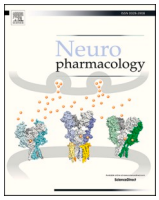
Neuropharmacology 251 (2024) 109930

Contents lists available at ScienceDirect

Neuropharmacology

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



Oxytocin and social learning in socially anxious men and women

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ARTICLE INFO

Handling editor: Prof D. Slattery

Keywords:
Social Phobia
Social Anxiety
Social Learning
Self-referential feedback
fMRI
Pupillometry
Amygdala

ABSTRACT

Objective: This study extended a classic self-referential learning paradigm by investigating the effects of intranasally-administered oxytocin in high and low socially anxious participants during social learning, as a function of social anxiety levels and sex.

Methods: In a randomized double-blinded design, 160 participants were either given intranasal oxytocin (24 I.U.) or placebo. Subsequently, while lying in an MR scanner, participants were shown neutral faces that were paired with positively, neutrally, or negatively valenced self-referential sentences, during which we measured self-reported arousal and sympathy of the facial stimuli, pupil dilation, and changes in the brain-oxygen-level dependent signal. Four-factor mixed analyses of variance with the between-subjects factors group (high socially anxious vs. low socially anxious), substance (oxytocin vs. placebo), and sex (male vs. female) and the within-subjects factor sentence valence (positive vs. neutral vs. negative) were conducted for each measure, respectively.

Results: Administration of intranasal oxytocin yielded an increase in sympathy ratings in high socially anxious compared to low socially anxious individuals and decreased arousal ratings for positively-conditioned faces in low socially anxious participants. As an objective physiological measure of arousal, pupil dilation mirrored the behavioral results. Oxytocin effects on neural activation in the insula interacted with anxiety levels and sex: low socially anxious individuals yielded lower activation under oxytocin than placebo; the converse was observed in high socially anxious individuals. This interaction also differed between sexes, as men yielded higher activation levels than women. These findings were more prominent for positively- and negatively-conditioned faces. Within the amygdala, high socially anxious men yielded higher activation than high socially anxious women in the left hemisphere, and low socially anxious men yielded higher activation than low socially anxious women from positively- and negatively-conditioned faces, though no influence of oxytocin was detected.

Conclusion: These results suggest oxytocin-induced behavioral, physiological, and neural changes as a function of social learning in socially low and high anxious individuals. These findings challenge the amygdalocentric view of the role of emotions in social learning, instead contributing to the growing body of findings implicating the insula therein, revealing an interaction between oxytocin, sex, and emotional valence. Such discoveries raise an interesting set of questions regarding the computational goals of regions such as the insula in emotional learning and how neural activity can play a diagnostic or prognostic role in social anxiety, potentially leading to new treatment opportunities that may combine oxytocin and neurofeedback differentially for men and women.

1. Introduction

If you remember the last time another person was rude to you – maybe even for no apparent reason – ask yourself if you attributed this aversive reaction to the other person ("they are having a bad day") or to yourself ("I did something wrong"). Individuals with social anxiety often attribute negative interpersonal experiences to their own person, instead of to others or the environment. Social anxiety disorder (SAD) represents

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https://doi.org/10.1016/j.neuropharm.2024.109930
Received 20 December 2023; Received in revised form 19 March 2024; Accepted 20 March 2024
Available online 25 March 2024
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the third most common mental disorder worldwide (Furmark, 2002). It is characterized by continuous fear of one or more social performance situations, and occurs on a wide spectrum ranging from mild adaptive fear to a maladaptive disorder, severely impacting an individual's life. According to cognitive behavioral models, this fear is associated with aberrant processing of threat cues and negative self-image, which in turn influences approach-avoidance-behavior and arousal (Clark and Wells, 1995; Rapee and Heimberg, 1997). Learning mechanisms relevant for the development and maintenance of SAD are thought to be related to fear conditioning (Mineka and Oehlberg, 2008; Mineka and Zinbarg, 2006; Öst and Hugdahl, 1981). In classical conditioning, a neutral cue (conditioned stimulus, CS) is paired with an aversive stimulus (unconditioned stimulus, US), which results in the expression of fear responses to the originally neutral stimulus (conditioned response, CR). In a social setting, the US may be an embarrassing experience in a social interaction and the CS may be associated persons (Mineka and Oehlberg, 2008; Mineka and Zinbarg, 2006; Öst and Hugdahl, 1981). A time-specific event (US) that results in fear related to the CS may then generalize to additional social situations (Lissek et al., 2008).

Previous studies have shown that, compared to low anxious individuals, high socially anxious individuals preferentially process threatening cues, such as angry facial expressions (see review of (Bar--Haim et al., 2007), and demonstrate more pronounced fear-conditioning responses (Etkin and Wager, 2007; Furmark, 2009), higher expectations of CS in the acquisition phase, and delayed extinction (Hermann et al., 2002; Schneider et al., 1999; Veit et al., 2002). To transfer fear-conditioning to a socially relevant setting for SAD, social conditioning paradigms have been developed using critical facial expressions or verbal feedback as aversive cues. These manipulations reliably elicit more negative ratings in participants with social anxiety, higher startle reflex, and higher amygdala reactivity for conditioned socially aversive cues (e.g., (Lissek et al., 2008; Pejic et al., 2013). Previous studies have also found sex-dependent differences in social anxiety with regard to social learning: Sutterby et al. (2012) for example, found that women with high social anxiety performed significantly better on social cognition measures than women with low social anxiety, while men with high and low levels of social anxiety did not differ. Women also generally display higher arousal ratings than men and reduced discrimination between conditioned and unconditioned stimuli (CS+ and CS-) in fear acquisition and extinction processes (Lonsdorf et al., 2015). In addition to ratings of arousal, it is of interest to investigate ratings of sympathy to tap into the aberrant self-referential processing and differences of self-belief in high socially anxious compared to low anxious individuals (Dixon et al., 2022).

Over the past two decades, the neuropeptide oxytocin has been widely studied and found to have beneficial effects on social interactions and associated cognitive processes (Lischke et al., 2012). However, the effects of oxytocin are highly dependent on external social cues as well as the participants' sex, personality traits, and/or psychopathology (Shamay-Tsoory and Abu-Akel, 2016). Behaviorally, oxytocin was mostly found to reduce anxiety, arousal, and stress reactivity (Cardoso et al., 2013; Ditzen et al., 2009; Heinrichs et al., 2003), increase socially reinforced learning (Hurlemann et al., 2010), and improve the ability to recognize and empathize with other's emotional states in healthy participants (see review of Barchi-Ferreira and Osório, 2021). Furthermore, oxytocin has also been found to facilitate fear learning (Eckstein et al., 2016) as well as conditioned fear extinction (Eckstein et al., 2015) in healthy men. Individuals with low social competence seem to especially profit from intranasal oxytocin administration with regard to their pro-social behavior (Bartz et al., 2011). On the other hand, oxytocin has also been associated with anti-social effects leading to jealousy and *Schadenfreude* during competitive games (Shamay-Tsoory et al., 2009). According to the 'social-saliency-hypothesis', oxytocin may not generally be anxiolytic and pro-socially encouraging, but rather its effects depend on the saliency of the social cue and the situation it is presented in Shamay-Tsoory (2010) and Shamay-Tsoory and Abu-Akel (2016). Pro-social effects of oxytocin disappear if a given cooperation partner is rated as untrustworthy (Mikolajczak et al., 2010), when important information about their person is lacking (Declerck et al., 2010), or when this person belongs to a perceived out-group (De Dreu and Kret, 2016). At the neural level, oxytocin seems to dampen activity in the amygdala (Baumgartner et al., 2008; Domes et al., 2007; Kirsch et al., 2005). Also, oxytocin has been reported to modulate activations in prefrontal, temporal, and insular cortices (Kanat et al., 2015; Wigton et al., 2015). However, the effects of oxytocin appear to be sex-dependent: while men yield a reduced amygdala reactivity and connectivity in response to negative facial expressions (Domes et al., 2007; Gamer et al., 2010; Kirsch et al., 2005), women tend to yield heightened amygdala reactivity (Domes et al., 2010; Lischke et al., 2012). More recently, Lieberz et al. (2020) suggested that oxytocin increases the salience of social signals by increasing the sensitivity for these signals in the amygdala and in the striatum in women, while it may primarily induce anxiolysis by reducing amygdala responses in men. There is further evidence for a dose-dependent effect on amygdala reactivity Spengler et al. (2017) that may not only depend on sex, but also on psychopathology. For example, oxytocin dampens anger-related amygdala reactivity in a number of mental disorders and differentially modulates the amygdala following social threatening or provoking cues based on psychopathology and sex (Jeung-Maarse et al., 2023). Also, several studies on polymorphisms in the oxytocin receptor as well as emerging epigenetic studies point to a link between oxytocinergic systems and psychiatric disorders (Londono Tobon et al., 2018). Even though there is empirical evidence that suggests functional brain asymmetry for emotion perception, research associated oxytonergic influences is scarce. Few findings suggest that oxytocin can influence hemispheric differences in neurotransmitter levels and brain activity, particularly in regions involved in social perception and cognition. According to Stanković and Nešić (2020), the brain is initially right-biased in emotional and neutral face perception by default, but subject to change under stress and respective psychophysiological activation ('hemispheric functional-equivalence model').

In 2015, Ziegler and colleagues found lower oxytocin receptor methylation in non-medicated female patients with SAD that correlated with symptom severity and higher amygdala reactivity. Under the assumption that lower oxytocin receptor methylation may lead to higher oxytocin receptor expression, the authors suggested that reduced oxytocin concentration may be causally relevant for higher oxytocin receptor activation found in SAD. Administration of oxytocin in SAD has been found to affect social learning processes in men, increasing reactivity of the amygdala in the presence of fearful facial expressions and the medial prefrontal cortex for sad facial expressions (Dodhia et al., 2014; Labuschagne et al., 2010). Such examples indicate a general dysregulation of the oxytonergic system in SAD. However, oxytocin receptors are not exclusive to the amygdala and previous studies have associated oxytocin with decreased activation of the insula in response to social cues (Yao et al., 2018). Task-based and resting-state functional neuroimaging studies have reported the insula to be a significant neural correlate of aberrant self-referential processing representing a core feature of SAD that is also related to therapeutic interventions (Yoon et al., 2019).

Given that individuals with SAD suffer from the psychological strain of social isolation and constant fear of evaluation by their peers, it is relevant to extend findings from fear-learning paradigms to those addressing social perception and processing. In light of the social and contextual importance of oxytocinergic effects, we specifically considered trait and sex differences in a double-blinded placebo-controlled social conditioning paradigm, adapted from Davis et al. (2010) in a cohort that varied in social anxiety levels. We extended this paradigm of pairing neutral faces with emotionally-charged self-referential sentences by measuring how oxytocin affects such socially learned contingencies (without altering visual input). Given the previous research, we predicted (1) that high socially anxious individuals would rate neutral facial expressions more negatively and more arousing than individuals with

low social anxiety, (2) that high socially anxious individuals would also show higher arousal to negative social conditioning, (3) that this tendency would be more pronounced in women, (4), that the amygdala (due to its role in the oxytocinergic system) and the insula (as a critical structure for self-referential social processing and saliency; see Shamay-Tsoory and Abu-Akel, 2016) would show sex-dependent higher activation levels in individuals with high social anxiety, and (5) that oxytocin administration would reduce these pronounced responses in the presence of aversively conditioned stimuli for individuals with high social anxiety as compared to their low socially anxious counterparts. We measured arousal and sympathy with a self-reported rating and an objective physiological response in the form of pupil dilation (see Bradley et al., 2008) and neural activity levels with functional magnetic resonance imaging (fMRI) using changes in the BOLD signal. Due to a lack of previous evidence, we consider potential hemispheric functional differences through oxytocin exploratorily.

2. Materials and methods

2.1. Subjects

Participants were asked to fill out the German version of the Liebowitz Social Anxiety Scale (LSAS; Liebowitz, 1987; Stangier and Heidenreich, 2005) online to assess social anxiety levels. Individuals with social anxiety scores above 27 and sum scores above 60 were ascribed to the high socially anxious group, while those with social anxiety scores under 13 were considered low socially anxious. This cut-off was chosen according to previous literature (see Heuer et al., 2007; Lange et al., 2008; Roelofs et al., 2010) and considering the highest and lowest 10% scores of the general German population (Heuer et al., 2007). Participants received monetary compensation after participation.

Participants were excluded in cases of: age below 18 or above 45 years, left-handedness, pregnancy, regular medication intake (with the exception of contraceptives), tobacco consumption of more than 5 cigarettes a day, chronic physical or neurological disorders, and current and/or lifetime mental disorders with the exception of SAD, mild depression, or avoidant personality disorder. Compatibility with neuroimaging techniques was another prerequisite (e.g., no pace maker or ferrometallic implants).

Ultimately, $n = 200$ participants were selected for the neuroimaging experiment (102 women, 98 men; $M = 23.71$ years; $SD = 4.55$). Eight participants (5 women and 3 men) had to be excluded due to bad signal-to-noise ratio in the MRI, four men had to be additionally excluded due to neurological aberrations in the structural scans, and another 28 participants were excluded (17 women, 11 men) due to incomplete mapping of the amygdala caused by susceptibility artefacts or a field of view that was too small. The final data set consisted of $n = 160$ participants (80 women and 80 men; $M = 23.62$ years; $SD = 4.45$; see CONSORT flow chart in Fig. 1 as well as Table 1 below). For the pupillometry analyses, we had to exclude 47 participants (19 women and 20 men; 19 LSA and 20 HSA), due to lack of pupil information recorded. All women were tested in their early follicular phase (2nd-8th day after menstruation), as this phase can be reliably established due to the onset of the menstruation and is related to findings of largest sex differences on self-reported and physiological stress responses (Kirschbaum et al., 1999).

Diagnostic interviews (DSM-IV, SKID-I und –II; (Wittchen et al., 1997); and International Personality Disorder Examination, IPDE; (Loranger et al., 1997) were conducted with all participants after a telephone screening to ensure eligibility. Depressivity was assessed via Beck's Depression Inventory (BDI-II; (Beck et al., 1996) for which internal consistency was average to good overall (BDI-II: Cronbach's $\alpha = 0.81$).

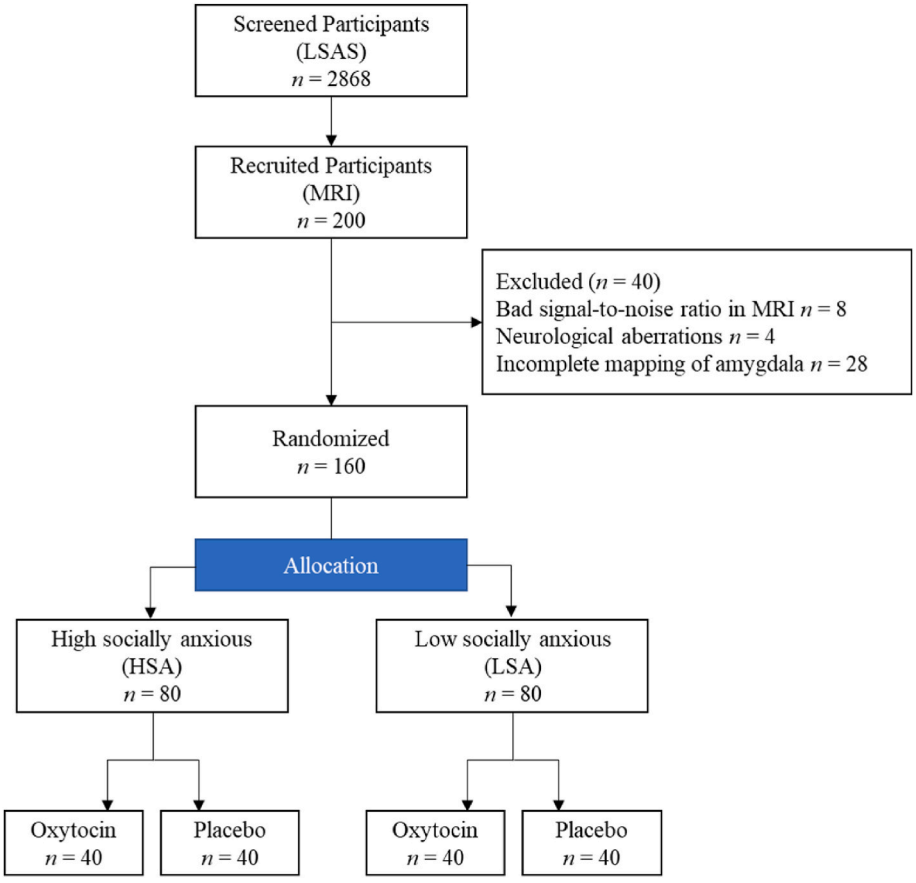

Fig. 1. CONSORT flow chart of eligible participants.

Table 1
Demographics of the total sample of participants ($n = 200$) divided into high and low socially anxious individuals according to the score in the Liebowitz Social Anxiety Scale (LSAS-score.) Means are reported with standard deviations in brackets.

	High socially anxious ($n = 100$)	Low socially anxious ($n = 100$)	Statistic
Age (years)	23.65 (5.24)	23.77 (3.74)	$t_{(198)} = -0.18$, $p = 0.860$
Sex (m/f)	49/51	49/51	$\chi^2 = 0$, $p = 1$
Years of Education	16.05 (2.91)	16.58 (2.82)	$t_{(191)} = -1.30$, $p = 0.195$
Plasma oxytocin (pg/ml)	4.22 (3.43)	4.74 (3.56)	$t_{(184)} = -0.99$, $p = 0.320$
LSAS (sum score)	74.66 (13.51)	19.26 (6.89)	$t_{(198)} = 36.42$, $p < 0.001$
STAI-State	41.89 (10.05)	31.83 (6.84)	$t_{(194)} = 7.34$, $p < 0.001$
STAI-Trait	38.83 (10.17)	26.45 (6.14)	$t_{(198)} = 10.39$, $p < 0.001$
BDI-II (sum score)	6.72 (6.14)	1.86 (2.22)	$t_{(198)} = 7.43$, $p < 0.001$

Note: Statistics refer to independent-sample t-tests; group differences for sex was analyzed with a chi-squared test. Group differences for the reduced sample of $n = 160$ do not differ from the ones of the whole sample $n = 200$. m: male, f: female; LSAS: Liebowitz Social Anxiety Scale; STAI: State-Trait Anxiety Inventory; BDI-II: Beck's Depression Inventory.

The study was approved by the Ethics Committee of the Medical Faculty of the University of Heidelberg and all experimental procedures aligned with the guidelines of the Declaration of Helsinki. Participation was voluntary and all participants provided written informed consent.

2.2. Procedure

Before starting the MRI session, participants were asked to carry out a training session to familiarize themselves with the paradigm. Before and 30 min after intranasal oxytocin administration, participants were asked to fill out a short version of the State-Trait-Anxiety-Inventory (STAI-S; Laux et al., 1981) to provide a further anxiety measure related to the experimental procedure. Before the experiment started, blood samples were drawn for the assessment of peripheral oxytocin plasma concentration (analyzed with sensitive radio-immune-assay) as well as estradiol and progesterone concentrations (chemiluminescence immunoassay) of female participants in order to verify the menstrual cycle phase. The data of these peripheral hormonal data have been published elsewere (Müller et al., 2019; Schneider et al., 2021) without any referral to the experimental data presented here. Importantly for the current study, no group differences in contraceptive intake or in peripheral oxytocin data were found and women with and without contraception did not significantly differ regarding baseline oxytocin, progesterone, or estradiol, levels (for details, see Schneider et al., 2021). Women were asked to self-administer a pregnancy test („viola®" CARE diagnostica®, Voerde, Germany) before taking the blood sample to ensure eligibility for the study.

In a randomized double-blinded design, participants were either given intranasal oxytocin (Syntocinon®-Spray, Novartis, Basel, Switzerland) or intranasal placebo, which consisted of the same components with the exception of the neuropeptide. Participants were instructed to administer 12 alternating spray applications (24 international units (I.U.) in total) each for the oxytocin group) at an interval of 45 s. Subsequently, they were asked to rest for 10 min lying down. The substance was administered 45 min before the MRI session, as this is the required time for oxytocin to accumulate at a plateau in the central nervous system following intranasal application (Born et al., 2002; Dal Monte et al., 2014). In a dose-response study of intranasal oxytocin, the strongest neuronal effect was seen at a dose of 24 IU and in the time interval of 45–70 min after application (Spengler et al., 2017). Experiments were performed at the afternoon when endocrine fluctuations are expected to be lower.

During the experiment, subjects were instructed to move as little as possible inside the bore of the MRI. Visual stimuli were presented using Presentation 12.2 software (Neurobehavioral systems, Inc) on a screen that subjects viewed via a mirror mounted on their head coil. The face stimuli consisted of three male and three female faces with neutral facial expressions acquired from an established set of face stimuli (Ebner et al., 2010). The sentence stimuli consisted of self-relevant sentences that were selected for valence (−4 very negative to +4 very positive) and matched for arousal (1 ″not emotionally arousing at all" to 9 ″as emotionally arousing as possible") in an independent pilot study of $n = 12$ raters. For the current study, 24 self-relevant sentences were selected: eight positive (e.g., "She thinks you are attractive"), eight neutral (e.g., "She thinks your T-shirt is blue"), and eight negative. ("She thinks you are stupid"). For negative and positive sentences, the respective opposites were used (e.g., smart vs. stupid).

The social conditioning paradigm (SCP) corresponded to a modified version of the paradigm presented by (Davis et al., 2010). It consisted of an orientation phase and a conditioning phase. In the *orientation phase*, the three male and three female faces with neutral facial expressions were presented 20 times each for 1 s to familiarize subjects with the faces. The faces were presented in a pseudorandomized order in which the same face could never be shown more than two times in succession. There was an inter-trial-interval of 1.5–2.5 s between two faces, during which a gray screen with a white fixation cross was shown. For this phase, the subjects were only given the task of attending to the faces.

In the *conditioning phase*, each face was paired with four positive, neutral, or negative self-relevant sentences. In each case, a male and a female face formed a valence pair that always predicted the same social consequence (e.g., insult, compliment, or neutral statement) after the faces were presented. Faces were presented for 1 s, in a period of 1.5–2.5 s before each sentence, in a pseudorandomized order. The sentence was presented for 2 s, with another 2.5–3.5 s until the next face presentation. This temporal "jittering" allowed us to separate overlapping hemodynamic responses to the modulation of the face-phrase pairings (Friston et al., 2003) (see Fig. 2).

The face-sentence pairings were counterbalanced across subjects. Each face-sentence pairing was presented 12 times, so that each valence was presented a total of 24 times. After both the orientation phase and the conditioning phase, subjects rated each face on a visual analog scale, first, how likable they found the face and, second, how emotionally aroused they were by the face. The corresponding questions were "How likeable do you find this person" (1: "not at all" to 5: "very strongly") and "How strongly are you emotionally aroused by this person?" (1: "not at all" to 5: "very strongly"). The experimental paradigm amounted to 20 min, to which another 8 min were added for an anatomical T1 recording (total MRI duration: 30 min). The total duration of the experiment was 1.5–2 h. To exclude possible influences of the circadian rhythm, all measurements were performed after 1 p.m.

2.3. Neuroimaging acquisition parameters

The MRI session was carried out 45 min after substance application with a 3 T S Tim Trio scanner equipped with a 32-channel standard head coil. Functional images were acquired using an T2*-weighted echoplanar (EPI) sequence, with a total of 145 vol for the orientation phase and 256 vol for the conditioning phase. One volume consisted of 40 transverse slices (descending order, slice thickness 2.3 mm, voxel size 2.3 × 2.3 × 3 mm, field of view 220 × 220 mm^2, echo time 27 ms, repetition time 2350 ms, flip angle 90°). The scans were centered at the amygdala, over the temporal lobe. The first four scans of the EPI sequence were removed before further analysis to minimize T1 saturation effects. After completing the functional measurements, we also acquired a high-resolution anatomical image for each subject using a T1-weighted 3D magnetization prepared rapid acquisition gradient echo sequence

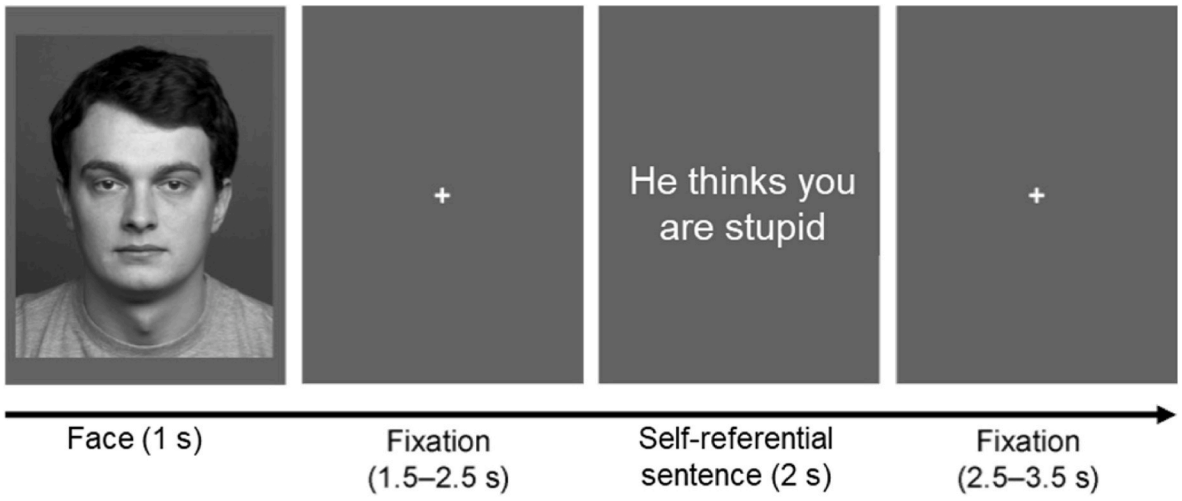

Fig. 2. Example of a trial sequence depicting a negative social conditioning to a neutral male face.

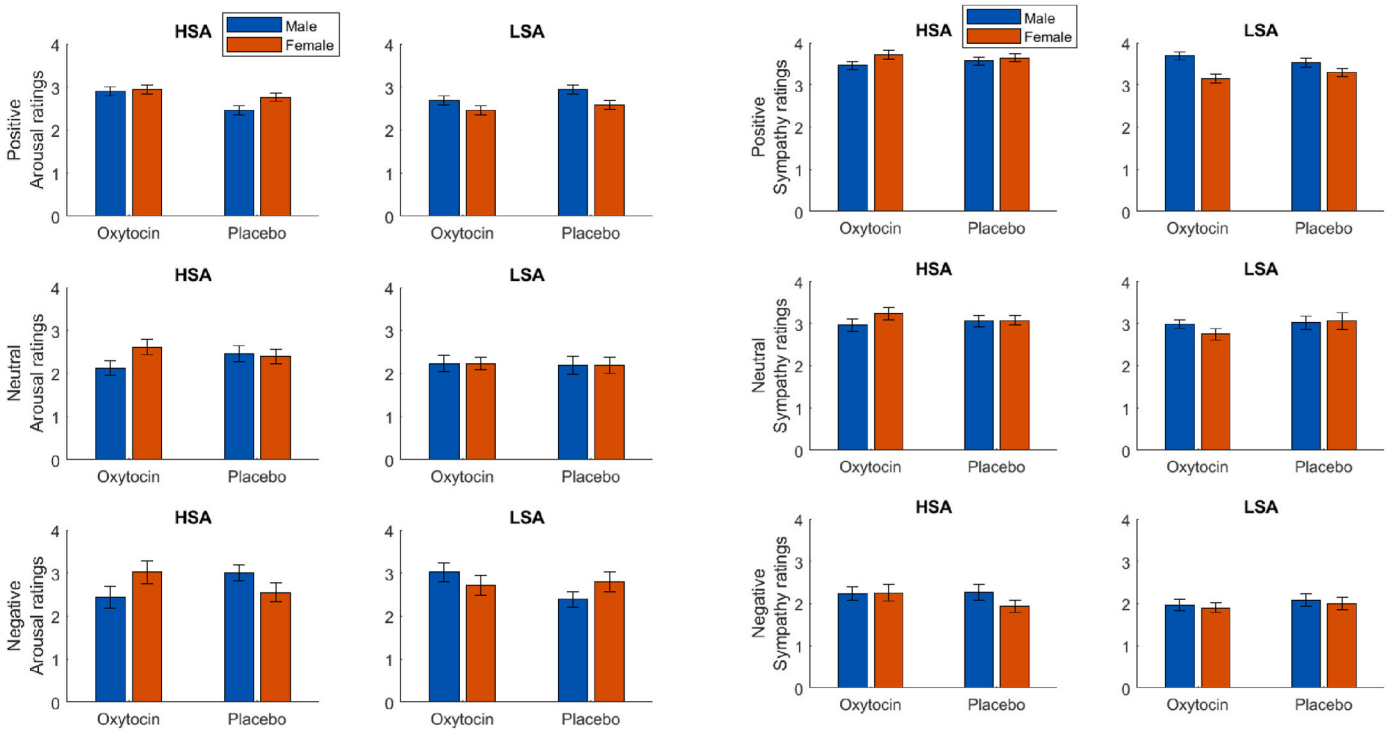

Fig. 3. ***Left***: Arousal ratings and ***right***: sympathy ratings for high socially anxious (HSA) and low socially anxious (LSA) men (blue) and women (red) as a function of substance administered (oxytocin or placebo) for the three sentence valences (positive, neutral, negative) conditioned to the presented faces. Error bars indicate standard errors of the mean.

(MPRAGE; voxel size 1 mm^3 isomorphic, field of view 256 × 256 mm^2, echo time 2.52 ms, repetition time 1900 ms, flip angle 9°) to serve as an individual template for coregistration during subsequent preprocessing. The total duration of the MRI measurements was approximately 30 min.

2.4. Data analysis

2.4.1. Behavioral data

The sympathy and arousal ratings that participants provided during the conditioning phase were analyzed using four-factor mixed analyses of variance (ANOVAs) with the between-subjects factors group (high socially anxious × low socially anxious), substance (oxytocin × placebo), and sex (male × female) and the within-subjects factor valence (positive × neutral × negative). Statistical thresholds were set at the conventional critical alpha level of 0.05; Greenhouse-Geisser sphericity corrections were applied when factors contained more than two levels.

2.4.2. Pupillometry

Monocular pupil height and width of the right eye were recorded in the scanner via a ViewPoint Eye-Tracker® (Arrington Research Inc., Scottsdale, USA). A 16-point calibration and validation were completed before recording to ensure measurement accuracy. For data preprocessing and analysis, we used the guidelines suggested by (Kret and Sjak-Shie, 2019). Using R (R studio©; (Team, 2020), raw data was loaded, blinks were detected and marked, timestamps set relative to the onset of the stimulus presentation and positional data transformed with

regard to the resolution. Time frames 300 ms before stimulus onset until 5000 ms after stimulus onset were considered for pupil measures. For blink detection, aspect ratios were defined (critical standard deviation of the aspect ration at 0.08; critical standard deviation of gaze position at 0.10; critical maximal aspect ratio at 0.99) and points selected, in which the aspect ratio was below the critical value or if standard deviations were above the critical standard deviation of gaze position. Pupil size changes were considered if they were higher than 1.5 mm and lower than 9 mm dilation. We then smoothed across blinks using linear interpolation and a median filter was applied and baselines corrected.

The preprocessed pupillometry data were then analyzed with a four-factor mixed ANOVA with the within-subjects factor sentence valence (positive × neutral × negative) and the between-subjects factors group (high socially anxious × low socially anxious), substance (oxytocin × placebo), and sex (male × female). Statistical thresholds followed the same conventions as for the analysis of the behavioral data.

2.4.3. Neuroimaging data

We analyzed the neuroimaging data from the conditioning phase (in correspondence with Davis et al., 2010) using SPM12 (https://www.fil.ion.ucl.ac.uk/spm/software/spm12/) and CoSMoMVPA (Oosterhof et al., 2016), built on MATLAB R2020a (The Mathworks, Natick, MA, USA). Preprocessing of the neuroimaging data employed the default settings of the SPM12 pipeline including slice timing correction, spatial realignment of the functional images to the mean image in the time series using a six-parameter rigid body transformation, coregistration of the functional images to each subject's structural image, normalization of the functional images to a standard 2 mm MNI template, and spatial smoothing of the images with a Gaussian kernel (8 mm FWHM).

The preprocessed functional images were analyzed using the general linear model containing one regressor for the onset (and duration) of the face stimulus in a given condition and one regression for the onset (and duration) of the sentence stimulus in a given condition. Motion correction parameters were included as regressors of non-interest, as well as a constant term.

2.4.4. Region-of-interest definition

Following from our hypotheses, we obtained left- and right-hemisphere regions-of-interest (ROIs) for the amygdala and the insula from the probabilistic Harvard-Oxford atlas (Frazier et al., 2005), thresholded at a probability of 0.5, yielding region sizes of 240 voxels (left amygdala), 280 voxels (right amygdala), 518 voxels (left insula), and 529 voxels (right insula).

2.4.5. Region-of-interest analysis

For each subject, we used the above-mentioned ROI masks to extract the corresponding beta-weight maps for the conditions face-positive, face-neutral, and face-negative and then averaged the voxels of a given ROI per condition, yielding 12 values (4 ROIs × 3 valence levels) that reflect a region's average activation for a given valence of a particular subject.

These subject-level values were submitted to a four-factor mixed ANOVA containing the within-subjects factors valence (positive × neutral × negative) and the between-subjects factors diagnosis (high social anxiety × low social anxiety), substance (oxytocin × placebo), and sex (male × female) and hemisphere (left × right). The ANOVA was run separately for the amygdalar data and the insular data. Statistical thresholds followed the same conventions as for the analysis of the behavioral and pupillometry data. We report post-hoc t-tests and corrected for Holm-Bonferroni for effects of a three-way interaction.

3. Results

3.1. Questionnaire data

Investigating relationships between the behavioral data from the conditioning phase and the questionnaire data from the STAI-S, STAI-T, and BDI-II using Spearman rank correlations did not reveal any particular associations that surpassed conventional statistical thresholds (all $|r| < 0.129$, all $p > 0.071$. A full table of the null results can be found in Table A1 of the Appendix.

3.2. Behavioral data

Analyzing the *arousal data* (Fig. 3, left panel) revealed.

(1) that participants rated positively- and negatively-conditioned faces higher than neutrally-conditioned faces (Valence: $F_{(2,384)} = 18.77$, $p = 1.66 \times 10^{-8}$; positive vs. neutral: $t_{(199)} = 5.93$, $p = 1.33 \times 10^{-8}$; neutral vs. negative: $t_{(199)} = -4.87$, $p = 2.28 \times 10^{-6}$; positive vs. negative: $t_{(199)} = -0.30$, $p = 0.763$),
(2) that arousal ratings were modulated by an interaction between oxytocin and social anxiety depending on the valence of conditioned faces (Group × Substance × Valence: $F_{(2,384)} = 3.42$, $p = 0.034$), in that with respect to positively-conditioned faces, arousal was higher for participants in the HSA group under oxytocin compared to placebo, and this difference differed from the corresponding effect for participants in the LSA group ($t_{(78)} = 3.528$, $p = 0.0007$, FWER-corrected). This interaction between Group and Substance was not observed with respect to the neutrally-conditioned faces ($t_{(78)} = 0.496$, $p = 0.621$), or the negatively-conditioned faces ($t_{(78)} = -0.771$, $p = 0.443$), and this interaction effect for the positively-conditioned faces was greater than that for the neutrally- ($t_{(78)} = 2.68$, $p = 0.0087$, FWER-corrected) and negatively-conditioned faces ($t_{(78)} = 3.37$, $p = 0.0012$, FWER-corrected). We did not detect a difference for the corresponding interaction between neutrally-conditioned faces compared to negatively-conditioned faces ($t_{(78)} = 0.97$, $p = 0.333$).
(3) that this three-way interaction was more pronounced in men than in women (Group × Substance × Sex × Valence: $F_{(2,304)} = 5.52$ 6.52, $p = 0.004$).

With respect to the *sympathy ratings* (Fig. 3, right panel), we found.

(1) that participants rated positively-conditioned faces highest and negatively-conditioned faces lowest (Valence: $F_{(2,384)} = 180.13$, $p = 6.623 \times 10^{-56}$; positive vs. neutral: $t_{(199)} = 7.12$, $p = 1.92 \times 10^{-11}$; neutral vs. negative: $t_{(199)} = 13.14$, $p = 4.27 \times 10^{-29}$; positive vs. negative: $t_{(199)} = 16.83$, $p = 2.14 \times 10^{-40}$), indicating that the conditioning was successful.
(2) participants with high social anxiety provided higher sympathy ratings ~~under oxytocin~~ than participants with low social anxiety (Group: $F_{(1,192)} = 7.05$, $p = 0.009$). Descriptively, socially high anxious women under oxytocin provided the highest sympathy ratings, while sympathy ratings from low anxious women under oxytocin appeared to be lowest. However, the correspondent Group by Substance by Sex interactions did not reach statistical significance ($F_{(1,192)} = 3.26$, $p = 0.073$).

Full ANOVA tables for the arousal and sympathy ratings can be found in Table A2 and Table A3 of the appendix, respectively.

3.3. Pupillometry

The *pupillometry data* (Fig. 4) during the conditioning phase of the experiment revealed a general increase in pupil size under oxytocin compared to placebo independent of sex, anxiety, and valence (Substance: $F_{(1,112)} = 6.22$, $p = 0.014$). The full ANOVA table for the pupillometry data can be found in Table A4 of the appendix.

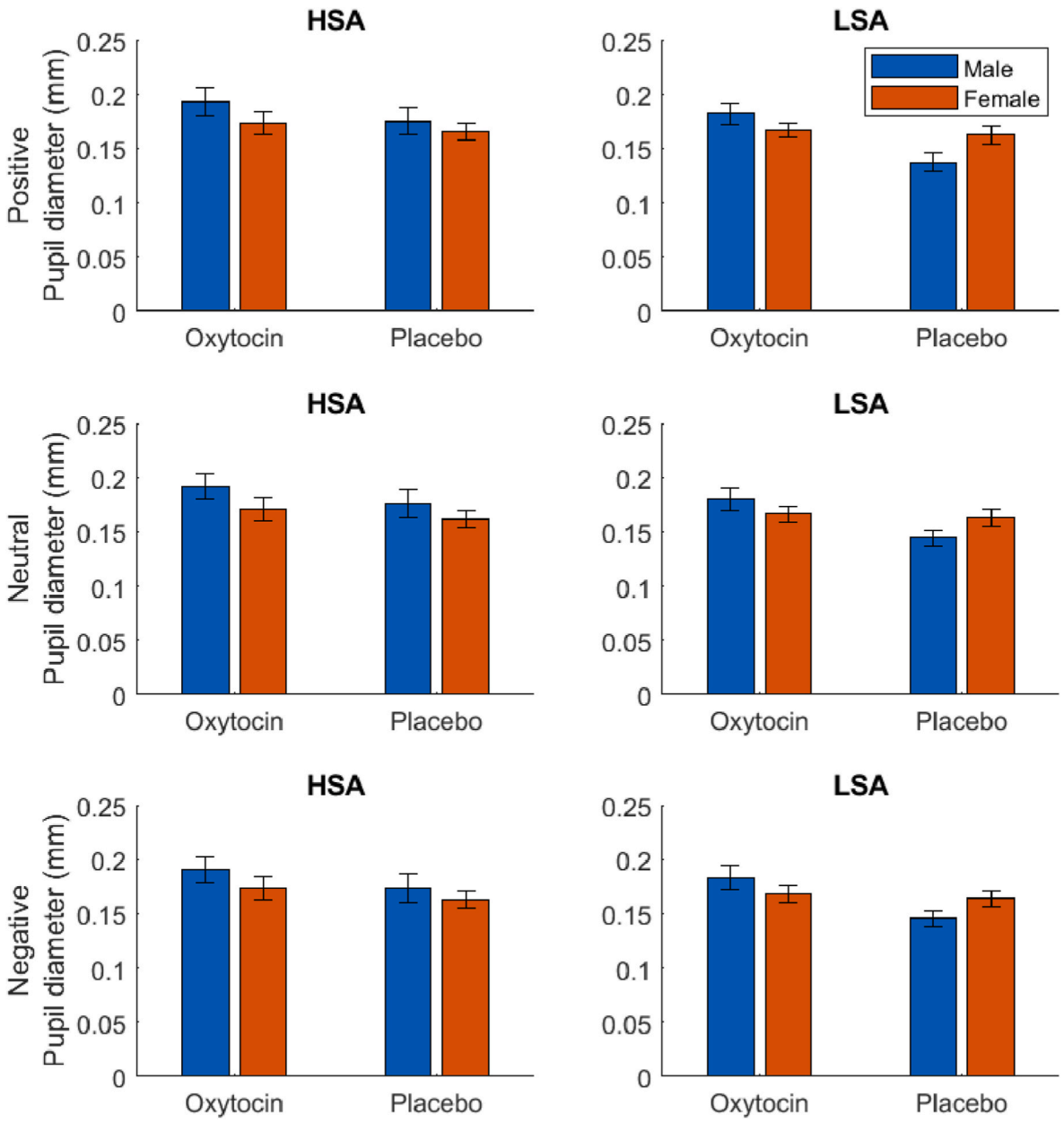

Fig. 4. Pupil size changes in diameter (mm) for high socially anxious (HSA) and low socially anxious (LSA) men (blue) and women (red) as a function of substance administered (oxytocin or placebo) for the three sentence valences (positive, neutral, negative) conditioned to the presented faces. Error bars indicate standard errors of the mean.

3.4. Neuroimaging data

3.4.1. Amygdala

Analyzing the functional neuroimaging data of the conditioning phase from the *amygdala* (Fig. 5) revealed.

(1) that high socially anxious men yielded higher parameter estimates than high socially anxious women in the left hemisphere, as compared to the right hemisphere, and
(2) that low socially anxious men yielded higher parameter estimates than low socially anxious women from positively- and negatively-conditioned faces (Group × Sex × Hemisphere × Valence: $F_{(2,304)} = 5.02$, $p = 0.007$).

3.4.2. Insula

Analyzing the functional neuroimaging data of the *insula* (Fig. 6) yielded.

(1) lower parameter estimates for low socially anxious participants compared to high socially anxious participants (Group: $F_{(1,152)} = 6.592$, $p = 0.011$).
(2) This effect was further explained by low socially anxious participants in the oxytocin group tending to yield lower parameter estimates than those in the placebo group, the effect of which was reversed in high socially anxious participants (Group × Substance: $F_{(1,152)} = 5.21$, $p = 0.024$).
(3) This group-by-substance interaction was itself further explained by participants' sex (in that high socially anxious men in the oxytocin group tended to yield higher parameter estimates than their female counterparts [Group × Substance × Sex: $F_{(1,152)} = 4.803$, $p = 0.030$]) and

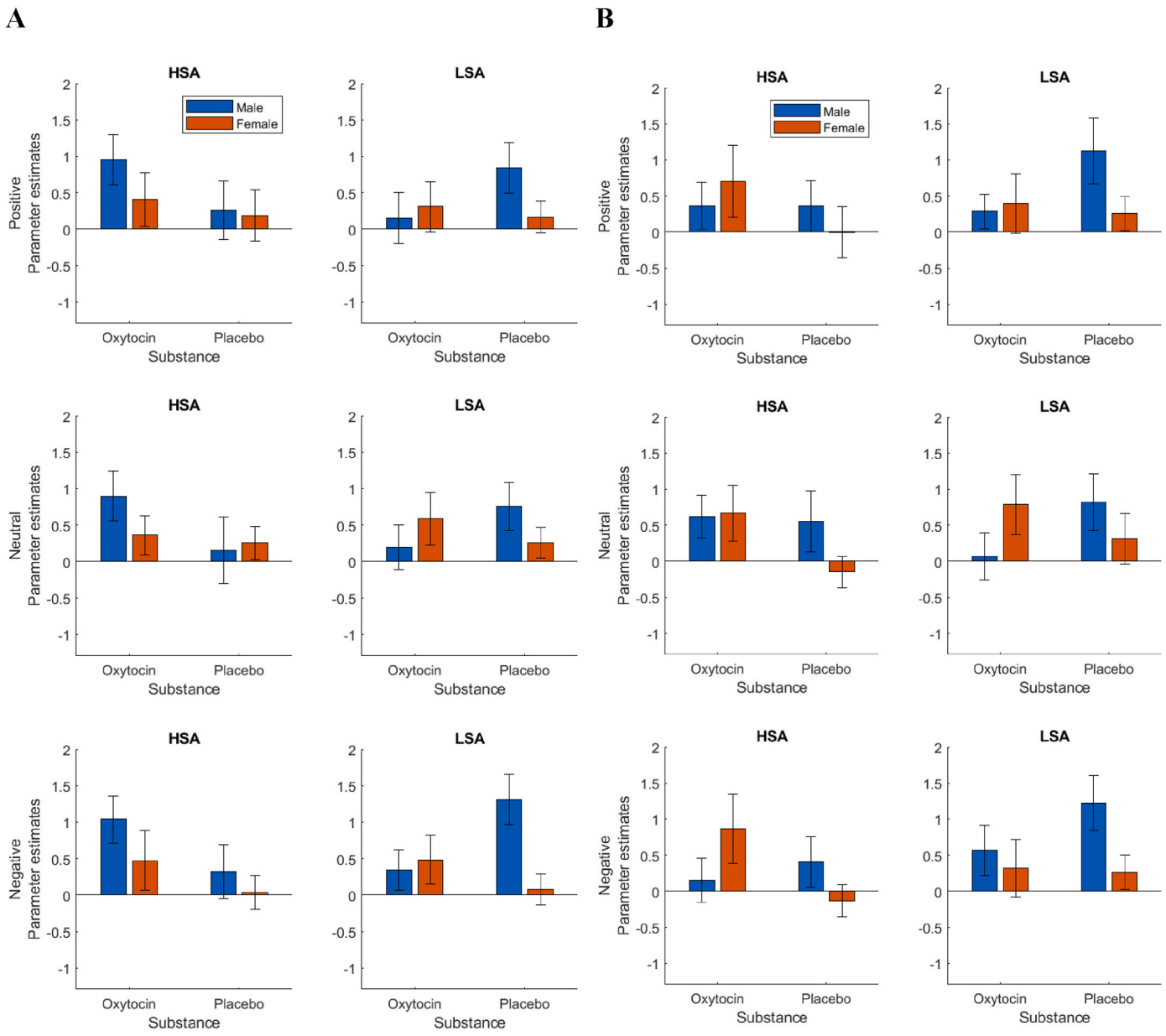

Fig. 5. Parameter estimates for **A**. the left and **B**. the right amygdala for high socially anxious (HSA) and low socially anxious (LSA) men (blue) and women (red) as a function of substance administered (oxytocin or placebo) for the three sentence valences (positive, neutral, negative) conditioned to the presented faces. Error bars indicate standard errors of the mean.

(4) the stimulus' conditioned valence (in that the effect was more pronounced for positively- and negatively-conditioned faces: Group × Substance × Valence: $F_{(2,304)} = 3.118$, $p = 0.0483$).
(5) there also appeared to be hemispheric differences, as the lower parameter estimates for the positively- and negatively-conditioned faces by low socially anxious participants were more distinct in the right insula compared to the left (Group × Hemisphere × Valence: $F_{(2,304)} = 3.754$, $p = 0.025$).

Full ANOVA tables for the parameter estimates of the amygdala and insula can be found in Table A5 and Table A6 of the appendix. Basal oxytocin levels of participants were not associated with either ROI activation patterns (all $r < 0.1158$ | all $p > 0.1641$).

4. Discussion

The anxiolytic effects of oxytocin suggested by previous research may have a therapeutic role in the treatment of social anxiety disorders (SAD). This study sought to investigate the relationship between social anxiety, oxytocin, and sex on behavioral and neurophysiological responses to social learning. To this end, we engaged participants in a social conditioning experiment while measuring their neurophysiological, pupillometric, and self-reported responses to images of individuals whose countenances had been paired with self-referential sentences expressing positive, neutral, or negative statements.

By exploring the interaction of social anxiety levels, intranasally-administered oxytocin, and sex, our primary results were four-fold: (1) oxytocin affected high and low socially anxious individuals differentially in how they reported the emotional arousal elicited by positively-conditioned faces, (2) interestingly, participants with high social anxiety tended to rate faces as more sympathetic, regardless of the emotional valence, than their low socially anxious counterparts under oxytocin, (3) oxytocin increased overall physiological arousal levels as measured by pupil dilation, (4) men and women showed different activation profiles

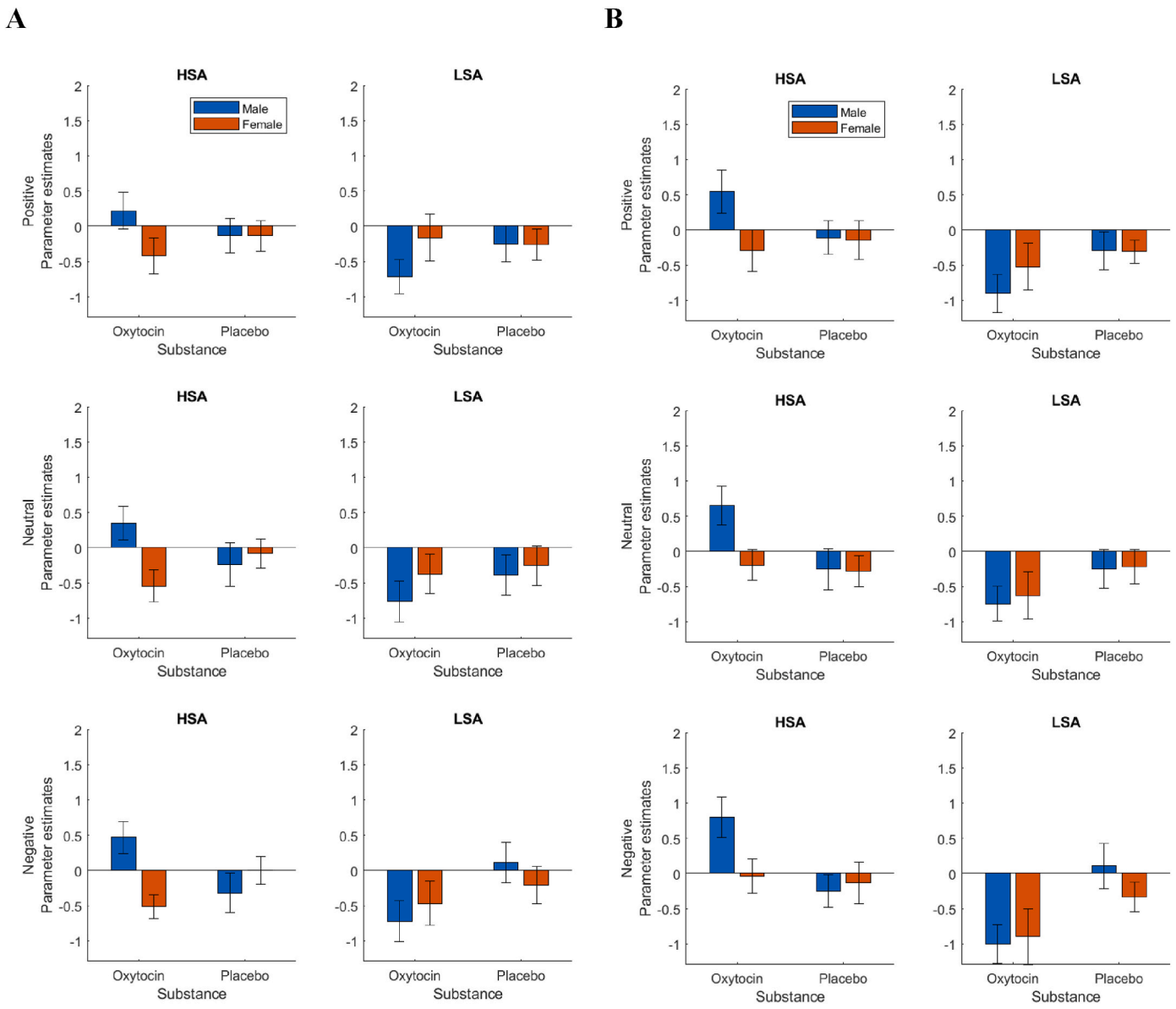

Fig. 6. Parameter estimates for **A**. the left and **B**. the right insula for high socially anxious (HSA) and low socially anxious (LSA) men (blue) and women (red) as a function of substance administered (oxytocin or placebo) for the three sentence valences (positive, neutral, negative) conditioned to the presented faces. Error bars indicate standard errors of the mean.

within the amygdala, depending on their anxiety levels and the hemisphere, and (5) the activation levels of the insula depended on an interaction between participants' anxiety levels and their oxytocin intake, the effect of which differed further by the participants' sex and was more prominent for the positively- and negatively-conditioned faces.

With respect to our hypotheses, these findings do not confirm that high socially anxious individuals rate neutral facial expressions more negatively and more arousing than individuals with low social anxiety. We were unable to discern a group difference with regard to neutral faces; instead, high socially anxious participants rated positively-conditioned faces as more arousing under oxytocin than their low socially anxious counterparts. Oxytocin therefore potentially induced a *social enhancement* of positive cues for individuals with high social anxiety, rather than a normalization of attentional bias for emotional faces in general. These results also speak against the assumption that high socially anxious individuals would be more susceptible to negative social conditioning and that this would be more pronounced in men, as we did not find sex differences.

Additionally, participants with high social anxiety provided higher sympathy ratings than participants with low social anxiety. This increased sympathy in the presence of social anxiety stands in contrast to the notion that individuals with social anxiety have difficulties with aspects of social interaction, including empathy and sympathy (Holt et al., 2018), but are in line with their desire, yet lack of courage to engage socially. Furthermore, (Weerdmeester and Lange, 2019) found that people with high social anxiety respond to rejection in a non-compensatory manner, compared to pro-social reactivity found in low socially anxious individuals. Higher sympathy ratings for others may be related to the perception of strength of the individuals judging them. Purdon et al. (2001) found that individuals with elevated social anxiety were more likely to judge others who appear anxious to have less strength of character and to be less attractive and more compassionate compared to others who do not appear anxious. Since all of the unconditioned stimuli in the present experiment (i.e., the sentences) were statements, the conditioned faces were most likely rather perceived as

vigorous rather than insecure.

Concerning our hypotheses at the neural level, our results are confirmatory with regard to the insula: we found higher activation for high socially anxious participants compared to low socially anxious participants. This finding substantiates previous research showing that SAD is associated with increased activation in brain regions involved in salience/threat processing, including the insula (Duval et al., 2018; Shah et al., 2009). However, the expected sex differences were contrary to the results found: namely, high socially anxious men in the oxytocin group showed higher activation of insula and amygdala from positively- and negatively-conditioned faces than their female counterparts. Even though this finding speaks against our predictions, the impact of sex on insula activation in social anxiety has not yet been specifically investigated. One study found that oxytocin attenuated the amygdala and anterior insula response to negative social interactions in men, but not in women (Chen et al., 2016). The fact that we do not find similar effects in the amygdala is disputable, as related research mostly finds reduced amygdala reactivity and connectivity in men in response to negative facial expressions (Domes et al., 2007; Gamer et al., 2010; Kirsch et al., 2005), while women show heightened amygdala reactivity (Domes et al., 2010; Lischke et al., 2012). However, these effects concern samples from the healthy population and may not transfer to individuals high in social anxiety; as such, neural activity and functional connectivity may depend on an individual's emotional or physiological state (see review of (van den Burg and Hegoburu, 2020).

The expectation that oxytocin would reduce neural hyperreactivity in individuals with social anxiety was also not met: high socially anxious participants in the oxytocin group showed higher activation of the insula under oxytocin than those in the placebo group (patterns were reversed for low socially anxious individuals). As mentioned above, the lack of oxytocin-related effects on amygdala activation was surprising and is inconsistent with previous research (Baumgartner et al., 2008; Domes et al., 2007; Kirsch et al., 2005). Oxytocin administration has also been shown to decrease activity in the anterior insula in response to positive social stimuli (Yao et al., 2018), dynamic angry faces (Ma et al., 2020), and empathy for embarrassment (Geng et al., 2018). Oxytocin has also been found to facilitate left insula responses for subsequently remembered negative items and increase functional coupling between the left amygdala, left anterior insula, and left inferior frontal gyrus in response to aversive social stimuli (Striepens et al., 2012). Our results instead suggest decreased activation of the insula through oxytocin as a function of social anxiety for social learning.

Specific to SAD, administration of oxytocin has been found to affect social learning processes, increasing reactivity of the amygdala, in the presence of fearful facial expressions, and the medial prefrontal cortex, in the presence of sad facial expressions (Dodhia et al., 2014; Labuschagne et al., 2010). The diverging evidence in our study may be explained by the fact that the presented faces were exclusively neutral and merely *paired* with valenced sentences, which ascribed to them a positive or negative value. Therefore, the visual information in the face stimuli remained neutral across all conditions, potentially explaining the lack of activation in the amygdala, which is said to primarily respond to emotional facial expressions (Thomas et al., 2001). Moreover, researchers such as (Hooker et al., 2006) specifically state that the response of the amygdala to facial expressions reflects the processing of primary reinforcement or emotional learning and is more responsive to learning object-emotion associations from happy and fearful facial expressions rather than the presentation of happy and fearful facial expressions alone. Considering the fact that our results are least driven by the neutral conditions, the question arises as to whether such a non-value condition can even be assigned in social learning (or learning, in general). Studies specifically addressing this notion would shed more light on differential effects in amygdala reactivity to emotional valence regarding associative learning (Damasio et al., 1991).

4.1. Limitations

One prominent limitation of our study is the small sample size following random assignment of participants into eight subgroups with regard to the variables of interest (social anxiety level, substance, sex). This limitation likely increased the within-group variance, thereby reducing the sensitivity of our measurements to detect between-group differences. Additionally, with respect to the computational level of analysis (Marr, 1982), there remains the underlying question of *what* is being processed in these regions during emotion-related tasks, such that an increase or decrease in regional activation (especially in the presence of substances such as oxytocin) can be attributed to a particular process or mechanism. Lastly, the study was not preregistered although analyses followed a protocol that was a priori approved by independent reviewers within the review process of the German Research Foundation as well as the Ethics Committee of the Medical Faculty of the Heidelberg University.

4.2. Conclusion

In sum, our study emphasizes the consideration of context, personal parameters, and task with regard to the effects of intranasal oxytocin. Oxytocin increased sympathy in socially high anxious, while decreasing arousal in socially less anxious individuals. Neural activation patterns in response to oxytocin yielded differences in gender, but also hemisphere of the examined brain regions amygdala and insula. Hence, oxytocin affected self-referential social learning behaviorally and physiologically, yet with considerable complexity regarding the investigated variables. There are currently a number of modalities to treat social anxiety (e.g., cognitive behavioral therapy, selective serotonin reuptake inhibitors/selective norepinephrine reuptake inhibitors, benzodiazepines), yet only 60%–70% of the treated patients show a clinically significant response (e.g., Hedges et al., 2007). Researchers and clinicians have sought out other candidates for social anxiety treatment, such as oxytocin. Previous literature indicates associations between social anxiety and oxytocin receptor gene alleles, as well as oxytocin plasma levels and SAD symptoms (Jones et al., 2017). However, as a potential treatment facilitator, studies show limited effectiveness of oxytocin. Voncken et al. (2021) found that oxytocin improved observer-rated social behavior in SAD patients compared to placebo, but only in the getting-acquainted task. Our findings encourage the consideration of functional hemispheric differences in the mode of action of oxytocin. Furthermore, our study is one of few to show that the involvement of the insula with regard to intranasal oxytocin administration in individuals with social anxiety should be considered for future research. While there is evidence of insula hyper-reactivity in individuals with SAD, more research is needed to confirm the reported sex differences in insular activation in social anxiety, as well as to determine whether oxytocin can enhance treatment outcomes for SAD when used with greater frequency, with a wider variety of social learning experiences, and in conjunction with interventions that more specifically target change in broader dysfunctional cognitions (Guastella et al., 2009).

Funding

The study was funded by the German Research Council (DFG; BE 5292/1-1).

CRediT authorship contribution statement

Aleya Flechsenhar: Writing – review & editing, Writing – original draft, Methodology, Formal analysis. **Seth M. Levine:** Writing – review & editing, Writing – original draft, Methodology, Formal analysis. **Laura E. Müller:** Writing – review & editing, Data curation, Conceptualization. **Sabine C. Herpertz:** Writing – review & editing, Supervision, Conceptualization. **Katja Bertsch:** Writing – review & editing, Supervision,

Resources, Project administration, Conceptualization.

Declaration of competing interest

The authors declare no conflicts of interest. This study was funded by the German Research Council (DFG; BE 5292/1-1) from September 01, 2013 until December 31, 2016. At the time of submission, SML is a Senior Scientific Editor at Elsevier.

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.neuropharm.2024.109930.

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