)		85.2 (7.9)	
T2	84.9 (11.9)		77.0 (9.7)	
T3	95.2 (17.7) [n= 27]		88.75 (13.0) [n= 21]	
Cortisol (nmol/L)				
T1 (baseline)	10.4 (1.1)		10.2 (1.8)	
T2	10.7 (1.2)		9.5 (1.3)	
T3	6.5 (0.8) [n= 27]		7.1 (0.7) [n = 23]	
Social Anxiety				
LSAS	26.5 (3.4)		29.9 (3.3)	
Approach-avoidance task (RT, ms)	Approach	Avoid	Approach	Avoid
Happy	671 (27)	729 (26)	739 (20)	794 (25)
Angry	703 (25)	711 (29)	764 (21)	785 (22)
Approach-avoidance task (error rate, %)	Approach	Avoid	Approach	Avoid
Happy	5.4 (0.9)	6.8 (0.9)	7.6 (1.6)	10.8 (1.6)
Angry	6.5 (0.9)	5.7 (1.4)	9.9 (1.9)	8.4 (2.0)

involving the reversed Substance contrast (i.e., oxytocin > placebo), and interactions between Substance × Movement (e.g., [oxytocin > placebo] × [approach > avoid]), or Substance × Emotion (e.g., [oxytocin > placebo] × [happy > angry]), did not yield significant amygdala activation. Oxytocin thus attenuated amygdala activation during threat approach.

3.2. aPFC activation and connectivity

The activity of the aPFC was increased during affect-incongruent compared to affect-congruent responses (Emotion × Movement interaction: coordinates of local maxima, 26 58 8; z-value = 3.75; P_{FWE} = 0.01), as in previous studies using the AA task (Radke et al., 2015; Volman et al., 2011b; Volman et al., 2013). This activation was not modulated by substance, i.e., there was no Substance × Emotion × Movement effect within the aPFC. Similarly, there were no oxytocin-induced alterations in connectivity between the right aPFC and the amygdala during incongruent compared to congruent responses. Together, these results indicate that the substance-related differences in amygdala reactivity are spatially specific and not due to dissimilar aPFC involvement. Whole brain effects are presented in Supplementary Table S1.

3.3. Behavioral approach-avoidance reactions

Overall, the social AA task showed the same congruency effects reported in previous related studies (Radke et al., 2015; Volman et al., 2011a; Volman et al., 2011b; Volman et al., 2013). There were faster responses to affect-congruent trials (approach-happy, avoid-angry) compared to affect-incongruent trials (avoid-happy; approach-angry). Namely, there was an Emotion x Movement interaction on RTs, $F_{1,47}$ = 18.44, P < 0.001, partial $\eta 2$ = 0.28 (but not on ER, $F_{1,47}$ = 2.0, P = 0.163, partial $\eta 2$ = 0.04). Additional main effects of Movement on RTs, $F_{1,47}$ = 48.33, P < 0.001, η^2 = 0.51, and Emotion on RTs, $F_{1,47}$ = 6.15, P = 0.017, partial η^2 = 0.12, indicated faster approach than avoidance movements and faster reactions to happy than to angry faces, respectively. Oxytocin administration increased RTs and error rates (Main effect of Substance on RTs and error rates: $F_{1,47}$ = 4.7, P = 0.035, partial $\eta 2$ = 0.09, and $F_{1,47}$ = 4.28, P = 0.044, partial $\eta 2$ = 0.08; see Table 1 for means). Note that these effects drop below significance when correcting for multiple comparisons using P < 0.025. No other effects were significant (all Fs < 2.7 all Ps > 0.11).

3.4. Endocrine and psychometric control measurements

The two groups (oxytocin, placebo) did not differ in the LSAS score, T_{50} = 0.70, P = 0.487.Cortisol levels showed a decrease over time, $F_{1,92}$ = 13.80, P < 0.001, partial $\eta 2$ = 0.23, but no other effects were significant for cortisol (all Fs < 0.7 all Ps > 0.47) or testosterone (all Fs < 1.6 all Ps > 0.21; see Table 1 for all means).

4. Discussion

This study explored how oxytocin influences neural processing of social threat when that threat needs to be acted upon. We considered the possibility that oxytocin administration could either reduce amygdala activation to social threat (Domes et al., 2007; Gamer et al., 2010; Kirsch et al., 2005; Petrovic et al., 2008), or increase amygdala sensitivity to those salient social cues (Prehn et al., 2013; Shamay-Tsoory and Abu-Akel, 2016). Our novel empirical evidence shows that oxytocin decreased amygdala responses only during threat approach. This finding fits with the idea of oxytocin's anxiolytic properties (Heinrichs and Domes, 2008), and with the observation of reduced amygdala activation during the perception of threatening scenes and faces after oxytocin administration in males (Domes et al., 2007; Gamer et al., 2010; Kirsch et al., 2005; Petrovic et al., 2008). The present study extends and qualifies those observations by investigating oxytocinergic function in its behaviorally relevant context, namely motivational actions. Importantly, oxytocin did not increase motivation- or emotion-specific amygdala activation, rendering generally enhanced approach motivation (Harari-Dahan and Bernstein, 2014; Kemp and Guastella, 2010) or processing shifts to positive cues (Gamer et al., 2010) less likely mechanisms of actions.

In group-living animals, phenotypically similar threat approach has been shown to serve dissimilar purposes, such as deterring attacks or acquiring information (Dugatkin and Godin, 1992). Accordingly, these might be targeted by different neuromodulators. Importantly, here we show that the effects of oxytocin on the amygdala during threat approach were specific to that combination as there were no correspondingly altered amygdala responses during threat avoidance. This oxytocin-related reduced reactivity to threat when approach is required is clearly different from the facilitation of threat approach linked to testosterone, which is based on biasing amygdala responses away from threat avoidance toward threat approach (Radke et al., 2015). Testosterone promotes active dealing with competition by increasing vigilance (Terburg

et al., 2012; van Honk et al., 2000) and facilitating threat approach (Enter et al., 2014), thus increasing the threat value experienced from the stimuli (Radke et al., 2015; Terburg et al., 2012; van Honk et al., 2000). In contrast, oxytocin supports reduced processing of fear, anger and stress (Eckstein et al., 2015; Gamer et al., 2010; Heinrichs and Domes, 2008), and decreases attention to social threats (e.g., Kanat et al., 2015). By decreasing the experienced threat value, oxytocin might facilitate focusing attention on social coping attempts (Tops et al., 2013) as well as socially favorable or safe situations (Bethlehem et al., 2014; Crespi, 2015). The latter hypothesis is substantiated by findings in rhesus macaques where exogenous oxytocin reduced social vigilance to potential social threats (Ebitz et al., 2013) and prolonged reaction times (Landman et al., 2014), thereby permitting social exploration (Chang and Platt, 2014). More generally, it appears that testosterone promotes a dominance–related approach strategy serving the individual's status defense, whereas oxytocin is rather linked to social exploration and in-group protection (Chang and Platt, 2014; De Dreu and Kret, 2016).

4.1. Interpretational issues

Future studies need to consider sex differences in the processing of emotional stimuli (Domes et al., 2010b; Whittle et al., 2011) as well as the sexually dimorphic effects of oxytocin administration on amygdala reactivity to threat (Domes et al., 2010a; Lischke et al., 2012). Interestingly, after oxytocin administration, males' neural responses appear to become more similar to those of females receiving placebo and vice-versa (Rilling et al., 2014), which might provide an explanatory framework for opposing amygdala responses between males and females in the placebo groups of the current and our testosterone administration study (Radke et al., 2015). Furthermore, given the rising concerns on limited statistical power and effect-size inflation of oxytocin studies (e.g., Walum et al., 2016), replication of the present results is crucial, preferably in a within-subject instead of the current between-subject design. In fact, this study builds on a closely related report that observed within-subject behavioral differences following oxytocin administration outside the scanner (Radke et al., 2013). Having previously shown the relevance and sensitivity of this task for capturing modulations of responses to threat, here we used milder affective manipulations in order to evoke between-group differences in cerebral effects unconfounded by behavioral differences in emotional processing (Radke et al., 2015; Volman et al., 2011b; Volman et al., 2013; Volman et al., 2016). Stronger affective responses, as those evoked by the zooming faces covering a larger range of emotional expressions and gaze directions, used in Radke et al. (2013), might result in stronger amygdala responses and thus corresponding behavioral differences that go beyond the previously reported overall slowed evaluation times of faces after oxytocin administration (Petrovic et al., 2008). In particular, our current behavioral findings drop below significance level after correction and should thus be interpreted with caution. Given that dose-response relationships of intranasal oxytocin administration have hardly been systematically investigated in humans (but see Cardoso et al., 2013), we cannot exclude that the 'booster' spray might have raised oxytocin levels outside its physiological range, which might underlie the 'maladaptive' increase of reaction times and error rates. Quantifying oxytocin levels might have aided in determining the time frame of its neurobiological impact.

4.2. Conclusions

This study shows that oxytocin decreases amygdala responses during threat approach. The finding fits with a social anxiolytic function of oxytocin, leading to enhanced social exploration, which is further underlined by distinct social phenotypes of hypersociability and increased approach to strangers (Egger et al., 2013; Järvinen et al., 2013). For instance, Williams syndrome has been associated with increased basal oxytocin levels and peak release (Dai et al., 2012) and decreased amygdala reactivity to threat-related facial expressions (Meyer-Lindenberg et al., 2005). These neurobehavioral mechanisms underlying social approach may have implications for psychiatric disorders associated with social motivational deficits. For instance, in patients with social anxiety disorder, oxytocin has been shown to normalize hyperactive amygdala responses and connectivity to threatening social cues (Gorka et al., 2015; Labuschagne et al., 2010). The current findings suggest that oxytocin might also prove helpful in normalizing motivational actions to threat and anxiety, possibly leading to increased social engagement.

Funding and disclosure

IV was supported by a Marie Curie Individual Fellowship (MSCA-IF-2014_EF_660397) within the European Union's Horizon 2020 Framework Programme. KR was supported by a VICI grant (453-12-001) from the Netherlands Organization for Scientific Research (NWO; http://www.nwo.nl/) and a starting grant from the European Research Council (ERC_StG2012_313749). EDB was supported by a VIDI grant from the NWO (452-12-005). IT was supported by a VICI grant from the NWO (453-08-002). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript. The authors declare no conflicts of interest.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.psyneuen.2017.02.028.

References

Adolphs, R., 2010. What does the amygdala contribute to social cognition? Ann. New York Acad. Sci. 1191, 42–61.
Ashburner, J., Friston, K.J., 2005. Unified segmentation. Neuroimage 26, 839–851.
Bale, T.L., Davis, A.M., Auger, A.P., Dorsa, D.M., McCarthy, M.M., 2001. CNS region-specific oxytocin receptor expression: importance in regulation of anxiety and sex behavior. J. Neurosci. 21, 2546–2552.
Bethlehem, R.A., Baron-Cohen, S., van Honk, J., Auyeung, B., Bos, P.A., 2014. The oxytocin paradox. Front. Behav. Neurosci. 8, 48.
Cardoso, C., Ellenbogen, M.A., Orlando, M.A., Bacon, S.L., Joober, R., 2013. Intranasal oxytocin attenuates the cortisol response to physical stress: a dose-response study. Psychoneuroendocrinology 38, 399–407.
Chang, S.W.C., Platt, M.L., 2014. Oxytocin and social cognition in rhesus macaques: implications for understanding and treating human psychopathology. Brain Res. 0, 57–68.
Crespi, B.J., 2015. Oxytocin, testosterone, and human social cognition. Biol. Rev. Camb. Philos. Soc. 91, 390–408.
Dai, L., Carter, C.S., Ying, J., Bellugi, U., Pournajafi-Nazarloo, H., Korenberg, J.R., 2012. Oxytocin and vasopressin are dysregulated in Williams Syndrome, a genetic disorder affecting social behavior. PLoS One 7, e38513.
Davis, M.H., Whalen, P.J., 2001. The amygdala: vigilance and emotion. Mol. Psychiatry 6, 13–34.
De Dreu, C.K.W., Kret, M.E., 2016. Oxytocin conditions intergroup relations through upregulated in-group empathy, cooperation, conformity, and defense. Biol. Psychiatry 79, 165–173.
Domes, G., Heinrichs, M., Glascher, J., Büchel, C., Braus, D.F., Herpertz, S.C., 2007. Oxytocin attenuates amygdala responses to emotional faces regardless of valence. Biol. Psychiatry 62, 1187–1190.
Domes, G., Lischke, A., Berger, C., Grossmann, A., Hauenstein, K., Heinrichs, M., Herpertz, S.C., 2010a. Effects of intranasal oxytocin on emotional face processing in women. Psychoneuroendocrinology 35, 83–93.
Domes, G., Schulze, L., Bottger, M., Grossmann, A., Hauenstein, K., Wirtz, P.H., Heinrichs, M., Herpertz, S.C., 2010b. The neural correlates of sex differences in emotional reactivity and emotion regulation. Hum. Brain Mapp. 31, 758–769.
Dugatkin, L.A., Godin, J.-G.J., 1992. Prey approaching predators: a cost-benefit perspective. Annales Zoologici Fennici 29, 233–252.

Ebitz, R.B., Watson, K.K., Platt, M.L., 2013. Oxytocin blunts social vigilance in the rhesus macaque. Proc. Natl. Acad. Sci. U. S. A. 110, 11630–11635.
Eckstein, M., Becker, B., Scheele, D., Scholz, C., Preckel, K., Schlaepfer, T.E., Grinevich, V., Kendrick, K.M., Maier, W., Hurlemann, R., 2015. Oxytocin facilitates the extinction of conditioned fear in humans. Biol. Psychiatry 78, 194–202.
Egger, J.I.M., Wingbermühle, E., Verhoeven, W.M.A., Dijkman, M., Radke, S., de Bruijn, E.R.A., de Vries, B., Kessels, R.P.C., Koolen, D., 2013. Hypersociability in the behavioral phenotype of 17q21.31 microdeletion syndrome. Am. J. Med. Genet. A 161, 21–26.
Ekman, P., Friesen, W.V., 1976. Pictures of Facial Affect. Consulting Psychologist Press, Palo Alto, CA.
Enter, D., Spinhoven, P., Roelofs, K., 2014. Alleviating social avoidance: effects of single dose testosterone administration on approach-avoidance action. Horm. Behav. 65, 351–354.
Enter, D., Spinhoven, P., Roelofs, K., 2016. Dare to approach: single dose testosterone administration promotes threat approach in patients with social anxiety disorder. Clin. Psychol. Sci. 4, 1073–1079.
Friston, K.J., Büchel, C., Fink, G.R., Morris, J., Rolls, E.T., Dolan, R.J., 1997. Psychophysiological and modulatory interactions in neuroimaging. Neuroimage 6, 218–229.
Friston, K.J., 1997. Testing for anatomically specified regional effects. Hum. Brain Mapp. 5, 133–136.
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.
Gorka, S.M., Fitzgerald, D.A., Labuschagne, I., Hosanagar, A., Wood, A.G., Nathan, P.J., Phan, K.L., 2015. Oxytocin modulation of amygdala functional connectivity to fearful faces in generalized social anxiety disorder. Neuropsychopharmacology 40, 278–286.
Harari-Dahan, O., Bernstein, A., 2014. A general approach-avoidance hypothesis of oxytocin: accounting for social and non-social effects of oxytocin. Neurosci. Biobehav. Rev. 47, 506–519.
Heinrichs, M., Domes, G., 2008. Neuropeptides and social behaviour: effects of oxytocin and vasopressin in humans. Prog. Brain Res. 170, 337–350.
Insel, T.R., Young, L.J., 2000. Neuropeptides and the evolution of social behavior. Curr. Opin. Neurobiol. 10, 784–789.
Järvinen, A., Korenberg, J.R., Bellugi, U., 2013. The social phenotype of Williams syndrome. Curr. Opin. Neurobiol. 23, 414–422.
Kanat, M., Heinrichs, M., Schwarzwald, R., Domes, G., 2015. Oxytocin attenuates neural reactivity to masked threat cues from the eyes. Neuropsychopharmacology 40, 287–295.
Kemp, A.H., Guastella, A.J., 2010. The role of oxytocin in human affect: a novel hypothesis. Curr. Dir. Psychol. Sci. 20, 222–231.
Kirsch, P., Esslinger, C., Chen, Q., Mier, D., Lis, S., Siddhanti, S., Gruppe, H., Mattay, V.S., Gallhofer, B., Meyer-Lindenberg, A., 2005. Oxytocin modulates neural circuitry for social cognition and fear in humans. J. Neurosci. 25, 11489–11493.
Labuschagne, I., Phan, K.L., Wood, A., Angstadt, M., Chua, P., Heinrichs, M., Stout, J.C., Nathan, P.J., 2010. Oxytocin attenuates amygdala reactivity to fear in generalized social anxiety disorder. Neuropsychopharmacology 35, 2403–2413.
Landman, R., Sharma, J., Sur, M., Desimone, R., 2014. Effect of distracting faces on visual selective attention in the monkey. Proc. Natl. Acad. Sci. U. S. A. 111, 18037–18042.
Liebowitz, M.R., 1987. Social phobia. Mod. Probl. Pharmacopsychiatry 22, 141–173.
Lischke, A., Gamer, M., Berger, C., Grossmann, A., Hauenstein, K., Heinrichs, M., Herpertz, S.C., Domes, G., 2012. Oxytocin increases amygdala reactivity to threatening scenes in females. Psychoneuroendocrinology 37, 1431–1438.
Lund, T.E., Norgaard, M.D., Rostrup, E., Rowe, J.B., Paulson, O.B., 2005. Motion or activity: their role in intra- and inter-subject variation in fMRI. Neuroimage 26, 960–964.
Lundqvist, D., Flykt, A., Öhman, A., 1998. The Karolinska Directed Emotional Faces (KDEF), CD ROM from Department of Clinical Neuroscience. Psychology Section, Karolinska Institute, Stockholm.
Maldjian, J.A., Laurienti, P.J., Kraft, R.A., Burdette, J.H., 2003. An automated method for neuroanatomic and cytoarchitectonic atlas-based interrogation of fMRI data sets. Neuroimage 19, 1233–1239.
Meyer-Lindenberg, A., Hariri, A.R., Munoz, K.E., Mervis, C.B., Mattay, V.S., Morris, C.A., Berman, K.F., 2005. Neural correlates of genetically abnormal social cognition in Williams syndrome. Nat. Neurosci. 8, 991–993.
Mitre, M., Marlin, B.J., Schiavo, J.K., Morina, E., Norden, S.E., Hackett, T.A., Aoki, C.J., Chao, M.V., Froemke, R.C., 2016. A distributed network for social cognition enriched for oxytocin receptors. J. Neurosci. 36, 2517–2535.
Petrovic, P., Kalisch, R., Singer, T., Dolan, R.J., 2008. Oxytocin attenuates affective evaluations of conditioned faces and amygdala activity. J. Neurosci. 28, 6607–6615.
Phaf, R.H., Mohr, S.E., Rotteveel, M., Wicherts, J.M., 2014. Approach, avoidance, and affect: a meta-analysis of approach-avoidance tendencies in manual reaction time tasks. Front. Psychol. 5.
Poser, B.A., Versluis, M.J., Hoogduin, J.M., Norris, D.G., 2006. BOLD contrast sensitivity enhancement and artifact reduction with multiecho EPI: Parallel-acquired inhomogeneity-desensitized fMRI. Magn. Reson. Med. 55, 1227–1235.
Prehn, K., Kazzer, P., Lischke, A., Heinrichs, M., Herpertz, S.C., Domes, G., 2013. Effects of intranasal oxytocin on pupil dilation indicate increased salience of socioaffective stimuli. Psychophysiology 50, 528–537.
Radke, S., Roelofs, K., De Bruijn, E.R.A., 2013. Acting on anger: social anxiety modulates approach-avoidance tendencies after oxytocin administration. Psychol. Sci. 24, 1573–1578.
Radke, S., Volman, I., Mehta, P.H., van Son, V., Enter, D., Sanfey, A.G., Toni, I., De Bruijn, E.R.A., Roelofs, K., 2015. Testosterone biases the amygdala toward social threat approach. Sci. Adv. 1, e1400074.
Rilling, J.K., DeMarco, A.C., Hackett, P.D., Chen, X., Gautam, P., Stair, S., Haroon, E., Thompson, R., Ditzen, B., Patel, R., Pagnoni, G., 2014. Sex differences in the neural and behavioral response to intranasal oxytocin and vasopressin during human social interaction. Psychoneuroendocrinology 39 (10), http://dx.doi.org/10.1016/j.psyneuen.2013.09.022.
Shahrestani, S., Kemp, A.H., Guastella, A.J., 2013. The impact of a single administration of intranasal oxytocin on the recognition of basic emotions in humans: a meta-Analysis. Neuropsychopharmacology 38, 1929–1936.
Shamay-Tsoory, S.G., Abu-Akel, A., 2016. The social salience hypothesis of oxytocin. Biol. Psychiatry 79, 194–202.
Terburg, D., Aarts, H., van Honk, J., 2012. Testosterone affects gaze aversion from angry faces outside of conscious awareness. Psychol. Sci. 23, 459–463.
Tops, M., Huffmeijer, R., Linting, M., Grewen, K.M., Light, K.C., Koole, S.L., Bakermans-Kranenburg, M.J., van Ijzendoorn, M.H., 2013. The role of oxytocin in familiarization-habituation responses to social novelty. Front. Psychol. 4, 761.
Tzourio-Mazoyer, N., Landeau, B., Papathanassiou, D., Crivello, F., Etard, O., Delcroix, N., Mazoyer, B., Joliot, M., 2002. Automated anatomical labeling of activations in SPM using a macroscopic anatomical parcellation of the MNI MRI single-subject brain. Neuroimage 15, 273–289.
Veinante, P., Freund-Mercier, M.J., 1997. Distribution of oxytocin- and vasopressin-binding sites in the rat extended amygdala: a histoautoradiographic study. J. Comp. Neurol. 383, 305–325.
Volman, I., Roelofs, K., Koch, S., Verhagen, L., Toni, I., 2011a. Anterior prefrontal cortex inhibition impairs control over social emotional actions. Curr. Biol. 21, 1766–1770.
Volman, I., Toni, I., Verhagen, L., Roelofs, K., 2011b. Endogenous testosterone modulates prefrontal-amygdala connectivity during social emotional behavior. Cereb. Cortex 21, 2282–2290.
Volman, I., Verhagen, L., Den Ouden, H., Fernandez, G., Rijpkema, M., Franke, B., Toni, I., Roelofs, K., 2013. Reduced serotonin transporter availability decreases prefrontal control of the amygdala. J. Neurosci. 33, 8974–8979.
Volman, I., von Borries, A.K.L., Bulten, B.H., Verkes, R.J., Toni, I., Roelofs, K., 2016. Testosterone modulates altered prefrontal control of emotional actions in psychopathic offenders. eNeuro 3, ENEURO (0107-0115.2016).
Von Borries, A.K.L., Volman, I., de Bruijn, E.R.A., Bulten, B.H., Verkes, R.J., Roelofs, K., 2012. Psychopaths lack the automatic avoidance of social threat: relation to instrumental aggression. Psychiatry Res. 200, 761–766.
Walum, H., Waldman, I.D., Young, L.J., 2016. Statistical and methodological considerations for the interpretation of intranasal oxytocin studies. Biol. Psychiatry 79, 251–257.
Whittle, S., Yucel, M., Yap, M.B., Allen, N.B., 2011. Sex differences in the neural correlates of emotion: evidence from neuroimaging. Biol. Psychol. 87, 319–333.
van Honk, J., Tuiten, A., van den Hout, M., Koppeschaar, H., Thijssen, J., de Haan, E., Verbaten, R., 2000. Conscious and preconscious selective attention to social threat: different neuroendocrine response patterns. Psychoneuroendocrinology 25, 577–591.
van Peer, J.M., Roelofs, K., Rotteveel, M., van Dijk, J.G., Spinhoven, P., Ridderinkhof, K.R., 2007. The effects of cortisol administration on approach-avoidance behavior: an event-related potential study. Biol. Psychol. 76, 135–146.
Ye, Z., Stolk, A., Toni, I., Hagoort, P., 2016. Oxytocin modulates semantic integration in speech comprehension. J. Cogn. Neurosci, 1–10.