Oxytocin enhances basolateral amygdala activation and functional connectivity while processing emotional faces: preliminary findings in autistic versus non-autistic women

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Abstract

Oxytocin is hypothesized to promote social interactions by enhancing the salience of social stimuli. While previous neuroimaging studies have reported that oxytocin enhances amygdala activation to face stimuli in autistic men, effects in autistic women remain unclear. In this study, the influence of intranasal oxytocin on activation and functional connectivity of the basolateral amygdala – the brain's "salience detector" – while processing emotional faces vs. shapes was tested in 16 autistic and 21 non-autistic women by fMRI in a placebo-controlled, within-subjects, cross-over design. In the placebo condition, minimal activation differences were observed between autistic and non-autistic women. However, significant drug × group interactions were observed for both basolateral amygdala activation and functional connectivity. Oxytocin increased left basolateral amygdala activation among autistic women (35 voxel cluster, MNI coordinates of peak voxel= -22 -10 -28; mean change=+0.079%, t=3.159, p_{tukey}=0.0166), but not non-autistic women (mean change =+0.003%, t=0.153, p_{tukey}=0.999). Furthermore, oxytocin increased functional connectivity of the right basolateral amygdala with brain regions associated with socio-emotional information processing in autistic women, but not non-autistic women, attenuating group differences in the placebo condition. Taken together, these findings extend evidence of oxytocin's effects on the amygdala to specifically include autistic women and specify the subregion of the effect.

Keywords: autism**,** basolateral amygdala, emotional face processing, oxytocin, salience

Background

For more than a decade, the neuropeptide oxytocin has been considered a candidate treatment for promoting social well-being in people with psychiatric conditions (1). The effects of oxytocin among autistic people have attracted particular attention, with early studies reporting that intranasal administration of oxytocin enhances emotion recognition (2) and eye contact during social interactions (3), among various effects. In efforts to clarify the clinical applications of oxytocin, neuroimaging studies have sought to identify the neurological underpinnings of oxytocin's effects on social behavior and cognition. Such studies have reported that a single dose of intranasal oxytocin influences amygdala activation in neurotypical participants (4–9), people with affective or anxiety-related disorders (10–15), and in animal models (16,17). Effects on the amygdala may help to explain two of oxytocin's broad effects, namely increased attention and orientation to social stimuli (18) and reduced anxiety responses to social stimuli (19,20).

In light of its central role in social information processing, the amygdala was one of the first brain regions implicated in the neurobiology of autism and continues to be seen as key to understanding autistic behavioral phenotypes (21). The amygdala theory of autism (22) proposed that diminished amygdala activation underlies alterations in attention and response to social stimuli. The neuroimaging literature supports this theory to some degree, with evidence of lower amygdala activation to emotional faces among autistic participants relative to controls (e.g., 22–24; for exceptions see 25–27). Notably, intranasal oxytocin is reported to influence amygdala activation to social stimuli in autistic people. In a study involving 14 autistic men and 14 non-autistic men who performed a face-matching task, a 24 IU dose of oxytocin increased right amygdala activation specifically among autistic participants, attenuating the group difference observed in the placebo condition (28). Oxytocin was also reported to increase left amygdala

activation during an emotion recognition task involving the same sample of autistic men (29). In a study where 20 autistic adults (19 men, 1 woman) played a virtual ball-tossing game, amygdala activation increased in the oxytocin condition (24 IU) when the virtual partner behaved in an unfair manner, but decreased when the partner behaved in an equitable manner. By contrast, a study of 19 autistic youth (16 boys, 3 girls, aged 8–16.5 years) found no effect of oxytocin (12–24 IU, depending on participant age) on amygdala activation during an emotion recognition task (30). However, a positive correlation was observed between amygdala activation and pre- to post-administration salivary oxytocin levels, suggesting that participants who experienced larger oxytocin increases tended to show increased amygdala activation. Taken together, these studies support that oxytocin enhances amygdala activation to socially relevant stimuli in autistic people, with effects varying with context and individual differences.

In oxytocin studies involving neurotypical participants, biological sex has emerged as a likely variable moderating oxytocin's effects on brain and behavior. Importantly, several studies have found that the effect of oxytocin on amygdala activation in female participants to be opposite to that originally reported in all-male samples (31–33). While the precise cause of sex differential effects of oxytocin remains unclear, they may arise due to interactions with sex steroid hormones or baseline sex differences in the neural oxytocin system (34,35). As the few studies examining the effects of oxytocin on amygdala activation in autistic people have involved predominantly men, the ability of oxytocin to enhance amygdala response to social stimuli should not be assumed to generalize to autistic women.

Aiming to extend knowledge of oxytocin's effects on the amygdala to include autistic women, the present study used fMRI to examine the influence of a single 24 IU dose of intranasal oxytocin on neural activation to emotional face stimuli in autistic and non-autistic women. In

light of our specific interest in the effects of oxytocin on the salience of social stimuli, our analysis focused on the basolateral amygdala, which has been described as the brain's "salience detector" due to its responsivity to both reward and threat (36,37). Informed by the above-described studies, we predicted that autistic women would show lower basolateral amygdala activation relative to non-autistic women in the placebo condition and that oxytocin would enhance basolateral amygdala activation in both autistic and non-autistic women. Furthermore, we predicted that oxytocin would enhance functional connectivity of the basolateral amygdala with other brain regions involved in processing socio-emotional information. Lastly, to explore individual differences that may moderate oxytocin's effects, we explored the relationships of autistic-like traits, social anxiety, and salivary oxytocin levels with amygdala activation.

Materials and methods

Study design and drug administration

Adopting a double-blind, placebo-controlled, cross-over design, participants completed two experimental sessions with drug order randomly determined. Of the non-autistic participants, 10/21 received oxytocin first; of the autistic participants, 9/16 received oxytocin first. To minimize variation in endogenous hormone levels, both sessions were scheduled during the follicular phase of the menstrual cycle; for participants who self-reported use of hormonal contraceptives, sessions were scheduled at least one week apart. On each experiment day, participants underwent a health screening by a clinician and were then instructed to self-administer nasal spray containing oxytocin (4 IU per puff; total dose 24 IU; Syntocinon, Novartis, Switzerland) or placebo. Participants rested for approximately 20 minutes before scanning. The emotional face-matching task described here began approximately 55 minutes after administration. A time window of 45–70 minutes after administration of 24 IU oxytocin is

reported to be optimal for assessing effects on the amygdala (38). Saliva samples were collected pre- and post-administration (immediately and 90 min post-administration), and salivary oxytocin was quantified by radioimmunoassay performed by an external lab (full details of salivary hormone analyses in these participants are reported in (39)). In advance of the experimental sessions, participants completed the autism-spectrum quotient (AQ), which assesses autistic-like traits (40), and the Liebowitz social anxiety scale (LSAS), which assesses social phobia and avoidance across various situations (41). This work is part of a larger study of the effects of oxytocin in autistic and non-autistic women, and the effects of oxytocin on resting-state connectivity are reported elsewhere (42,43).

The *a priori* power calculation indicated that a sample size of 34 was needed for a repeated measures mixed effect design with 2 measures across 2 groups with $\alpha = 0.05$, an estimated effect size of 0.35, and a correlation between measures of 0.4. A recent meta-analysis of common fMRI tasks reported a intraclass correlation coefficient of 0.397, which is consistent with the value used in our *a priori* power calculation (44).

All participants provided written informed consent prior to participation. This work was approved by the NHS Research Ethics Service (NRES Committee East of England-Cambridge Central, 14/EE/0202) and conducted following the Declaration of Helsinki. The UK Medicines and Healthcare Regulatory Agency (MHRA) exempted this study from clinical trial status.

Participants

A total of 42 women were recruited from Cambridge, UK, and the surrounding area to participate in this study and completed both neuroimaging sessions. Four non-autistic women were excluded due to data acquisition errors and one non-autistic woman was excluded due to

excessive motion, as detailed in the fMRI preprocessing section below. Table 1 presents the demographic characteristics, hormonal contraceptive use, questionnaire scores, and salivary oxytocin levels for the final sample of 16 autistic and 21 non-autistic women. As shown, the groups did not differ significantly in age, full-scale IQ, or salivary oxytocin levels. Autistic women had significantly higher AQ and LSAS scores relative to non-autistic women.

Autism was determined as a clinical diagnosis of an autistic disorder/childhood autism or Asperger's disorder/syndrome based on DSM-IV or ICD-10 criteria. Exclusion criteria were pregnancy, cigarette smoking, substance dependence, epilepsy, a genetic syndrome related to autism, intellectual disability, and a diagnosis of bipolar, obsessive-compulsive, panic, or psychotic disorder.

fMRI task

Participants completed an established emotional face-matching paradigm (45,46). To minimize potential context-related effects of varying emotional expressions, we used a variant involving only negatively-valanced faces (angry or fearful) shown to elicit robust bilateral amygdala response in autistic and non-autistic participants (47). The task design is shown in Supplementary Figure S1. Participants completed four blocks of face-matching interspersed with five blocks of shape-matching. Each block comprised six trials. Participants were instructed to press a button corresponding to which of the two images at the bottom of the screen matched the image at the top of the screen. Response time was recorded for each trial. For face-matching trials, images were matched for the same emotion; for shape-matching trials, images were matched for the same orientation. As shown, the shape stimuli were scrambled images of the face stimuli.

fMRI data acquisition and preprocessing

Scanning was performed at the Wolfson Brain Imaging Centre in Cambridge, UK, using a 3T Siemens MAGNETOM Tim Trio MRI scanner. Functional images were acquired with a multi-echo EPI sequence (field-of-view (FOV) = 240 mm; flip angle = 80°; 3 echoes at TE = 12, 29, and 46 ms; repetition time (TR) = 2300 ms; 33 oblique slices, interleaved slice acquisition, slice thickness = 3.8 mm, 11% slice gap; GRAPPA acceleration factor = 2, BW = 2368 Hz pixel^{-1}). Anatomical images were acquired using a T1-weighted magnetization prepared rapid gradient echo (MPRAGE) sequence (FOV = 256 mm; flip angle = 9°; TE = 2.98 ms; TI = 900 ms; TR = 2250 ms; voxel size = 1 mm^3; acquisition matrix size = 256 × 256 × 256 mm).

Multi-echo functional images were combined and denoised using the AFNI-integrated multi-echo independent component analysis (ME-ICA, meica.py v3) pipeline (48). After deleting the first four volumes, which were dummy scans collected before task onset, each TE functional dataset was slice-time corrected and decomposed into independent components (ICs). ICs were categorized as representing BOLD or non-BOLD signals based on their linear relationship with TE (49). Removal of non-BOLD ICs increases the signal-to-noise ratio by systematic removal of motion, physiological, and scanner artifacts based on the characteristics of T2* decay, and has been shown to improve estimates of effect size (49,50). Additional pre-processing included removal of non-brain areas, co-registration of structural images to the first TE functional image, spatial smoothing with a 6 mm FWHM Gaussian kernel, and registration to the MNI152 2 mm template.

Four participants were excluded due to data acquisition issues and one participant was excluded due to excessive motion (mean framewise displacement (FD) = 0.75 mm, max FD = 6 mm) in

the placebo session. Average FD in the included 37 participants did not differ between drug conditions (placebo = 0.157 ± 0.09 mm, oxytocin = 0.155 ± 0.06 mm, p = 0.92).

Subject-level fMRI analysis

FEAT (FMRI Expert Analysis Tool), part of FSL 6.00 (FMRIB's Software Library, www.fmrib.ox.ac.uk/fsl) was used to fit a general linear model (GLM) for each participant for each session. Two regressors of interest were included, representing the onset times of the face-matching and shape-matching blocks. Neural response to emotional face stimuli was obtained as the contrast of activation to faces relative to shapes (Faces > Shapes). The generated contrast images for each participant were then used in the group-level analyses.

Group-level fMRI analyses

Planned analyses included: (i) group differences (autistic vs. non-autistic) in the baseline (placebo) condition; (ii) Drug × Group differences; and (iii) correlations between oxytocin-associated amygdala activation and autistic-like traits (AQ), social anxiety (LSAS), and salivary oxytocin levels.

Group analyses were conducted as higher-level mixed-effect analyses using the FMRIB Local Analysis of Mixed Effects (FLAME) tool in FSL. For analyses of group differences in the placebo condition, drug order (i.e., whether the placebo session occurred first or second) and hormonal contraceptive use were included as regressors of no interest. Analyses were performed separately for maps of the left and right basolateral amygdala, which were determined using probabilistic maps from the Juelich Histological Atlas implemented in FSLeyes. Voxels were included if ≥ 50% probability of belonging to the laterobasal nucleus of the amygdala complex

(based on (51); see Supplementary Figure S2). Significant results were determined as $Z > 2.3$ and a cluster-corrected threshold of $p < 0.05$. In the case of significant clusters, the contrast estimates for Faces > Shapes were extracted for the cluster using *featquery* as percent signal change and imported into R for post-hoc analyses. In addition to this region of interest (ROI) analysis focused on the basolateral amygdala, exploratory whole-brain analyses were conducted using an uncorrected statistical threshold of $p < 0.001$ and a cluster extent threshold $k > 10$ voxels.

Functional connectivity analysis

Task-based functional connectivity of the basolateral amygdala with other brain regions was examined using the psychophysiological interaction (PPI) approach. The left and right basolateral amygdala masks described above were used as the functional seeds. In FSL, PPI is modeled as the interaction between the task regressor (Faces > Shapes) and the timeseries of the ROI (extracted for each participant using *fslmeants;* see Supplementary Materials for further details). The subject-level results were then used in group-level random effects models (FLAME1) to compare connectivity of the amygdala seeds with other brain regions implemented in FEAT as a 2 (Drug) × 2 (Group) repeated measures ANOVA. PPI analyses were performed for the whole-brain and results were considered significant at $Z > 2.3$ and cluster-corrected $p < 0.05$. Post-hoc analyses were performed by extracting the parameter estimates for the contrast Faces > Shapes for the significant clusters using *featquery*.

Statistical analysis

Statistical analyses were performed using R software (52). The effects of oxytocin on task performance and clusters in autistic vs. non-autistic women were assessed using the 'lmerTest'

package (53), with drug and group as fixed effects and participant ID as a random effect, controlling for drug order and hormonal contraceptive use. Fixed effects are reported as type III ANOVA with Satterwaithe's method. Post-hoc tests were performed by comparing pairwise differences in estimated marginal means ('emmeans' package) with Tukey adjustment for multiple comparisons. Change in activation of the basolateral amygdala ROI between drug conditions was computed as $activation_{OXYTOCIN}$ - $activation_{PLACEBO;}$ thus, a positive value indicates increased activation in the oxytocin condition. Relationships between activation and psychological/hormonal variables were then assessed using correlation analyses.

Results

Behavioral results

Participants' reaction times were recorded for all trials and compared between groups (autistic vs. non-autistic), drug conditions (oxytocin vs. placebo), and stimulus type (shapes vs. faces). As shown in Figure 1, reaction times were significantly faster for matching shapes than matching faces ($F_{(1,112)} = 844$, $p < 0.001$). No other effects or their interaction were statistically significant.

Functional activation analyses

Group differences

Among all participants, the contrast Faces > Shapes resulted in significant bilateral amygdala activation as well as activation of the frontal cortex, visual areas, motor areas, temporal occipital fusiform cortex, and cerebellum (Supplementary Figure S3). No significant group differences in basolateral amygdala activation were observed between autistic and non-autistic women in the baseline (placebo) condition (Supplementary Figure S4, S5). Exploratory whole-brain analyses (Supplementary Table S1) indicated greater angular gyrus and cerebellum activation in non-

autistic women relative to autistic women, while autistic women relative to non-autistic women showed greater activation of the middle frontal gyrus.

Effect of oxytocin on basolateral amygdala activation

The ROI analysis indicated a significant Drug × Group interaction for the left basolateral amygdala ($Z = 2.96$, $p = 0.0266$, $k = 35$ voxels, MNI coordinates of peak activation = -22 -10 -28; Figure 2). Similar findings were obtained when the 6 women taking hormonal contraceptives (all non-autistic) were excluded (see Supplementary Materials). Post-hoc tests showed higher activation in the oxytocin relative to placebo condition among autistic women (pairwise difference = 0.079, $t = 3.159$, $p_{tukey} = 0.0166$), but no significant change among non-autistic women (pairwise difference = 0.003, $t = 0.153$, $p_{tukey} = 0.999$). As show in Figure 2, 14 of the 16 (88%) autistic women participants showed an activation increase in the oxytocin condition; by contrast, 13 of the 21 (62%) non-autistic women showed an activation increase in the oxytocin condition.

Relationship of amygdala activation with psychological and hormonal variables

With the aim of understanding individual variation in the effects of oxytocin on amygdala activation among our participants, we explored the relationships between activation of the left basolateral amygdala ROI and autistic-like traits, social anxiety, and salivary oxytocin levels. No relationship was observed between AQ score and left basolateral amygdala activation in non-autistic women (Supplementary Figure S6). A moderate, but not statistically significant, correlation was observed between self-reported social anxiety score and oxytocin-associated change in left basolateral amygdala activation in non-autistic women ($r = 0.34$, $p = 0.15$; Supplementary Figure S7), whereas autistic women showed a moderate, but not statistically significant, correlation between pre- to post-administration change in salivary oxytocin and left

basolateral amygdala activation (r = 0.48, p = 0.06; Supplementary Figure S8). Additional details and results are provided in the Supplementary Materials.

Functional connectivity analyses

Group differences

For the contrast Faces > Shapes in the baseline (placebo) condition, non-autistic women showed higher connectivity of bilateral basolateral amygdala seeds with large clusters that included the frontal lobe, temporal/occipital fusiform cortex, precuneus, putamen, and cerebellum ($Z > 2.3$, cluster-corrected $p < 0.05$; Supplementary Table S2, Supplementary Figure S9). No group differences were observed in the oxytocin condition.

Effect of oxytocin on functional connectivity of basolateral amygdala

Using the right basolateral amygdala as the seed, a significant main effect of Drug (Table 2a; Figure 3) was observed such that connectivity with two large clusters comprising frontal areas (cluster 2) and cerebellar/occipitotemporal areas associated with visual processing (cluster 1) was higher in the oxytocin relative to placebo condition in the combined participant sample. In addition, significant Drug × Group interactions were observed for four clusters (Table 2b; Figure 3). Post-hoc analyses showed that, for autistic women, the functional connectivity of all four clusters was significantly higher in the oxytocin relative to placebo condition (all $p_{tukey} < 0.01$; see Supplementary Table S3 for the mean value for each cluster); by contrast, non-autistic women showed non-significant decreases in connectivity of the right basolateral amygdala seed with the four clusters. Similar results were obtained when the 6 women taking hormonal contraceptives (all non-autistic) were excluded (see Supplementary Materials).

Discussion

Despite more than a decade of research efforts, intranasal oxytocin as a treatment for social cognition challenges has not been realized, in part due to remaining uncertainties about its mechanism of action, optimal treatment course, and heterogeneity in effects related to sex, age, and clinical phenotype (35,54,55). Given the underrepresentation of women in both oxytocin research (56) and autism research (57), the influence of oxytocin on amygdala activation in autistic women was essentially unknown prior to this study.

The present study used fMRI to test the effects of a single 24 IU dose of intranasal oxytocin on basolateral amygdala response to emotional face stimuli in a sample of autistic and non-autistic women matched for age and IQ. Autistic women, but not non-autistic women, showed a significant increase in left basolateral amygdala activation in the oxytocin condition. This result is broadly consistent with the findings of Domes et al. (29), who reported increased left amygdala activation (11 voxels, x = -18, y = -1, z = -23) to images of the eye or mouth area of emotional faces among autistic men, but no change among non-autistic men. Moreover, as was the case in the study by Domes et al., the oxytocin-associated increase in activation resulted in, on average, higher left amygdala activation in autistic vs. non-autistic participants in the oxytocin condition, rather than attenuation of a group difference. Domes et al. (29) interpreted increased left amygdala activation in autistic men as indicating greater salience of the emotional face stimuli, as there was a positive correlation between amygdala activation and performance on the emotion recognition task. Although oxytocin had no significant effect on performance of the emotional face-matching task in our study, we interpret the effect specific to the basolateral amygdala to reflect an alteration in salience processing. Domes et al. did not address the amygdala subregion of their finding, but we note that the peak voxel has a >20% probability of belonging to the basolateral group. Despite early oxytocin administration research reporting

amygdala subregion-specific effects (e.g., 9), many oxytocin- and autism-related studies have treated the amygdala as a homologous structure, potentially obscuring the findings. Whereas the "amygdala theory of autism", as detailed above, did not make amygdala subregion-related predictions, a model of amygdala alterations in psychiatric conditions specifically predicts hypoactivity of the basolateral amygdala (58) to be associated with reduced reflexive shifting of gaze to the eye area, manifesting in social and emotional challenges. Thus, we suggest that future studies consider amygdala subregions to clarify the functional significance of their findings and possible clinical implications.

As the oxytocin administration literature grows, it has become increasingly clear that its effects vary across individuals (59). Indeed, not all autistic women in our study showed increased basolateral amygdala activation in the oxytocin condition. However, the additional variables considered (AQ, LSAS) did not significantly explain the observed variation. Although there are no other studies of autistic women with which to compare our findings, several studies have reported that oxytocin decreased amygdala activation to social stimuli in women with borderline personality disorder (60, 61). While this result contrasts with our main finding that oxytocin increased amygdala activation in autistic women, the borderline personality neurophenotype also contrasts with autism, with the borderline personality participants reported to show higher amygdala activation to social stimuli than controls at baseline (and some evidence of lower amygdala activation in autism, as detailed above). Taken together, these findings suggest that the effects of oxytocin on amygdala activation in women vary with clinical condition, and may "normalize" activation only in cases of deviation from optimal amygdala responsivity to social and emotional stimuli.

In the present study, we also performed functional connectivity analyses to examine group differences and effects of oxytocin on the functional coupling of the amygdala with other brain regions during the emotional face-matching task. In contrast to the similarities in basolateral amygdala activation between groups, widespread differences in functional connectivity were observed in the placebo condition, with autistic women showing lower connectivity of bilateral basolateral amygdala seeds with frontal, striatal, and occipitotemporal areas associated with reward and processing of socio-emotional information (see Supplementary Table S2). In the combined sample, oxytocin increased functional connectivity of the right basolateral amygdala seed with frontal, cerebellar, and occipitotemporal areas. However, significant Drug × Group effects were also present, with post-hoc analyses indicating that oxytocin's enhancement of functional connectivity was limited to autistic women, with non-autistic women showing non-significant decreases in connectivity. Notably, these opposite directional changes in autistic and non-autistic women attenuated the group differences in functional connectivity present in the placebo condition. Several previous studies have reported lower amygdala functional connectivity during socially relevant tasks in autistic relative to non-autistic participants (62–64), and that oxytocin influences resting-state connectivity of the amygdala in autistic people (65,66). However, to the best of our knowledge, this is the first report of oxytocin's effects on task-associated amygdala functional connectivity in autistic people. A study using a comparable emotional face-matching task reported that oxytocin enhanced connectivity of the amygdala with the insula and cingulate while processing images of fearful faces in a sample of men with generalized social anxiety disorder, but not in controls (14). Similar to our findings, the oxytocin-associated increases in connectivity in men with anxiety attenuated the group

differences observed in the placebo condition. While our functional connectivity findings should be considered preliminary, they suggest that examining connectivity of an amygdala-related network, rather than amygdala activation in isolation, may be useful for understanding differences in how social-emotional information is processed between autistic and non-autistic people and how oxytocin influences coupling of relevant brain networks.

This study is subject to several limitations. Although this women-focused study has increased the representation of autistic women in oxytocin research, the sample size of 16 is relatively small. As a result, effects of oxytocin of a small effect size are unlikely to have been detected, which is a limitation of many intranasal oxytocin studies (67). Further studies in larger samples are warranted to confirm these findings. Second, we lack a true baseline condition for the comparison of amygdala activation between groups. Although we found no significant differences, it is possible that placebo administration exerted its own effect on the amygdala, obscuring group differences. Third, measures of salivary oxytocin following intranasal oxytocin administration are greatly elevated due to 'drip down' into the throat. In our analysis, changes in oxytocin were scaled relative to participants' pre-administration oxytocin level (% change) as an effort to account for inflated post-administration values. Nevertheless, the finding of a relationship between oxytocin-associated changes in the present study and previous work involving autistic people (68–70) highlights the need for a more reliable marker of individual differences in the oxytocin system than saliva or plasma levels (71). Lastly, this was a single administration study and we did not collect data on participants' subjective emotional responses to the face stimuli or effects of the experiment on mood or arousal levels. As the ultimate aim of oxytocin administration is to improve quality of life for people interested in a pharmacological intervention, studies examining the effects of long-term oxytocin use on amygdala reactivity to

naturalistic social stimuli, as well as subjective social well-being, are needed. While several studies have now reported positive effects of long-term oxytocin administration in autistic people (e.g., 72,73), autistic women continue to be underrepresented in such work. Future oxytocin administration studies need to include sex-balanced and gender-inclusive samples.

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Figure captions

Figure 1. Boxplots showing median response time (in milliseconds) for face-matching and shape-matching trials between groups (autistic vs. non-autistic) and drug conditions (placebo vs. oxytocin). Only the effect of task (matching faces vs. shapes) was significant. ** $p < 0.01$

Figure 2. Boxplots showing activation of the left basolateral amygdala cluster identified in the group analysis (35 voxels, peak activation = -22 -10 -28) between drug conditions and groups. Each dot represents one participant and the gray lines connect their activation values for the contrast Faces > Shapes between drug conditions. Image is shown such that the left side is the left hemisphere. * $p < 0.05$

Figure 3. Effects of oxytocin (vs. placebo) on functional connectivity. Using the right basolateral amygdala as the seed, oxytocin increased connectivity with temporal lobe and occipital lobe clusters. A significant Drug × Group was also observed such that oxytocin significantly increased connectivity with frontal lobe regions and the cerebellum among autistic women. All tests $Z > 2.3$, $p < 0.05$ cluster-corrected. Image is shown such that the left side is the left hemisphere.

Tables

Table 1. Characteristics of the autistic and non-autistic women included in the analysis**.**

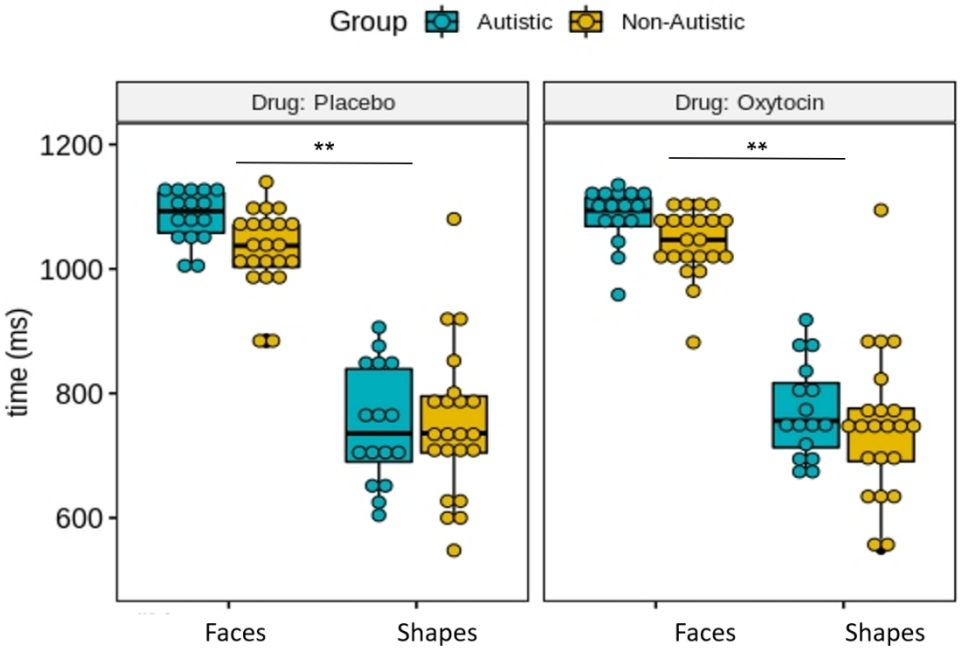
	Autistic	**Non-autistic**	***p***
N	16	21	
Age (years)	29.9 ± 8.4	26.4 ± 7.9	0.20
Full-scale IQ	121 ± 16.4	117.9 ± 13.7	0.52
Hormonal contraceptive use (n)	0	6	0.01*
Psychological traits			
AQ	37.1±5.2	13.2 ± 6.6	< 0.001**
LSAS	73.3±24.2	31.71±13.6	< 0.001**
Salivary oxytocin (pg/ml) [1]			
Placebo condition			
Baseline	3.3 ± 0.9	3.0 ± 0.9	0.17
Immediately post-administration	3.0 ± 0.9	2.7 ± 0.8	0.32
90 min post-administration	2.9 ± 1.0	2.6 ± 0.7	0.30
Oxytocin condition			
Baseline	2.8 ± 0.9	2.9 ± 0.8	0.89
Immediately post-administration	112.9 ± 15.1	113.5 ± 16.0	0.91
90 min post-administration	46.1 ± 15.6	35.3 ± 21.3	0.08

AQ = Autism-spectrum quotient, , LSAS = Liebowitz social anxiety scale; Full-scale IQ = Weschler abbreviated scale of intelligence, * $p < 0.05$, ** $p < 0.01$

Table 2. Summary of oxytocin effects on functional connectivity of right basolateral amygdala seed with other brain regions in autistic and non-autistic women during the experimental task (Faces > Shapes). Z > 2.3 and cluster-corrected p < 0.05.

	k	p		Z	x	y	z
(a) Main effect of Drug							
Cluster 1	944	0.0012	Cerebellum	3.82	-44	-60	-44
			Lateral Occipital Cortex	3.48	-48	-78	-14
			Inferior Temporal Gyrus/Temporal Occipital Fusiform Cortex	3.38	-48	-50	-22
			Cerebellum	3.26	-42	-56	-30
			Cerebellum	3.21	-48	-70	-38
			Lateral Occipital Cortex	3.21	-50	-72	-16
Cluster 2	1083	0.000423	Precentral Gyrus	4.05	-52	-10	38
			Superior Frontal Gyrus	3.36	-18	8	52
			Precentral Gyrus	3.31	-32	-6	54
			Superior Frontal Gyrus	3.3	-24	0	54
			Precentral Gyrus	3.15	-48	-4	42
(b) Group x Drug interaction							
Cluster 1	737	0.00626	Cerebellum	3.58	-32	-48	-46
			Cerebellum	3.32	-40	-68	-50
			Lateral Occipital Cortex	3.22	-52	-68	-22
			Cerebellum	3.21	-40	-50	-42
Cluster 2	1100	0.000374	Lateral Occipital Cortex	3.99	-30	-64	46
			Superior Parietal Lobule	3.57	-36	-56	56
			Lateral Occipital Cortex	3.47	-32	-76	42
			Lateral Occipital Cortex	3.39	-28	-74	42
			Supramarginal Gyrus/Superior Parietal Lobule	3.37	-44	-50	54
			Angular Gyrus	3.14	-36	-52	34
Cluster 3	2364	p<0.000001	Middle Frontal Gyrus	3.86	-42	36	20
			Middle Frontal Gyrus	3.74	-44	32	28
			Middle Frontal Gyrus	3.57	-38	22	30
			Frontal Pole	3.49	-50	40	4
			Superior Frontal Gyrus	3.41	-14	34	42
			Frontal Pole	3.31	-32	48	18
Cluster 4	4275	p<0.000001	Lingual Gyrus	3.98	-4	-78	-16
			Cerebellum	3.94	0	-76	-26
			Lingual Gyrus/Visual Cortex	3.73	12	-62	-4
			Occipital Fusiform Gyrus	3.71	2	-82	-24
			Lingual Gyrus/Visual Cortex	3.6	18	-58	-2
			Precuneus Cortex	3.59	22	-58	16

k = cluster size in voxels, xyz = MNI coordinates of peak activation.



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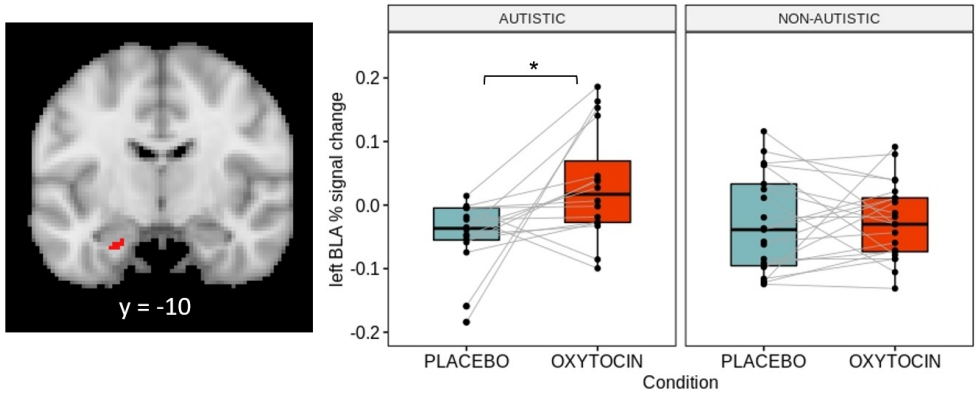

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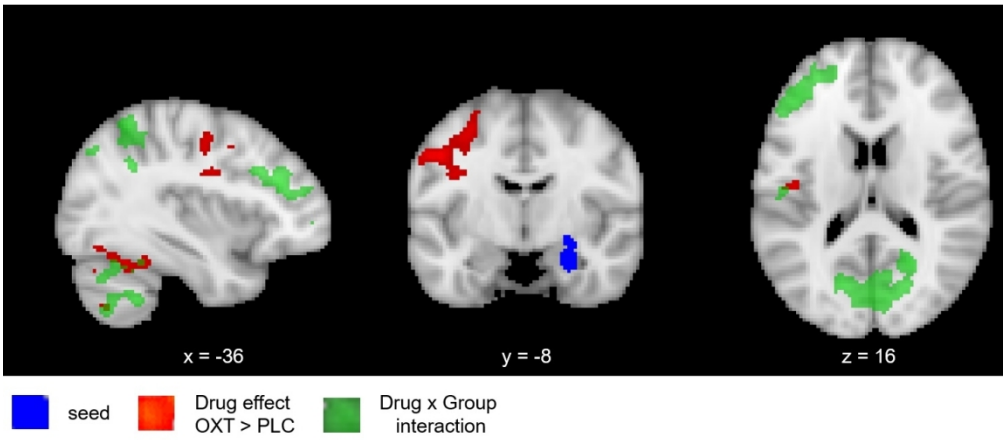

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123x55mm (300 x 300 DPI)

Supplementary Materials: *Oxytocin enhances basolateral amygdala activation and functional connectivity while processing emotional faces: preliminary findings in autistic women versus non-autistic women*

Procyshyn et al. 2022

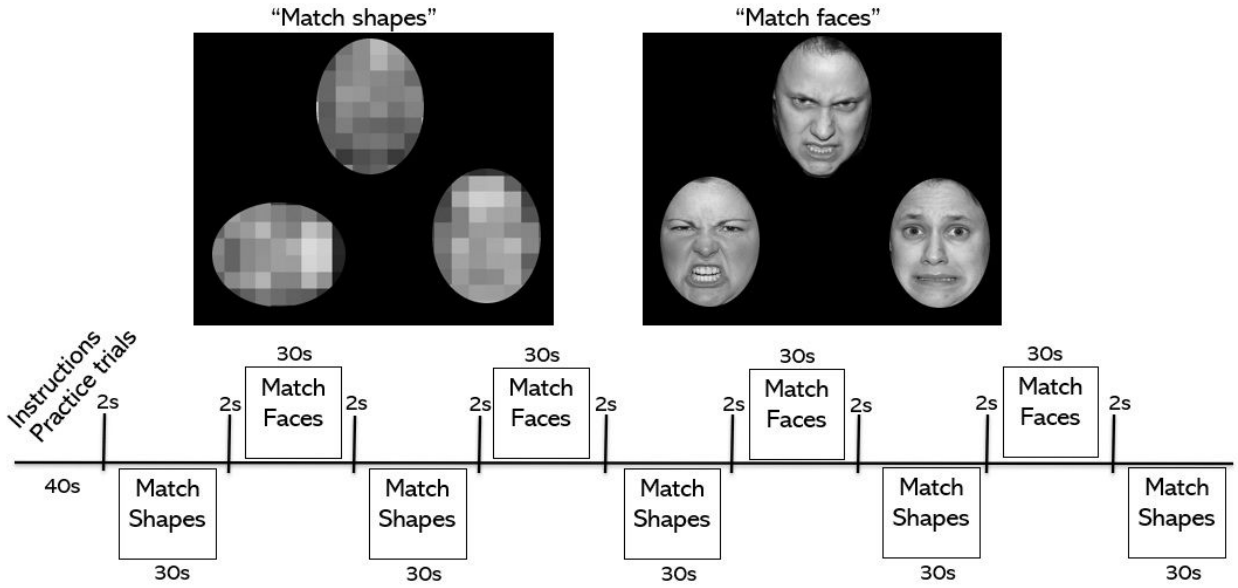

Figure S1. Schematic of the experimental task design. Participants completed five blocks of shape-matching and four blocks of face-matching, separated by 2 s of instructions. Images were matched for the same emotion or the same orientation. Each block comprised 6 matching trials lasting 5 s each, for a total duration of 30 s per block.

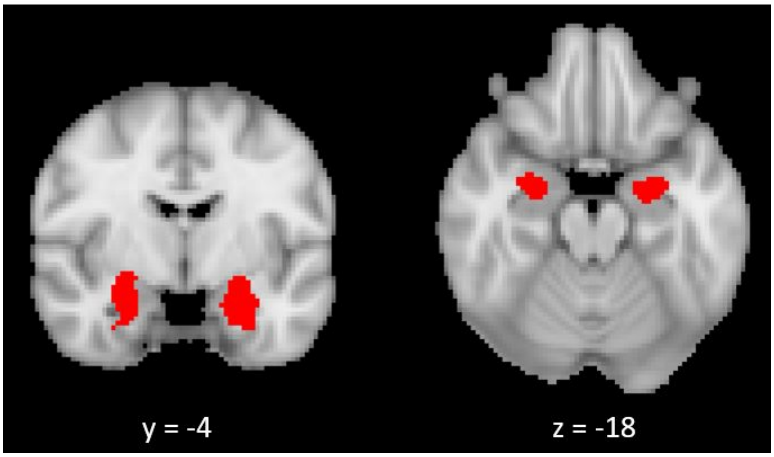

Figure S2. Region of interest was defined as voxels with ≥ 50% probability of belonging to the latero-basal nucleus of the amygdala complex in the Juelich Histological Atlas implemented in FSLeyes.

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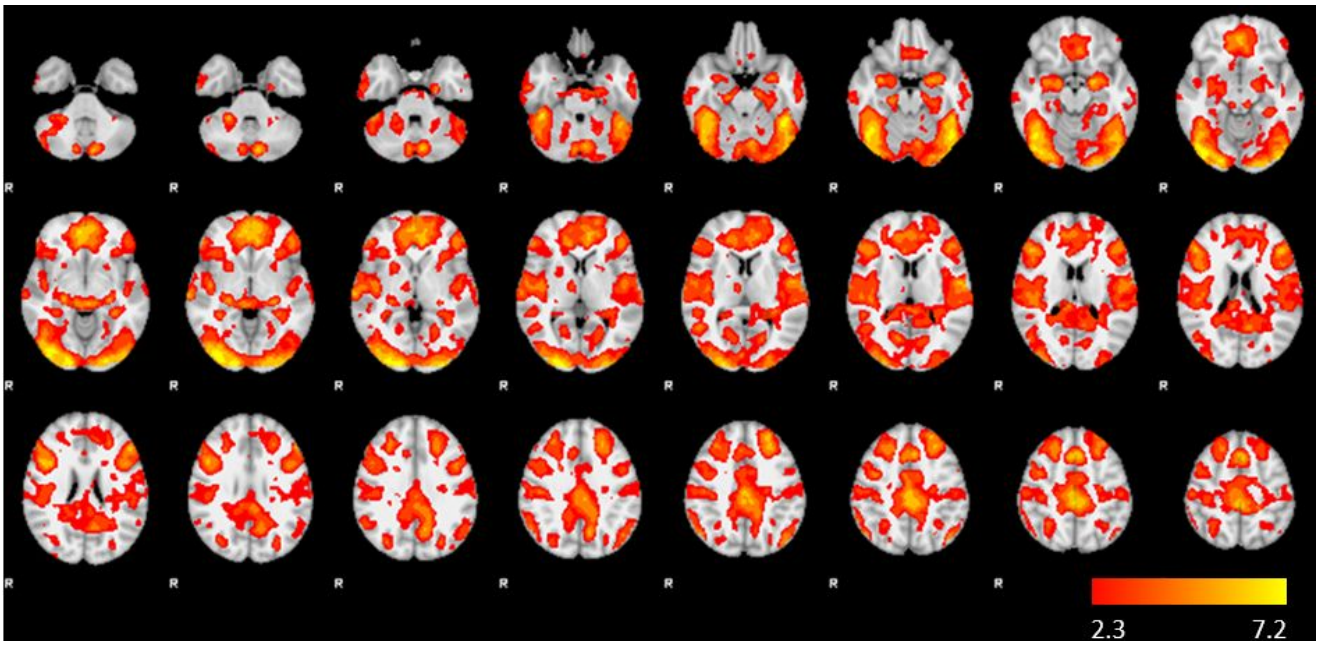

Figure S3. Whole-brain activation for the contrast Faces > Shapes in the baseline (placebo) condition for all participants (FLAME, Z > 2.3, cluster-corrected p < 0.05).

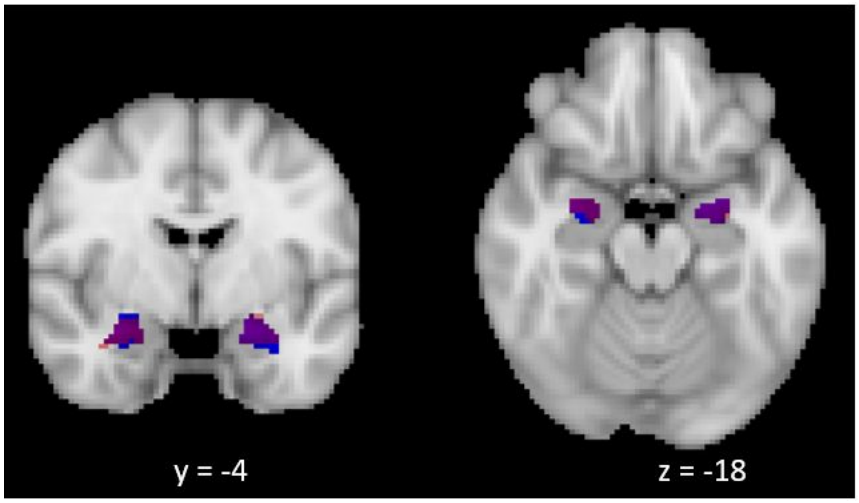

Figure S4. The area of basolateral amygdala activation to Faces > Shapes in the baseline (placebo) condition was spatially similar between autistic and non-autistic women (Z > 2.3, cluster-corrected p < 0.05). The area of activation for autistic women is indicated in red and activation for non-autistic women is indicated in blue; overlapping areas of activation are thus shown in purple.

Supplementary Materials, *Procyshyn et al. 2022*

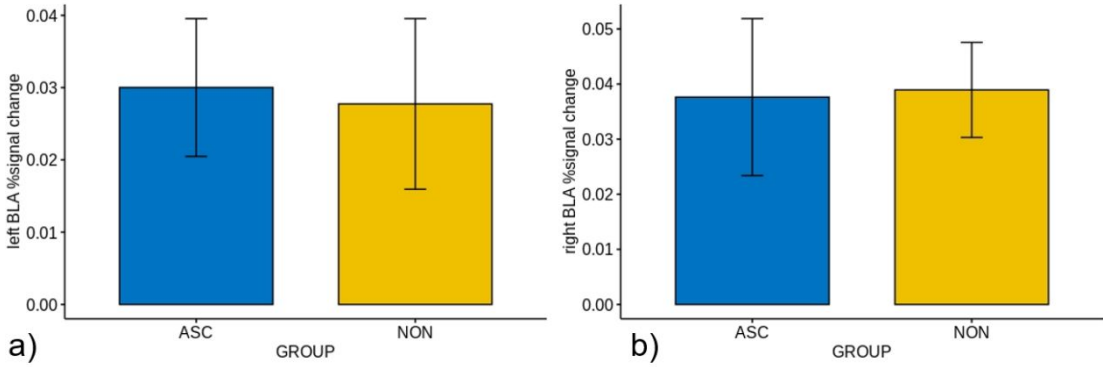

Figure S5 In the placebo condition, no significant differences in (a) left or (right) basolateral amygdala (BLA) activation were observed between autistic (ASC) and non-autistic (NON) women. Bar plots show mean and standard error.

Table S1. Summary of findings of group differences (autistic vs. non-autistic) in the placebo condition in whole-brain analysis of the contrast Faces > Shapes ($p < 0.001$, uncorrected, $k > 5$).

	side	region	Z	k	x	y	z
(a) NON > ASC	R	Angular gyrus	3.71	17	46	-56	28
	L	Cerebellum	3.48	17	-10	-80	-40
	R	White matter	4.21	8	22	6	34
(b) ASC > NON	L	Middle frontal gyrus	3.56	7	-50	32	26

ASC = autistic group, NON = non-autistic group; k = cluster size in voxels; x y z refer to MNI coordinates; region labelled according to anatomical atlases included in FSLeyes.

Supplementary Materials, *Procyshyn et al. 2022*

Relationship of amygdala activation with psychological and hormonal variables

AQ score

Given the significant Drug x Group difference in effects of oxytocin on activation of the left basolateral amygdala, we further predicted that non-autistic women with higher levels of autistic-like traits would tend to show an increase in activation of the left basolateral amygdala ROI (i.e., a positive correlation between AQ score and activation change). However, no relationship was observed in non-autistic women ($r = 0.08$, $p = 0.73$, Supplementary Figure S5) or autistic women ($r = -0.06$, $p = 0.84$).

LSAS score

Previous work has shown a relationship between amygdala activation to face stimuli and social anxiety (e.g., 1). A moderate, but not statistically significant, correlation was observed in non-autistic women ($r = 0.34$, $p = 0.15$, Supplementary Figure S6), whereas no relationship was present in autistic women ($r = -0.09$, $p = 0.76$).

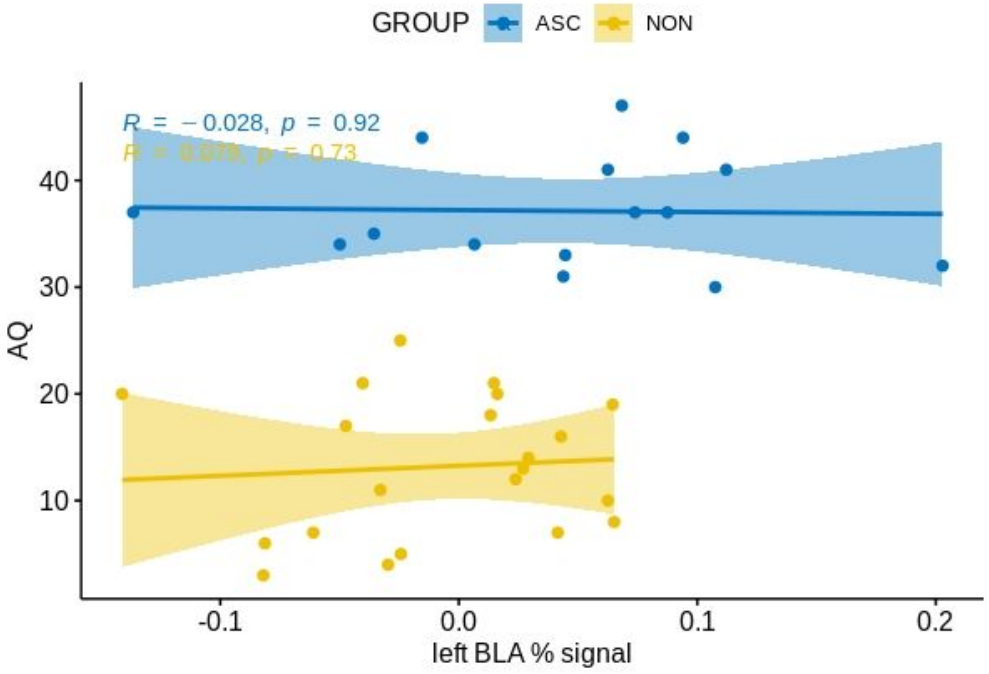

Figure S6. Correlation between self-reported autistic-like traits (AQ score) change in left basolateral amygdala activation between the oxytocin and placebo conditions. The autistic and non-autistic groups are shown in blue and yellow, respectively. Plots show regression line and 95% confidence intervals. Each dot represents one individual.

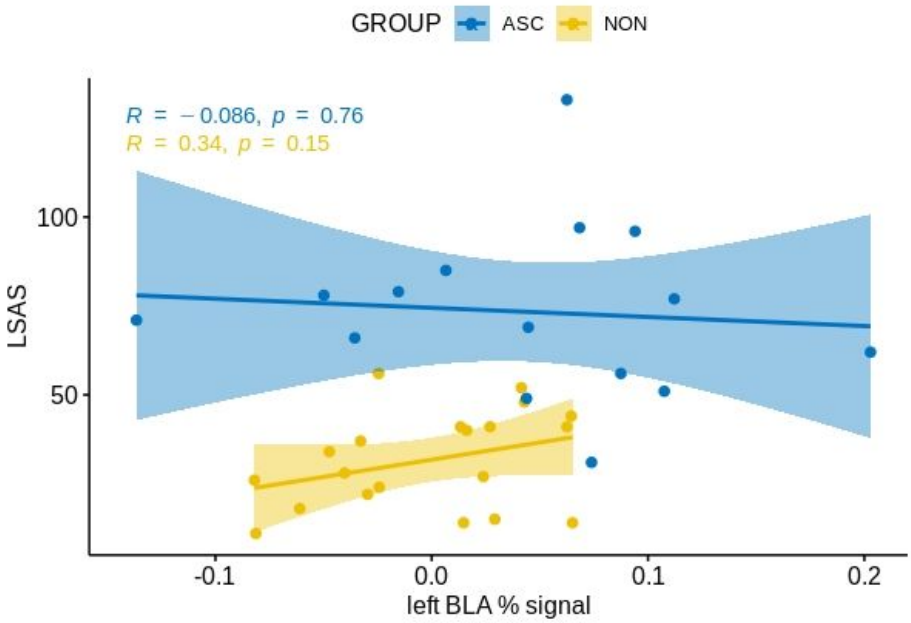

Figure S7. Correlation between self-reported anxiety (Liebowitz Social Anxiety Scale) change in left basolateral amygdala activation between the oxytocin and placebo conditions. The autistic and non-autistic groups are shown in blue and yellow, respectively. Plots show regression line and 95% confidence intervals. Each dot represents one individual.

Salivary oxytocin

In a study of effects of oxytocin in autistic youth, Gordon et al. (2) reported a positive correlation between right amygdala activation (x = 6, y = 8, z = −17) to social stimuli and change in salivary oxytocin level pre- to post- oxytocin administration changes in salivary OT levels (baseline to 30 min post-administration). To explore whether a similar relationship was present in our data, i.e., did participants whose oxytocin levels were more elevated from baseline by the manipulation tend to show activation increases, correlations were computed between %change oxytocin and activation of the left basolateral amygdala ROI. As saliva samples were collected at approximately 10 minutes and 90 minutes after oxytocin administration, and the task took place approximately 55 minutes after administration, the average of the two salivary oxytocin measures is used as the final value, i.e., %change salivary oxytocin was calculated as: (final value – initial value)/initial value * 100.

Supplementary Materials, *Procyshyn et al. 2022*

The change in salivary oxytocin was positively correlated with left amygdala activation to Faces > Shapes in the oxytocin condition among autistic women (r = 0.48, p = 0.06), but not in non-autistic women (Supplementary Figure S7). Using the %change oxytocin value from baseline to 10-min post-administration or 90-min post-administration did not change the direction of the relationship or the group difference (autistic group: r = 0.50, p = 0.05, and r = 0.33, p = 0.21, respectively; non-autistic group: r = 0.05, p = 0.83, and r = -0.11, p = 0.63, respectively). A similar relationship was present when the full atlas-defined left amygdala, not just the basolateral amygdala mask, was used (autistic women: r = 0.54, p = 0.03; non-autistic women: r = -0.0001, p = 1).

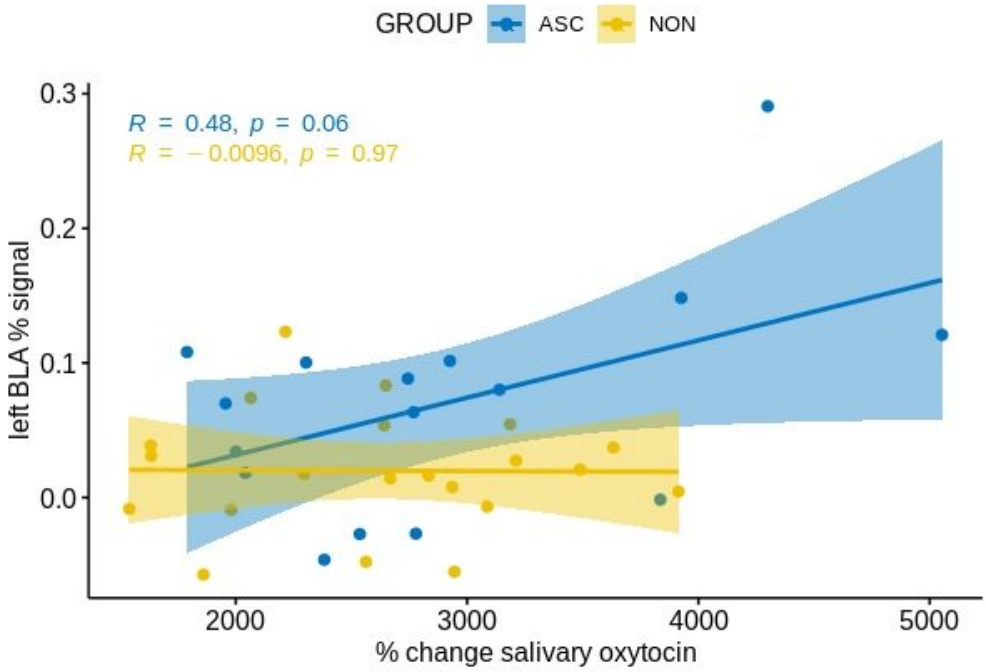

Figure S8. Correlation between pre- to post-administration change in salivary oxytocin and left basolateral amygdala activation in the oxytocin condition. The autistic and non-autistic groups are shown in blue and yellow, respectively. Plots show regression line and 95% confidence intervals. Each dot represents one individual.

Functional connectivity analyses

Psychophysiological interaction (PPI) analysis was performed to examine differences in the paired activation of the amygdala with other brain regions during the emotional face-matching task. To do so, the following steps were taken. First, the atlas-defined masks of the left and right basolateral amygdala were back-projected into native space for each participant using the FSL linear registration tool (FLIRT). The mean timeseries was then extracted for the mask for each participant for each drug condition using *fslmeants*. In FSL, the PPI interaction is modeled as the interaction between the task regressor and the extracted timeseries of the ROI. As PPI analysis can only generate an interaction between two regressors, the faces and shapes regressors were combined into one regressor that embodies the contrast Faces - Shapes. In line with recommended practices (https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/PPIFAQ), a regressor of no interest modeling the contrast Faces + Shapes was also included.

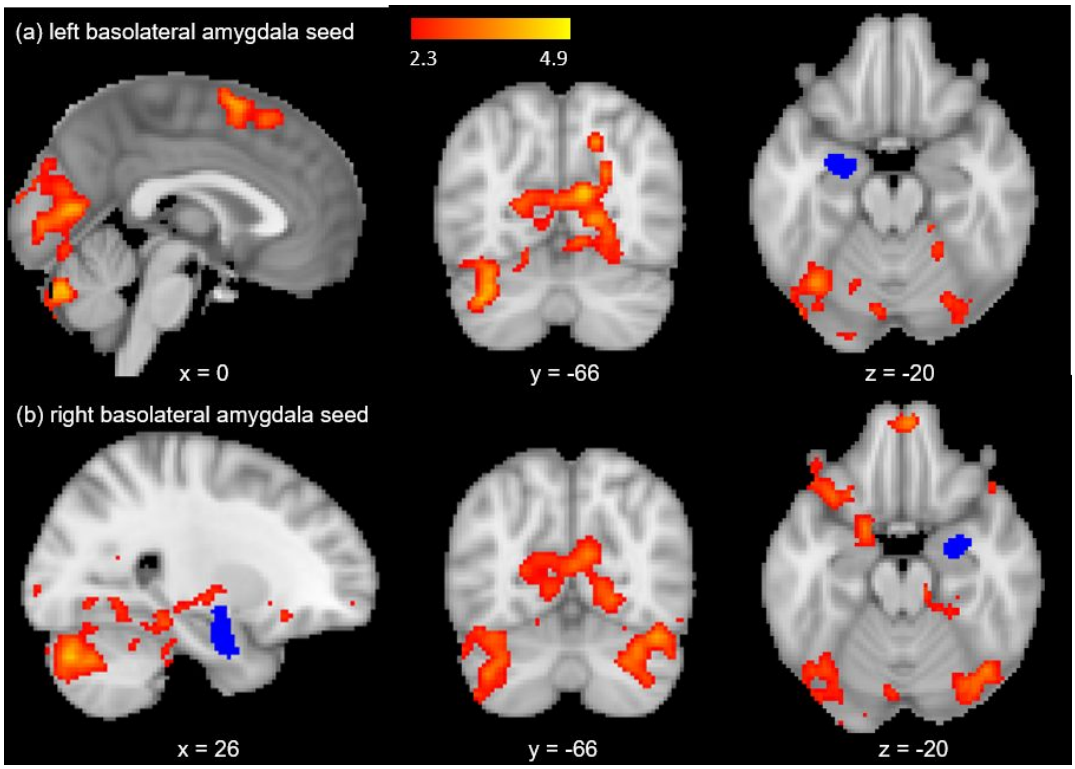

Figure S9. Group differences (Non-autistic > Autistic) in functional connectivity using the (a) left and (b) right basolateral amygdala ROIs as the seeds in the baseline (placebo) condition ($z > 2.3$, cluster-corrected $p < 0.05$). The basolateral amygdala seeds are shown in blue. Images are presented so that the left side is the left hemisphere.

Supplementary Materials, *Procyshyn et al. 2022*

Table S2. Summary of task-associated (Faces > Shapes) differences in functional connectivity in autistic vs. non-autistic women in the baseline (placebo) condition.

		k	p	Z	x	y	z
(a) Non-autistic > Autistic, left basolateral amygdala seed							
Cluster 1	Precuneus Cortex	4377	< 0.0001	3.98	14	-62	14
	Supracalcarine/Intracalcarine Cortex						
	Lingual Gyrus						
	Occipital Fusiform Gyrus						
	Right Putamen/Pallidum						
Cluster 2	Cerebellum	1716	< 0.0001	3.9	-38	-56	-28
	Temporal Occipital Fusiform Cortex						
	Occipital Fusiform Gyrus						
Cluster 3	Superior Frontal Gyrus/	855	0.0044	4.2	-6	24	54
	Juxtapositional Lobule Cortex (formerly Supplementary Motor Cortex)						
(a) Non-autistic > Autistic, right basolateral amygdala seed							
Cluster 1	Intracalcarine/Supracalcarine Cortex	3404	< 0.0001	4.08	-10	-72	18
	Precuneus Cortex				18	-56	16
Cluster 2	Thalamus	3008	< 0.0001	4.03	-6	-8	-2
	Pallidum				-10	-2	-6
	Putamen				-18	8	0
Cluster 3	Cerebellum	2149	< 0.0001	4.88	30	-78	-32
	Occipital Fusiform Gyrus				42	-66	-22
Cluster 4	Temporal Occipital Fusiform Cortex	1622	< 0.0001	3.74	-42	-60	-26
	Cerebellum				-40	-66	-40
Cluster 5	Inferior Frontal Gyrus	949	0.00193	3.4	-52	22	18
	Frontal Pole				-52	38	6
	Middle Frontal Gyrus				-42	36	22
Cluster 6	Inferior Frontal Gyrus/Frontal Pole	746	0.0089	3.45	50	34	8
	Frontal Orbital Cortex				50	32	-16
	Frontal Pole				54	36	-4
Cluster 7	Frontal Pole	737	0.00956	3.68	4	56	-20
	ACC				-12	40	8
	Frontal Medial Cortex				4	50	-8
	Paracingulate Gyrus				6	44	-8
Cluster 8	Frontal Pole	604	0.028	3.58	-20	56	28

Table S3. Estimated Z scores for the clusters identified in the functional connectivity analysis as showing significant Group or Drug x Group effects. Means obtained using the 'emmeans' package in R.

	Drug	**Group**	
			Z mean
(a) main Drug effect			
Cluster 1	PLC	all	-0.318
	OXT	all	0.374
Cluster 2	PLC	all	-0.432
	OXT	all	0.275
(b) Drug × Group interaction			
Cluster 1	PLC	Autistic	-0.8454
	OXT	Autistic	0.4087
	PLC	Non-autistic	0.2347
	OXT	Non-autistic	0.0459
Cluster 2	PLC	Autistic	-0.311
	OXT	Autistic	0.638
	PLC	Non-autistic	0.7
	OXT	Non-autistic	0.129
Cluster 3	PLC	Autistic	-0.5766
	OXT	Autistic	0.4358
	PLC	Non-autistic	0.5035
	OXT	Non-autistic	0.0114
Cluster 4	PLC	Autistic	-0.95
	OXT	Autistic	0.063
	PLC	Non-autistic	0.173
	OXT	Non-autistic	-0.273

Analyses excluding women taking hormonal contraceptives

Previous studies have found that the use of hormonal contraceptives may influence the effects of oxytocin and neural response to face processing (e.g., Scheele et al. 2015). Thus, further analyses were performed excluding these women.

Of the non-autistic women not taking hormonal contraceptives, 9/15 (60%) showed an increase in left basolateral amygdala activation in the oxytocin condition. Furthermore, among non-autistic women, the change in left basolateral amygdala activation did not differ significantly between women taking hormonal contraceptives and those not taking hormonal contraceptives (t-test, mean change in non-HC group = +0.001%, mean change in HC group = +1.3%, p = 0.758).

All main analyses in FSL were performed excluding the 6 women taking hormonal contraceptives (who were all non-autistic). Similar to the findings for the full sample, a significant Drug x Group interaction effect was observed for the left basolateral amygdala (k = 16, Z = 2.8, p = 0.04, MNI coordinates of peak difference: x = -22 y = -10 z = -28). Note that the coordinates of the peak difference are unchanged.

The functional connectivity analyses were also performed excluding the 6 women taking hormonal contraceptives. Similar to the findings for the overall sample, a significant main effect of Drug was found such that right basolateral amygdala connectivity increased with a large cluster showing peak activation in the precentral gyrus (k = 570, Z_{max} = 4.0, p = 0.021, coordinates of peak: x = -52 y = -10 z = 38). Significant Drug x Group interactions were also found, such that autistic women showed increased connectivity of the right basolateral amygdala seed with frontal, occipital, and cerebellar areas in the oxytocin relative to placebo condition. Similar to the findings for the full sample, four clusters were identified (see Table S4 for full details).

Table S4. Summary of oxytocin effects on functional connectivity of right basolateral amygdala seed with other brain regions in autistic and non-autistic women during the experimental task (Faces > Shapes), excluding women taking hormonal contraceptives. $Z > 2.3$ and cluster-corrected $p < 0.05$.

	k	p	Z	x	y	z
(a) Main effect of Drug						
Cluster 1	570	0. 0.0214	4	-52	-10	38
			3.13	-18	6	54
			3.08	-38	-12	46
			3	-26	0	50
			2.94	-30	-6	54
			2.85	-48	-22	38
(b) Group x Drug interaction						
Cluster 1	581	0.0193	3.21	-42	38	-20
			3.14	-44	28	-18
			3.12	-40	18	-26
			3.11	-26	26	-14
Cluster 2	756	0.00408	3.62	48	24	18
			3.29	52	28	2
			3.25	38	60	-2
			3.14	56	30	22
			3.02	54	26	26
			3	46	54	-4
Cluster 3	3256	4.66e-10	3.73	-8	24	50
			3.69	-30	48	24
			3.62	0	26	46
			3.62	-32	48	20
			3.59	0	34	44
			3.55	-32	52	24
Cluster 4	7973	3.59e-19	3.95	26	-34	-42
			3.91	-50	-58	-24
			3.8	-4	-78	-16
			3.77	14	-72	22
			3.72	2	-74	-26
			3.72	36	-78	-18

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1. Herrington JD, Miller JS, Pandey J, Schultz RT (2016): Anxiety and social deficits have distinct relationships with amygdala function in autism spectrum disorder. 907–914.

2. Gordon I, Vander Wyk BC, Bennett RH, Cordeaux C, Lucas M V., Eilbott JA, *et al.* (2013): Oxytocin enhances brain function in children with autism. *Proc Natl Acad Sci* 110: 20953–20958.

3. Scheele, D., Plota, J., Stoffel-Wagner, B., Maier, W., & Hurlemann, R. (2015). Hormonal contraceptives suppress oxytocin-induced brain reward responses to the partner's face. Social Cognitive and Affective Neuroscience, 11(5), 767-774. doi:10.1093/scan/nsv157