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Gabriella A. Caceres Gamundi April 8, 2019

Intranasal oxytocin enhances visual fixation to the face in individuals with Autism Spectrum Disorder: a dose-response study

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There are currently no pharmacological treatments targeting the core social deficits in Autism Spectrum Disorder (ASD). Previous studies have examined the effects of intranasal oxytocin (IN-OXT), a neuropeptide known to be a key modulator in social cognition, as a possible treatment. While acute administration of IN-OXT showed promising results on several primary outcomes related to social cognition, chronic intake led to inconclusive results in terms of efficacy of treatment. Research calls for a dose-dependent study to determine the specific actions of IN-OXT on brain and behavior to optimize its use as hormonal treatment for future studies in patients with ASD. In this present randomized, double-blind, placebo-controlled, dose-response, within-subject study, we examined the effects of different doses of IN-OXT (8 IU, 24 IU, 48 IU) and placebo on eye gaze in adults with high-functioning ASD (18- 45 years old), and compared it to healthy controls' baseline (with placebo intake) during an fMRI study. Outcome measures included visual fixation time on different areas of interest (AOI) (face, eyes, mouth, forehead, outside face) while observing video stimuli of morphed emotional faces during a Face Perception Task (FPT). We found that IN-OXT enhances visual fixation time to the face in a dose-dependent way, with higher doses of IN-OXT leading to more time looking at faces. In addition, visual fixation time to the face (but not to the other AOIs) was significantly different between healthy controls and ASD individuals who received placebo and low dose (8IU) of IN-OXT. Middle (24 IU) and high (48 IU) doses of IN-OXT were able to normalize their visual fixation to the face similar to healthy controls. This study shows promising results of IN-OXT in enhancing saliency to social stimuli and,

possibly, the motivation to attend to faces. It also emphasizes the need for future studies investigating individual differences and the best treatment approaches to alleviate the social deficits in ASD.

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Abstract

There are currently no pharmacological treatments targeting the core social deficits in Autism Spectrum Disorder (ASD). Previous studies have examined the effects of intranasal oxytocin (IN-OXT), a neuropeptide known to be a key modulator in social cognition, as a possible treatment. While acute administration of IN-OXT showed promising results on several primary outcomes related to social cognition, chronic intake led to inconclusive results in terms of efficacy of treatment. Research calls for a dose-dependent study to determine the specific actions of IN-OXT on brain and behavior to optimize its use as hormonal treatment for future studies in patients with ASD. In this present randomized, double-blind, placebo-controlled, dose-response, within-subject study, we examined the effects of different doses of IN-OXT (8 IU, 24 IU, 48 IU) and placebo on eye gaze in adults with high-functioning ASD (18- 45 years old), and compared it to healthy controls' baseline (with placebo intake) during an fMRI study. Outcome measures included visual fixation time on different areas of interest (AOI) (face, eyes, mouth, forehead, outside face) while observing video stimuli of morphed emotional faces during a Face Perception Task (FPT). We found that IN-OXT enhances visual fixation time to the face in a dose-dependent way, with higher doses of IN-OXT leading to more time looking at faces. In addition, visual fixation time to the face (but not to the other AOIs) was significantly different between healthy controls and ASD individuals who received placebo and low dose (8IU) of IN-OXT. Middle (24 IU) and high (48 IU) doses of IN-OXT were able to normalize their visual fixation to the face similar to healthy controls. This study shows promising results of IN-OXT in enhancing saliency to social stimuli and, possibly, the motivation to attend to faces. It also emphasizes the need for

future studies investigating individual differences and the best treatment approaches to alleviate the social deficits in ASD.

1. Background

Autism Spectrum Disorder (ASD) is a continuum of lifelong neurodevelopmental disorders characterized by core deficits in social behavior and communication, as well as repetitive and stereotyped behaviors (American Psychiatric Association, 2013). The latest estimate reports by the CDC indicate that 1 in 59 children will develop autism (CDC, 2018). High functioning ASD individuals show pronounced selective social deficits despite preserved intellectual and linguistic capacities. Particularly, individuals with ASD experience difficulties in establishing and maintaining eye contact as well as in processing other's facial information (Guillon et al., 2014), and alterations in responding to social rewards (Izuma et al., 2011). At a neurobiological level, researchers have found a decreased functional connectivity between cortical networks involved in social cognition and theory of mind (Cheng et al., 2017). In addition, ASD patients show lower BOLD activity in response to faces in areas that are involved in social and reward processing (such as the ventral striatum, amygdala, fusiform gyrus, anterior cingulate cortex, and superior temporal sulcus) (Hasegawa et al., 2013; Tseng et al., 2015), and have abnormal brain responses while looking at faces (Shultz et al. 2000). This decrease in BOLD activity in response to faces was found to be correlated with a decrease in eye gaze (Hadjikhani et al., 2017).

In particular, atypical eye gaze patterns have become a hallmark of their social deficits. A substantial amount of studies have indicated that ASD individuals exhibit decreased eye-to eye contact and impairments in orienting attention towards social stimuli. In a sample of fifteen cognitively able adolescents and adults with ASD, Klin et al. (2002) found that the best predictor for autism was reduced eye-region fixation time. They also found that fixation on mouth and external objects significantly correlated with social functioning: fixation to the mouth indicated improved sociality while fixation to objects predicted greater social impairments. Other studies

have indicated that children, adolescents, and adults demonstrate decreased attention to faces (Riby and Hancock, 2009) and are slower in first fixating on a face compared to controls (Freeth et al., 2010; Riby and Hancock 2009). Bird et al. (2011) found that high functioning ASD individuals spent significantly less time observing face than non-face areas compared to control-matched peers. ASD individuals also show significant impairments during face and gaze cue processing when observing emotional faces versus neutral faces (de Jong et al., 2008). They mainly avoid eye contact and look mostly at other surrounding areas of the head. In addition, early identification and one of the most early-emerging diagnostic symptoms of ASD in toddlers largely involves eye contact examinations, eye fixation, and preference to facial stimuli (Klin, 2008). It has been shown that infants later diagnosed with ASD exhibit mean decline in eye fixation from 2 to 6 months of age, a pattern not observed in infants who do not develop ASD (Jones & Klin, 2013).

1-1. Eye Tracking and ASD

Observing and quantifying visual fixation seems to be a useful biomarker of social cognition known to be deficient in ASD. Eye tracking offers a direct measure of visual social attention, notably other individual's face and eyes. A study by Murias et al. (2018) found strong associations in eye-gaze patterns in response to social stimuli with five well-validated care-giver reported assessments of social and communication abilities including the Vineland Adaptive Behavior Scales-II Socialization and Communication Subscales (VABS-II), behavior Assessment System for Children, Second Edition – Functional Communication and Social skills Subscales (BASC-2), and the Pervasive Developmental Disorder Behavior Inventory Expressive/Receptive Social Communication Subscale (PDD-BI). Other self-report based questionnaires have been highly correlated with eye gaze measures, such as the ADOS-2 symptom severity levels (Frazier et al., 2018) and the Mullen Scales of Early Learning (MSEL) (Moore et al., 2018). Validation of

eye tracking techniques and consideration as a potential biomarker can further be used as an endpoint that can substitute other subjective methods. Eye tracking is a non-invasive and objective assessment of gaze response to visual stimuli. This device can serve as both a diagnosing tool and as a research measure of sociality in clinical trials aiming to find potential treatments that alleviate the core symptoms of ASD.

1-2. Treatments and ASD – Oxytocin

Despite the growing research on ASD, we still don't have a pharmacological treatment for their social and emotional deficits. Currently approved pharmacotherapies target irritability and non-core phenotypes. Therefore, there is a substantial need for clinical trials testing novel treatments for core ASD symptoms. Treatments targeting social communication may act by improving individual's saliency and attention to social information, such as eye gaze attention to faces, and provide them with opportunities to gain social skills. Current evidence-based treatments include behavioral interventions targeted to social communication impairments, some of which include cognitive behavioral interventions, reinforcement training, modeling, parent-implemented interventions, structural play groups, and social skills training (Watkins et al., 2017). However, such behavioral therapies can be costly and time consuming, and multiple treatment programs may be necessary in order to see significant long-term improvements (Mendell, 2012).

One potential approach for treating social deficits in ASD is to target the neural systems involved in socio-emotional processing and potentially enhancing social cognition and function. In particular, oxytocin (OXT) has emerged as a key modulator of social behavior and is being considered as an intervention to enhance social functioning in ASD. OXT is a neuropeptide that is produced by the hypothalamus and is released to the blood and brain by magnocellular neurons (Figure 1) (Sofroniew, 1983; Swanson and Sawchenko, 1983; Landgraf and Neumann, 2004). It

acts as a neurotransmitter in the brain by binding to oxytocin receptors (OXTR) located in multiple brain regions important for social behavior (Mitre et al., 2016). It is known to modulate a variety of social behaviors such as maternal attachment, pair bonding formation, social recognition and prosocial behaviors (Ross and Young, 2009). For instance, OXT interacts with the dopamine system in the ventral striatum and facilitates the formation of social attachment (Young and Wang, 2004). In prairie voles, OXT fibers from the hypothalamus projects to the nucleus accumbens (NAcc) and polymorphisms in the OXTR gene have predicted individual variation in pair bonding (King et al., 2016). OXT's involvement in the modulation of various aspects of social behavior has been discussed as a potential role in ASD, and this is supported by genetic evidence. OXTR knockout mice show abnormal social behaviors, impaired social memory, and elevated aggressive behaviors (Nishimori et al., 2008). In addition, single nucleotide polymorphisms (SNPs) and microsatellites in the receptor genes of OXT are associated with ASD diagnosis and measures of social behaviors (Francis et al., 2016).

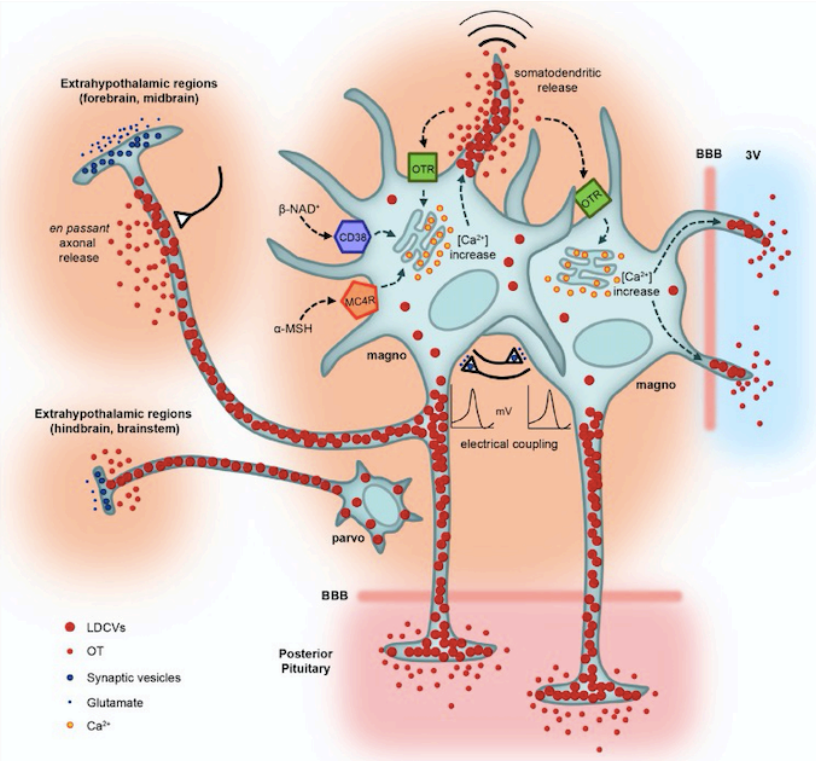

Figure 1. The OXT system. Morphology, neuronal projections, and release mechanisms of OXT neurons (Johnson and Young, 2017).

1-3. Intranasal Oxytocin and Social Cognition

It is believed that OXT affects complex social behaviors by modulating fundamental processes, such as the salience of social stimuli and their rewarding value (Yao et al., 2018). In humans, there are numerous studies indicating that delivering intranasal OXT (IN-OXT) (nasal spray, Novartis Syntocinon) has a similar prosocial effect (Domes et al., 2007; Rilling et al., 2012; Heinrichs et al., 2009; Rimmele et al., 2009; Chen et al., 2017; Hurlemann et al., 2010; Quintana et al., 2016). The delivery of oxytocin intranasally provides a non-invasive measure that allows direct delivery to the brain. Researchers have found that IN-OXT directly reaches the brain using a mass spectrometry assay distinguishing labeled exogenous OXT in the CSF (Lee et al., 2018). IN-OXT increases trust in others, enhances the ability to infer emotions of others from viewing

images of the eyes, and increases attention to the eye regions of faces. A study by Kotani et al. 2017 using marmosets found that IN-OXT significantly enhanced visual attention to eyes/face region, and this increase in visual attention was blocked by an oxytocin receptor antagonist L-368,899. Studies using fMRI have also shown that IN-OXT affects the BOLD activity of emotional brain regions relevant to saliency, such as the amygdala during face perception (Kirsch et al., 2005; Gamer, 2010), and the caudate nucleus to reward, during reciprocated cooperation (Rilling et al., 2012).

1-4. Intranasal Oxytocin and ASD

In light of the growing evidence of IN-OXT and social cognition in humans, researchers have asked the question of whether exogenous administration of oxytocin can alleviate some of the social deficits in ASD. Andari et al. 2010 demonstrated that IN-OXT enhanced the ability of ASD subjects to detect and respond to social reciprocity in social games. They also showed that IN-OXT enhances visual fixation to faces and to the eye region while patients perceived facial stimuli. Auyeung et al. 2015 replicated these findings showing enhanced gaze behavior after administration of IN-OXT, particularly to the face and eyes in autism and control groups. Today, there are at least 24 studies investigating the effects of oxytocin inhalation on social skills in ASD. Supplemental Table 1 shows a summary of earlier clinical trials investigating the effects on IN-OXT on ASD patients. After IN-OXT intake, patients show enhanced social abilities, BOLD activity in emotional brain networks in response to social cues, increased attentional preference to faces, and gaze fixation to social stimuli (Parker et al., 2017; Gordon et al., 2016; Andari et al., 2016; Kanat et al., 2017; Kruppa et al., 2019). The administration of oxytocin as a potential treatment is promising and shows little to no side effects. For instance, administering 24IU twice

a day for 6 weeks showed no serious adverse effects (Anagnostou et al. 2012). IN-OXT has been found to be safe to administer for long periods of time.

Despite these promising results, a few studies have found mixed evidence. For example, a study by Guastella et al. (2015) found no beneficial effects on the Social Responsiveness Scale (SRS) and Clinical Global Impression-Improvements subscale (CGI-I) following treatment of 18 IU or 24 IU of IN-OXT. Other studies found no significant improvements during the Reading the Mind in the Eyes Test (RMET), a widely used measure of theory of mind and recognizing emotional states of others (Quintana et al., 2017; Gordon et al., 2013). In addition, work by Dadds et al., 2014 showed that IN-OXT did not significantly improve emotion recognition and social interaction during a family interaction task. The experimenters did, however, find a significant increase in child-parent eye contact and non-verbal positive behaviors. This discrepancy in the results can be due to the limitations of objective outcome measures used and to a variety of inter-individual differences that can play a crucial role. Understanding the mechanisms of the effects of IN-OXT, to know when and how much to administer, can be crucial for future therapeutic applications.

One way to address this controversy is to study the effects of several doses of IN-OXT on brain function and behavior in patient population. The Autism-Oxytocin Brain (AOB) project that I am involved in consists of determining the effects of different doses of IN-OXT (8IU, 24IU, 48IU) and placebo on social behavior and fMRI BOLD activity of adult patients with high functioning ASD. This project also consists of studying brain and behavioral responses to social stimuli in healthy subjects after placebo as a baseline measurement. Indeed, several studies have shown strong placebo effects that can influence brain activity and behavioral responses. This will allow a direct comparison between healthy controls and patients who received placebo. The AOB

project includes a wide range of outcome measures and procedures such as collecting fMRI brain data during resting state, face perception task (FPT), an interactive social game, emotion and social learning tasks, and other behavioral outcomes.

1-4. Aims and Hypotheses

This study aims to investigate whether differing doses of intranasal oxytocin have a dose-dependent effect on visual fixation to the face and eyes during a face perception task. The project also aims to study whether visual fixation patterns differ between baseline ASD and control participants and whether oxytocin enhances visual fixation patterns to more closely resemble that of controls. Appropriate and comfortable levels of eye contact and visual attention to the face are important in all types of social interactions, communication, and building connections with other individuals. Individuals with ASD have sensory-perceptual processing deficits that weaken their abilities to attend to and perceive social stimuli. Daily experiences consist of countless changes in social information, and any failure to attend to or process social cues might lead to an inappropriate social interaction. For this reason, tracking eye gaze patterns during a face perception task provides a comparison for treatment improvements in social cognition.

Our first aim was to determine the effects of different doses of intranasal oxytocin on visual fixation during a face perception task in individuals with ASD. We hypothesized that IN-OXT would enhance gaze duration to the face and eyes in a dose-dependent manner, such that the higher doses of IN-OXT (48IU and 24IU) would have the most significant effect on visual fixation in patients with ASD.

Our second aim was to examine the differences between healthy controls' and ASD participants' gaze fixation patterns in response to social stimuli. We hypothesized that ASD

patients with placebo intake would look significantly less at the face and eyes when compared to controls.

Our third aim was to examine the differences between the effects of IN-OXT in ASD and normative activity in healthy subjects. We hypothesized that visual fixation to the face and eyes during a face perception task would not differ between the higher doses of IN-OXT (24 IU, 48 IU) in ASD and baseline healthy controls.

2. Methods

2-1. Participants

Participants were n = 32 patients with ASD and n = 19 healthy controls. All subjects were contacted by email and/or phone. ASD participants were recruited through the Emory Marcus Autism Center. They had already participated in a previous study (IRB00064623) and had agreed to be contacted for future studies. Participants with or without a family member present underwent a screening process over the phone to ensure their interest and eligibility for the study.

Inclusion criteria for the ASD group required a diagnosis of ASD based on the Autism Diagnostic Observation Schedule (ADOS) and the Autism Diagnostic Interview (ADI) criteria, considered gold standard tools of research-based autism diagnosis. General inclusion criteria were as follows: (1) males (women were excluded from participation due to the greater prevalence of ASD in males and to avoid confounding effects of hormonal changes of the menstrual cycle from IN-OXT administration); (2) of ages ranging between 18 and 45 years old; (3) Not color blind; (4) Normal or corrected-to-normal vision; (5) Intelligence Quotient (IQ) greater than 70 as measured by the Wechsler Adult Intelligent Scale-III (WAIS-III).

The exclusion criteria were as follows: (1) recent occurrent seizures (past 5 years); (2) brain damage or head trauma; (3) cardiovascular disease; (4) presence of a severe medical problem; (5)

history of alcoholism or substance abuse; (6) asthma (at the discretion of the nurse practitioner or the study physician); (7) migraine/headaches (at the discretion of the nurse practitioner or the study physician); (8) claustrophobia; (9) pacemakers, cochlear implants, surgical clips or metal fragments.

Participants were also asked to complete other screening forms involving demographic information and medical history (Table 1). 32 ASD and 19 healthy controls were included in the randomization procedure. One patient dropped out of the ASD group due to discomfort inside the MRI and two participants dropped out in the control group due to time and transportation conflicts, leaving 31 ASD and 17 healthy controls who completed all the experimental procedures.

Table 1. *Demographics and general characteristics of ASD participants randomized to receive IN-OXT*

	ASD
	(*n*=32)
Gender (%)	
Male	1 (100)
Female	0
Age (years, *M* ± *SD*)	28.84 ± 5.56
Race (%)	
Caucasian	0.81 (81)
African American	0.13 (13)
other	0.06 (6)
Occupation (%)	
unemployed	0.28 (28)
part-time job	0.44 (44)
full-time job	0.25 (25)
student	0.03 (3)
Medications (%)	
on medication	0.83 (83)
no medication	0.17 (17)

2-2. Study Design and Setting

The present randomized, double-blind, placebo-controlled, dose-response, within subject design was conducted at Emory University in Atlanta, GA, at the CRN unit of the Emory Hospital and BTCI Imaging Center MRI scanner. The study protocol was approved by the Institutional Review Board (IRB) for Emory University and registered (IRB00093455). Written informed consent was obtained from all participants before the start of each trial.

ASD subjects participated in five visits in order to receive all the experimental conditions. The first visit consisted of training and a baseline run inside the MRI. During the next four visits ASD participants received either a low (8IU), medium (24 IU) or high (48 IU) dose of IN-OXT or placebo in a randomized fashion. Healthy controls only received placebo and participated in one total visit in a single-blind fashion.

2-3. Procedure

2-3.1. Drug Protocol. The Syntocinon Spray (Novartis, 5ml) and the placebo were bought from a pharmacy in Switzerland. They were rebottled at the Investigational Drug Service pharmacy at Emory Hospital in similar glass bottles to preserve the double-blind design. The placebo solution contained all the inactive ingredients of the OXT solution except for the active oxytocin neuropeptide.

The spray bottles were prepared and labeled minutes before the start of each trial and stored in a dedicated storage refrigerator at the hospital. The pharmacy at IDS had access to the randomization sequence. Each patient with ASD received 12 puffs in the nostril (six puffs per nostril) for all the different doses (8 IU, 24 IU, 48 IU, and placebo) during four different clinical visits. Healthy controls also received 12 puffs of placebo spray in a single-blind trial. Control

subjects were blind to the treatment and believed that they could be receiving either placebo or oxytocin. They were debriefed at the end of the study and informed of having received placebo.

At the beginning of the administration period, nurses (who were blinded to the dose) provided participants with the placebo or oxytocin spray and followed the appropriate methods of successful administration. These included that: (1) the subject cleared his nose with tissue paper; (2) the nurse primed the bottle by spraying it in the air before administration; (3) the subject was instructed to sit with his head slightly tilted to the back and cover one nostril while the nurse administered the spray to the other nostril; (4) the nurse asked the patient to sniff and breathe in gently after each puff. Vital signs were taken before and after drug administration to check for any side effects of IN-OXT. Participants received the spray 40 minutes before going to the MRI and starting the experimental tasks.

2-3.2. Face Perception Task. Fifty minutes after receiving the spray, participants moved on to the Face Perception Task (FPT) inside the MRI, which consisted of presenting morphed emotional faces on the screen separated by a fixation cross that was either red or blue. Participants were asked to observe the faces displayed on the screen and to press a key when they detected the blue cross. This was mainly done to keep participants engaged. The faces were second video clip animations morphed from neutral to one of four emotional expressions (happy, angry, fear, disgust) or to neutral expression. Each participant underwent two different runs of the FPT that contained different randomizations of the facial stimuli. Each run consisted of eight blocks of seven minutes, and each image (eight images per block) was presented for five seconds. Fixation crosses were presented for one second within each block, and for nine seconds between blocks (Figure 2). There were a total of eight different individuals (four males, four females) for each emotion category. The research team collaborated with a professor at Harvard, Dr. Nouchine

Hadjikhani, who created the morphed video clip animations from the dataset (Hadjikhani et al., 2015). The task was created with and run through ExperimentCenter™ 3.6.40 design software system. Participants were not told that their eyes were being tracked during the task, and were debriefed about this at the end of the study.

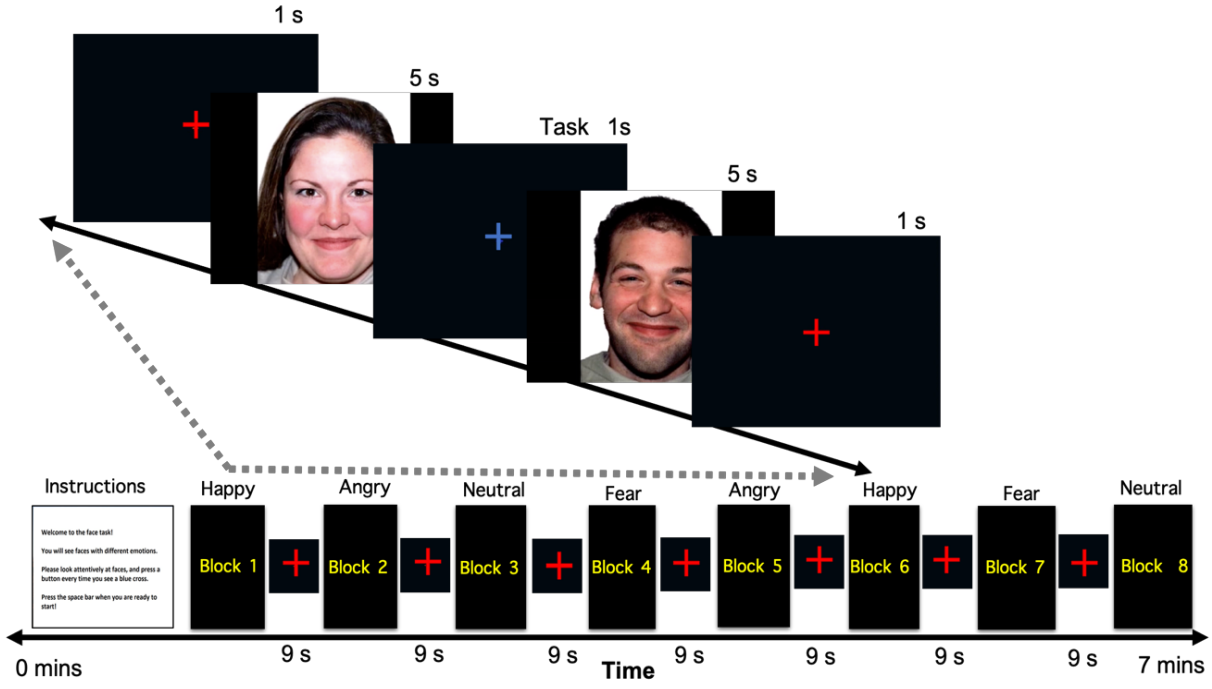

Figure 2. Face Perception Task (FPT). Representative slides displayed on the fMRI screen showing one of two runs, each of which consisted of eight blocks and eight morphed emotional faces within each block. Emotion categories were maintained for every one block, but the individuals being displayed were different. Each facial stimulus was followed by a one second fixation cross and each block was followed by a nine second fixation cross. Participants were asked to press a key whenever the blue fixation cross was displayed on the screen.

2-3.3. Eye tracking technology. During the FPT, a non-invasive eye tracking camera device pointing to participant's left eye was mounted to the fMRI head coil piece. We used SensoMotoric Instruments (SMI) iViewX™ recording software and ExperimentCenter™ 3.6.40 design software systems to record eye gaze data during the FPT. The system relied on a pupil/corneal reflection technique for estimating the point of gaze and tracking participants' eye movements inside the scanner. Prior to the eye tracking test, a calibration procedure was carried out. Participants were instructed to follow with their eyes (without moving their head) a series of fixating points of known coordinates that moved around the screen. After calibration, the FPT was presented and the software could then apply a function to transform the absolute gaze location into the screen's x and y coordinates system of every measured point of gaze, associated timestamps, pupil size, and validity code and ultimately generate output gaze data.

2-3.4. Data Analysis. Data was uploaded to SMI BeGaze™ 3.6.40 program for analyzing behavioral and gaze data. For ASD, out of the 124 sessions (31 total patients x 4 visits), 110 were successfully loaded into the analysis software. Different settings in calibration or problems with data acquisition impacted the quality of acquired data such as it was not possible to upload them into the software. All the 17 sessions of healthy controls (17 healthy controls x 1 visit) were successfully loaded for both runs. We drew areas of interest (AOI) on each facial stimuli indicating the eyes, face, forehead, mouth, and outside the face (Figure 3). The AOI-based approach was used to quantify spatial distribution of visual attention and specify gaze duration for specific regions of the stimuli. Out of the 110 sessions, 53 sessions were included in the analysis. 57 sessions were excluded before conducting unblinded analysis. These sessions were mainly excluded because of loss of data (visual fixation and saccades). This loss of data was observed during the gaze replay of each of the videos of each visit (or session) and also confirmed with zero data points in the excel

exported file. Gaze replay consisted of a recording of eye movements during the face perception task. A few sessions were excluded because of partial loss of data where the fixation cross appeared only in parts of the gaze replay and was lost or highly jittery for most of the video. The partial loss of data was also associated to the visual fixation being stuck in one part of the stimulus for the whole task. For healthy controls, 13 sessions were included. Table 2 illustrates a summary of excluded trials for each run.

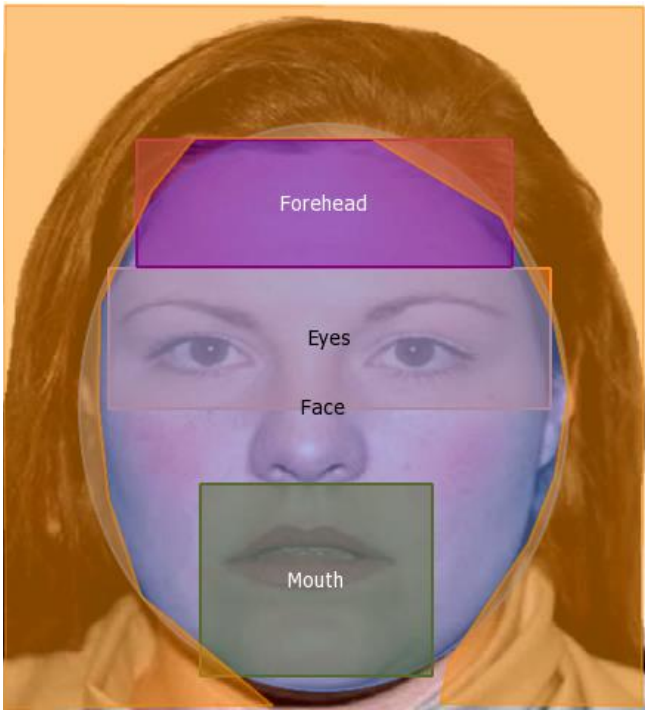

Figure 3. Representation of determined AOI regions for a female face stimuli. The same regions of interests were drawn for every stimuli. There were a total of 8 individuals (4 males, 4 females) for each emotion category.

Table 2. *Summary of data excluded from analysis*

	ASD (N=31)		**Controls (N=17)**	
	(124 sessions)		(17 sessions)	
	Data acquisition –calibration	Visual fixation – saccades (missing data points)	Data acquisition – calibration	Visual fixation – saccades (missing data points)
RUN 1	sessions =13	sessions = 56	sessions = 0	sessions = 7
RUN 2	sessions =15	sessions = 58	sessions = 0	sessions = 4

*Data acquisition – calibration = data failed to be loaded into the BeGaze problem due to calibration error, data collection or drop outs
*Visual Fixation – saccades (missing data points) = Data was excluded based on the pre-determined exclusion criteria

For the analysis, we calculated sum of fixation time to each AOI for each subject during both runs and then averaged fixation times across both runs (within each individual) resulting in N=13 for the high dose group (48 IU), N=14 trials for the medium dose group (24IU), N= 21 trials for the low dose group (8 IU), N=12 trials for the placebo group (0 IU) and N=13 for healthy controls.

Gaze data was analyzed using IBM SPSS® software (IBM Corporation, Armonk, NY, USA). Given that we had a within-subject design but eliminations for data processing, we had several missing data per individual for all four doses. We conducted a Linear Mixed Model with a repeated function (with different doses of IN-OXT as a fixed effect and with visual fixation times to each AOIs as dependent variables) to compare the effects of different doses in ASD. We used post-doc t-test to compare between the different doses. We used an independent samples t-test to compare between healthy controls and ASD for different doses on IN-OXT.

3. Results

3-1. Tests of Main Hypotheses

3-1.1. Aim 1. To examine the hypothesis that there is a dose-dependent effect of IN-OXT on visual fixation time to the face and eyes, a linear mixed model was performed to study the difference between different doses of IN-OXT in terms of fixation time on the face and eye area. There was a significant dose dependent effect (F=3.44, p=0.024) of IN-OXT on eye gaze to the face. No significant dose-dependent effects were found to the eyes (F=1.00, p=0.398), forehead (F=1.053, p=0.377), mouth (F=0.868, p=0.465), or outside face (F=1.245, p= 0.306) (Figure 4). Pair wise comparisons (one-sided (p_{corr})) show that higher doses of IN-OXT (48IU, 24IU) enhances gaze fixation on the face area as compared to lower dose (8IU) (p_{corr}= 0.01; p_{corr}= 0.005; respectively) and to placebo (p_{corr}= 0.04; p_{corr}= 0.027; respectively). Figure 5 shows qualitative heat maps of eye gaze recordings for each dose of IN-OXT. In addition, no differences were found between 8IU and placebo and no differences were found between 24IU and 48IU. When comparing the higher doses (48IU and 24IU) to lower dose (8IU) and placebo (0 IU), a significant increase was found with the higher doses (t(60)=3.187, p=0.002). No significant effects were found between the different doses of IN-OXT when separating visual fixation times by emotion expression.

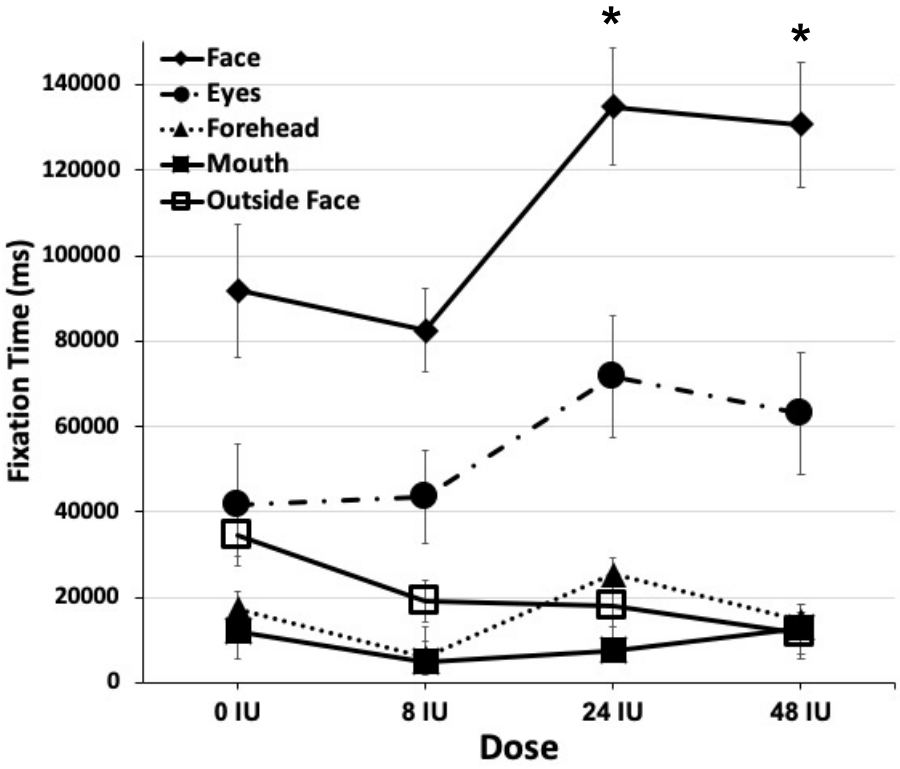

Figure 4. Effects of different doses of IN-OXT on visual fixation time in ASD. Each point on the graph represents the average fixation time spent at each pre-determined AOI during administration of placebo (0 IU), low dose (8 IU), medium dose (24 IU) and high dose (48 IU) of IN-OXT.

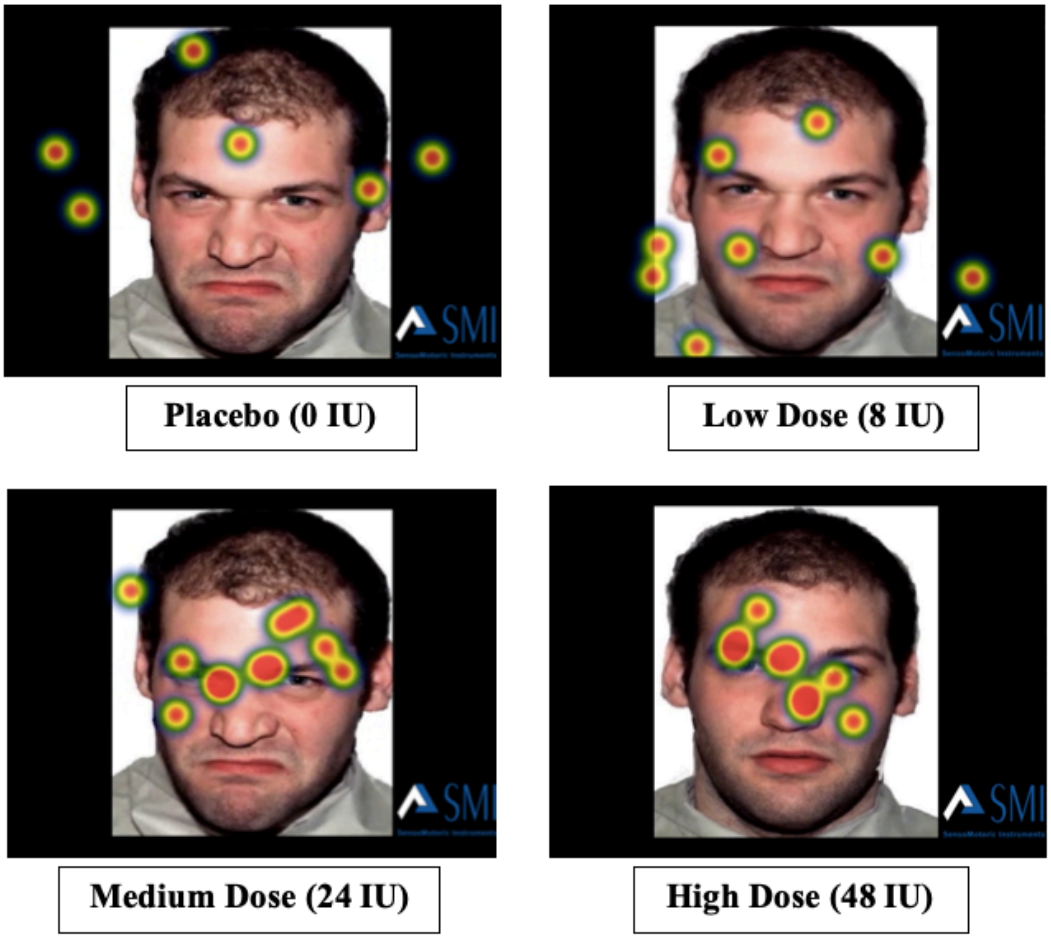

Figure 5. Heat map demonstrating eye gaze recording during different doses of IN-OXT and placebo in ASD participants.

3-1.2. Aim 2. To examine the hypothesis that visual fixation patterns of healthy controls will differ from that of ASD participants during saline administration and low doses of IN-OXT, an independent samples t-test was conducted. We found that healthy controls had significantly more visual fixation on the face area when compared to ASD patients administered placebo (t(25)=2.371; p=0.026), and when compared with the low dose of IN-OXT (8IU) (t(32(=3.151; p=0.001) (Figure 6). Figure 7 displays qualitative heat maps displaying eye gaze of healthy controls and ASD after placebo and low dose of IN-OXT. We did not find any differences between healthy controls and ASD with placebo and low doses on IN-OXT in terms of visual fixation to the eyes, forehead, mouth and outside face.

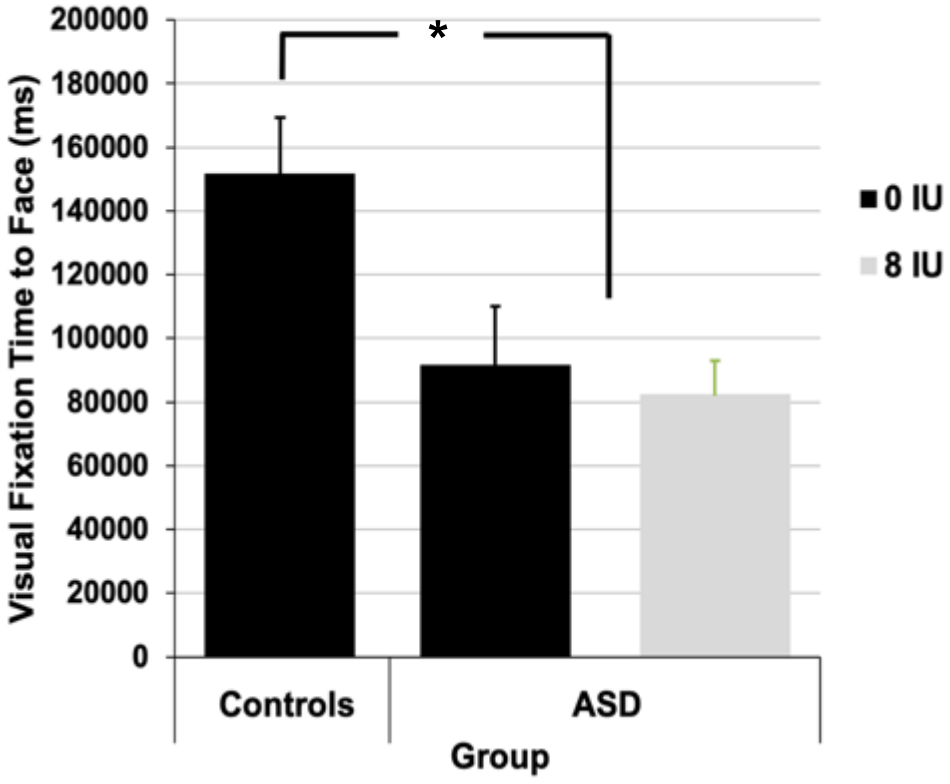

Figure 6. Visual fixation time (ms) to the face comparing healthy controls to ASD patients after placebo (0 IU) and low dose (8 IU) of IN-OXT administration.

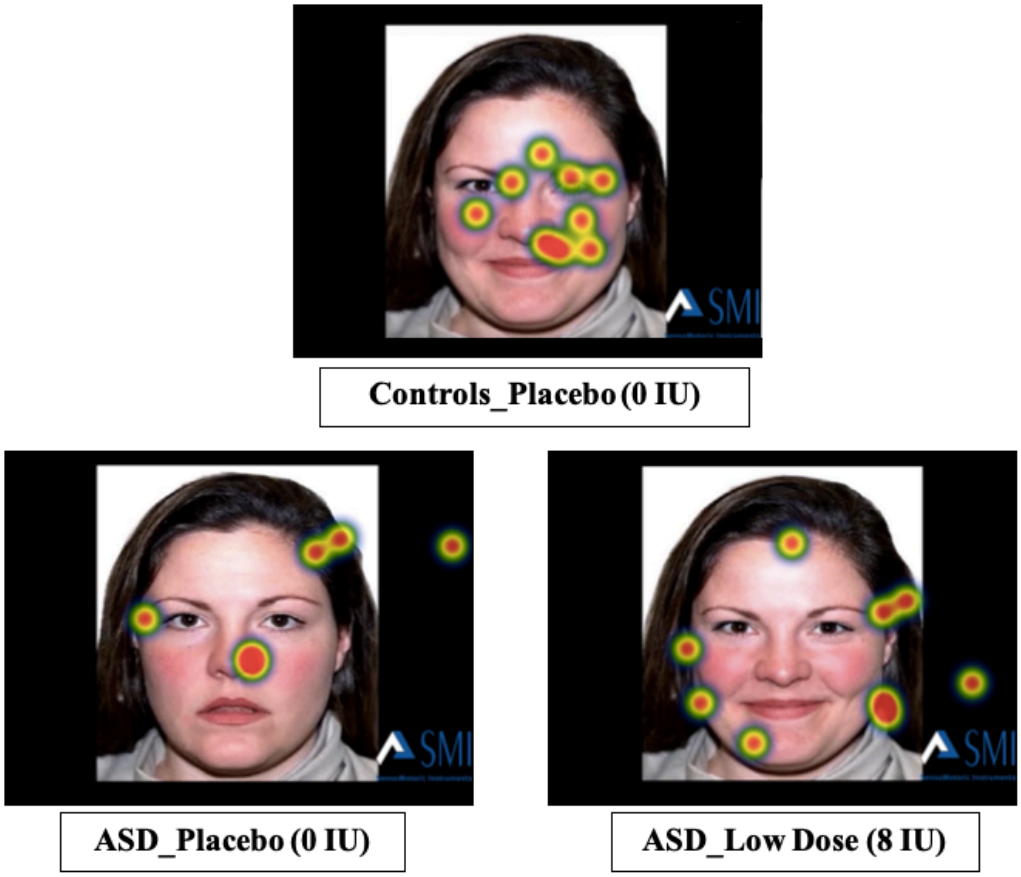

Figure 7. Heat map comparing baseline (placebo, 0 IU) activity of controls and ASD individuals administered placebo (0 IU) and low dose (8 IU) of IN-OXT.

3-1.3. Aim 3. To examine the hypothesis that visual fixation patterns in healthy controls will not differ from that of ASD participants during middle and high doses of IN-OXT, an independent samples t-test was conducted. We found that healthy controls did not differ in terms of visual fixation to the face when compared to patients who received 24IU ($t(25)=0.725$, p-0.475) or 48IU ($t(23)=0.819$, $p=0.421$) (Figure 8). Figure 9 shows heat maps displaying eye gaze of healthy controls and ASD participants after middle and high dose of IN-OXT. Given that no significant differences to other AOIs were found between healthy controls and ASD participants administered placebo and low dose (8IU) of IN-OXT, we did not continue further analyzing visual fixation times to other AOIs for middle and high doses of IN-OXT.

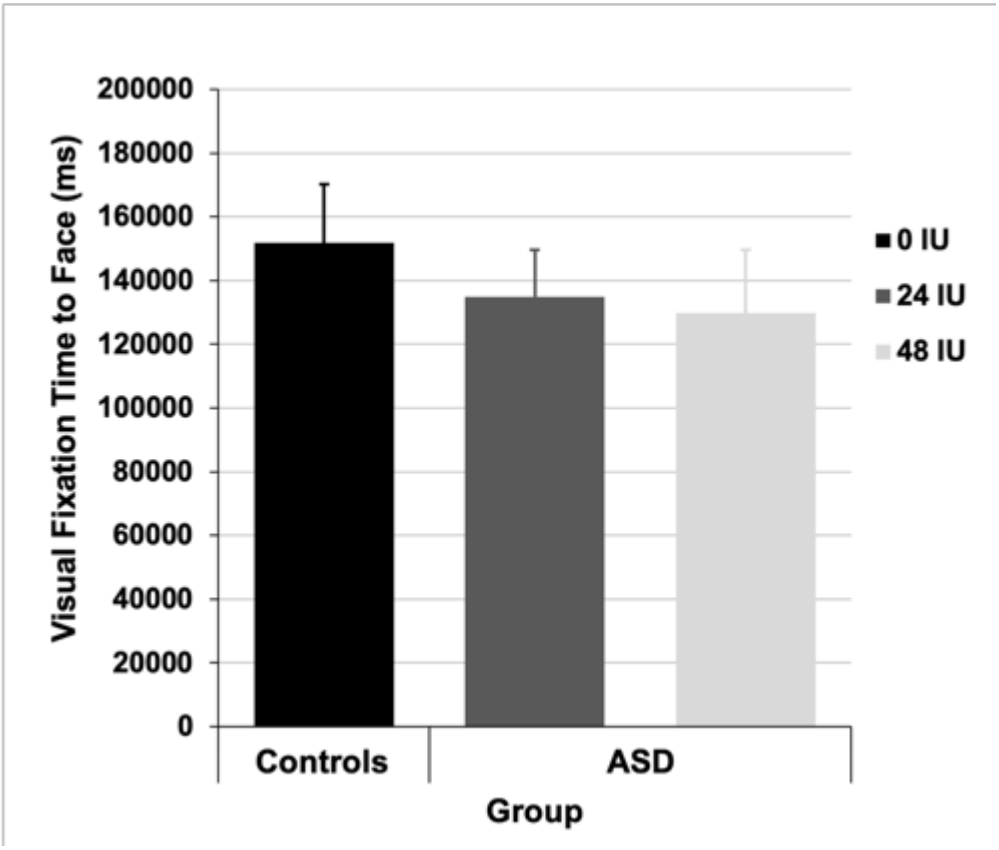

Figure 8. Visual fixation time (ms) to the face comparing healthy controls with ASD patients after middle (24 IU) and high dose (48 IU) of IN-OXT administration.

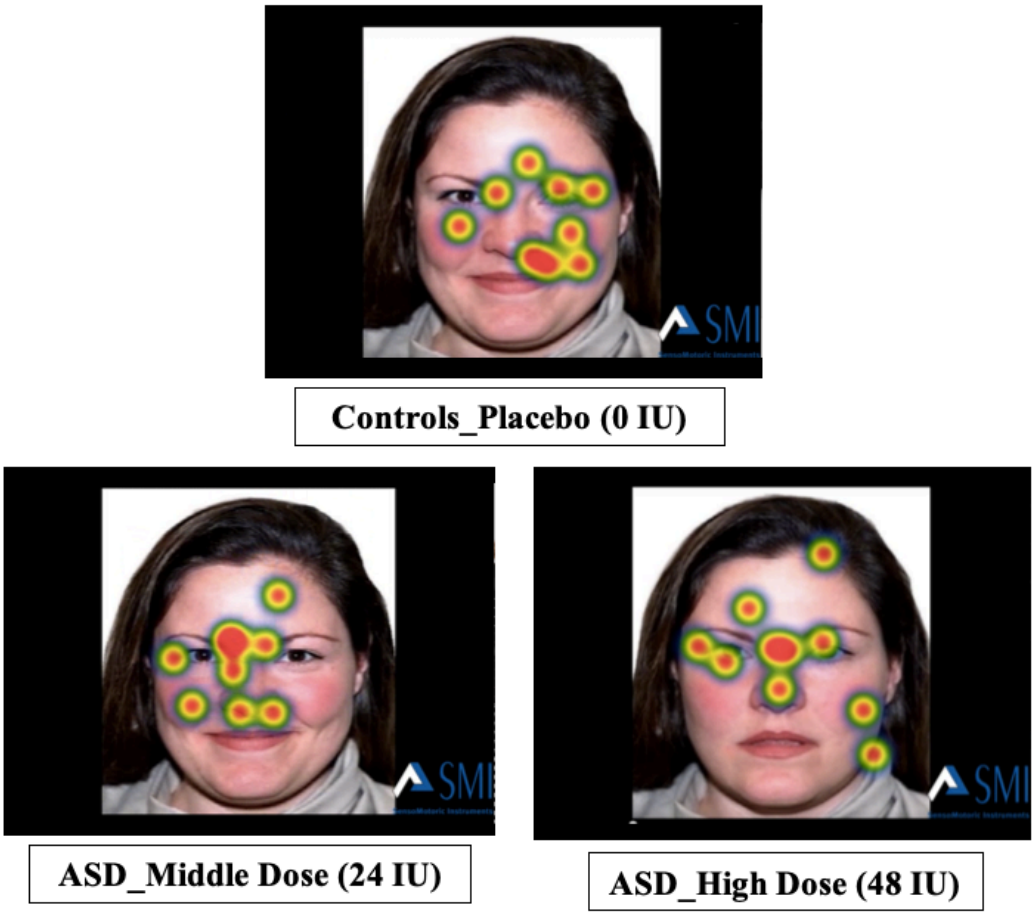

Figure 9. Heat maps demonstrating baseline activity of controls administered placebo and ASD individuals administered middle dose (24 IU) and high dose (48 IU) of IN-OXT.

4. Discussion

The present study examined the differences in visual fixation patterns between baseline ASD and control participants, and whether different doses of IN-OXT had an effect on visual fixation in ASD. Three hypotheses were examined: (1) there would be a dose-dependent enhancement effect of IN-OXT to the face and eyes in individuals with ASD; (2) ASD individuals administered placebo and low dose of intranasal oxytocin will look significantly less to the face and eyes than healthy controls; (3) ASD individuals administered middle and high dose of IN-OXT will not differ from controls in visual fixation patterns. The current investigation and AOB project aims to understand how oxytocin affects social cognition in ASD with the purpose that it may provide clues for understanding its potential role in the etiology and treatment of ASD.

4-1. Results and Implications

Our study delivers promising results and initial evidence of a dose-dependent effect of IN-OXT on enhancement of visual fixation to the face in patients with ASD. There was a significant increase in visual fixation to the face area following middle and high dose of IN-OXT, but not after receiving placebo or low dose. No significant differences were identified between placebo and low dose, or between medium and high dose of IN-OXT.

In addition, such positive effects during middle and high dose of IN-OXT rose to the level of visual fixation observed in healthy controls, whom initially showed significant differences in visual fixation to the face after placebo and low dose of IN-OXT administration. These findings are consistent with other studies examining the effects of IN-OXT on eye gaze in patients. Kanat et al. (2017) found that during a dot probe task assessing attentional preference to a face vs. non-social stimulus, IN-OXT significantly increased the allocation of attention towards faces in ASD to the level observed in controls. Another study by Yamasue et al. (2018) found that after receiving

48 IU of IN-OXT, ASD participants fixated on the eye region of a talking face for a greater percentage of time than after receiving placebo.

However, our findings did not show a significant dose-dependent effect of IN-OXT on the eye region, a result that we expected originally. There was an apparent trend of a linear increase of visual fixation with higher doses of IN-OXT. The lack of significant results on the eye region is not in line with the original hypothesis and the increasing evidence in the literature. We also did not find a difference between healthy controls and patients with placebo (baseline) in terms of visual fixation to the eyes during the face perception task.

The absence of a significant increase to the eyes from IN-OXT administration may be due to the fact that normal eye gaze patterns involve a more holistic view of the entire face. Previous research on face perception revealed that healthy participants instructed to rely on their intuitive "gut feelings" for facial judgements of differing emotions had a visual fixation pattern resembling a more global/holistic view of the face (Mega and Volz, 2017). Furthermore, participants in our study observed faces that morphed into different emotional expressions. Such task may require judgement of all internal facial features (mouth, forehead, eyes) in order to identify the emotion being presented. It seems that we adopt a strategy that draws out the expressive facial cues from all internal facial features when processing facial expressions. Guo et al. (2012) revealed that the proportion of fixation times directed at internal features of the face was not affected by varying degrees of emotional intensity during an expression categorization task, and the overall gaze distribution was similar across different expressions at different intensities. Such results are consistent with our findings in that we observed no differences in visual fixation patterns between different emotions.

Although our findings are consistent with studies investigating the effects of IN-OXT on visual fixation in ASD, they differ from other studies using alternative outcome measures of sociality. A lack of assessment tools to evaluate sociality changes might explain this variability. Common measures that have previously been used and produced both positive and negative IN-OXT effects on social cognition include: (1) ADOS, Module 4, a semi-standardized assessment for diagnosing individuals suspected of ASD; (2) the Social Responsiveness Scale (SRS), a 65-item measure designed to assess autistic traits, primarily reciprocal social behavior; (3) Autism Quotient, a standardized assessment of social aptitudes in ASD; (AQ) the Clinical Global Impression-Improvements Scale (CGI-I), which assesses how much the patient's condition has improved or worsened; and (4) the Reading the Mind and the Eyes Task (RMET), which tests the ability to read emotions from photographs of eye regions (Parker et al., 2017; Quintana et al., 2017; Hirosawa et al., 2017; Kosaka et al., 2016; Guastella et al., 2015; Gordon et al. 2013; Yamasue et al., 2018; Watanabe et al., 2015; Yatawara et al., 2016; Anagnostou et al., 2016; Munesue et al., 2016). A previous report by Morrier et al., (2017) found no correlation between ADOS-2 Module 4 Calibrated Severity Scores and SRS-2 self and parent report scores, or with AQ scores. Variability exists between findings when using more self-reported measures compared to more objective measures such as neuroimaging, plasma OXT levels, and eye gaze technologies. For example, studies using fMRI have found that IN-OXT increases BOLD activity in brain regions important for processing socio-emotional information such as the amygdala, insula, anterior cingulate gyrus, fusiform gyrus, medial prefrontal cortex, and striatum, including NAcc and putamen. It also enhances functional connectivity between the brain reward and socioemotional processing systems (Kruppa et al., 2017; Gordon et al., 2013; Domes et al., 2013; Andari et al., 2010; Gordon et al., 2016; Aoki et al., 2015). In addition, previous work revealed a significant IN-

OXT effect on functional connectivity between ACC and dmPFC but not on outcome measures such as the SRS and AQ (Watanabe et al. 2015), and found no correlation between eye gaze and ADOS (Yamasue et al., 2018). Future studies warrant the need of using more objective tools to precisely quantify treatment efficacy.

4-2. Limitations and Future Directions

Several limitations of this study need to be considered. Using the FPT to measure visual fixation to the face without other non-social distractors may not provide as much space for different attentional preferences as other social stimuli that more closely resemble real-world scenarios. In the real world, individuals are presented with complex scenes in which many different people, objects, and sounds are involved and are competing for attention. Previous eye-tracking studies have used video scenes of actors interacting with other individuals or objects (Klin et al. 2002) or pictures of social stimuli next to a picture of non-social distractor stimuli (Kanat et at., 2017). Due to the dual utilization of the FPT to examine both eye gaze and BOLD activity (AOB project) while observing facial stimuli, other tasks that more closely resemble everyday situations containing distracting stimuli were not suitable. By using morphed videos instead of emotional images, we attempted to more closely resemble a real world example of an emotion expression. In addition, by observing different doses of IN-OXT, we sought to control for these confounding effects. Future studies call for a dose-dependent IN-OXT study on visual fixation while observing more naturalistic social scenes.

Another limitation in our study is the loss of data during acquisition due to the complexity of the system that we used and high sensitivity to pupil sizes. Several criteria can impact the quality of acquisition: eye lobule size, position in the coil, and other factors.

More sophisticated software can be used in future MRI/eye tracking studies that can have a higher detection rate and more flexibility with regards to subjects' movements.

Future studies should also attempt to look at individual differences in terms of IN-OXT effects. In our ASD sample, we observed variation in baseline visual fixation performance during the FPT. It has been suggested that an optimal range may exist for oxytocin functioning, with its behavioral effects dependent on individual differences such as baseline oxytocin levels, oxytocin receptor gene polymorphisms, or its interactions with other biological factors. This model, first proposed by Zoghbi and Bear (2012) during observations of "high" and "low" levels of gene expression and synaptic plasticity effects, has been supported by subsequent studies in the field. Such have suggested that individuals with ASD may have lower baseline levels of OXT compared to controls (Feldman et al., 2014; Green et al., 2001), and that intranasally administered oxytocin increases plasma oxytocin levels (Althaus et al., 2016; Yamasue et al., 2018; Munesue et al., 2016; Andari et al., 2010). In addition, previous work has demonstrated that individuals with lower oxytocin concentrations and OXT signaling deficits have benefited more from OXT treatment (Parker et al., 2017). ASD individuals fall within a spectrum, so profiling individuals based on biological and behavioral characteristics may bring insight onto whether IN-OXT will have a treatment effect and provide better target therapies for treating the core deficits of the disorder. For example, Groppe et al. (2013) was able to demonstrate the effect of IN-OXT on performance during a social reward and punishment task depended on whether the individuals had high or low sociability ratings. IN-OXT increased social reward and avoided punishment in the low sociability group, but had the opposite effect on the high sociability group.

4-3. Conclusions

There are no currently approved pharmacological therapies available to treat the core social deficits of ASD. As the prevalence rises, understanding the biological basis and obtaining a treatment is of vital importance. Early studies have shown mixed results of whether chronic administration of IN-OXT could have a beneficial effect on social cognition for this patient population. In this study we were able to demonstrate enhancing effects of IN-OXT on visual fixation to the face using an eye gaze tracking measure, indicating greater preference to attend to social stimuli. During placebo administration, individuals with ASD appear to avoid observing facial stimuli by looking around the face or to surrounding areas. It has been proposed that decreased attention to faces in ASD may be due to inadequate salience and lack of motivation towards social cues, or to an avoidance behavior as a strategy to regulate magnified arousal (Kleinhans et al. 2010). Both reasons could go hand in hand, where inadequate salience and motivational decrease to social stimuli leads to abnormal brain development and response to social stimuli. Our results suggest that after administration of IN-OXT there is an enhancement on the attention to social stimuli. This provides a window of opportunity for reinforcement learning in social situations. Thereby, future studies should consider that IN-OXT might be effective in the long-term when combined with other behavioral therapies focused on social learning. This study not only provides evidence of beneficial clinical effects of IN-OXT administration but also calls attention to seek the most advantageous approaches to use IN-OXT in future studies.

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Supplemental Table 1. *Results of earlier clinical trials investigating the effects of IN-OXT on ASD participants*

	Study	N	Age	Dose of IN-OT	Experimental Design	Acute vs. Chronic	Measures	Results
1	Watanabe et al. 2014	n= 33 ASD	M = 28.5	24IU or placebo 40 mins before scan	randomized, double-blind, placebo-controlled, within-subject crossover	two visits, 1 week apart	fMRI during a friend or foe judgement task depicting 80 original monochrome movies	OT increased ability to make non-verbal information based judgements more frequently and with shorter response time
2	Parker et al. 2017	n= 32 ASD	6-12	24IU and placebo, twice daily	double-blind, randomized, placebo-controlled, parallel design	4-wk intranasal OXT treatment (24 IU, twice daily)	Social Responsiveness Scale (SRS), blood OXT concentrations	INOT enhanced social abilities in the Social Responsiveness Scale SRS and showed that individuals with pretreatment OXT signaling deficits (lower OXT concentrations) benefited more from OXT treatment
3	Andari et al. 2010	n= 13 ASD	M = 26	single dose of 24 IU or placebo 50 mins before tasks	randomized, placebo-controlled, double-blind within-subject experimental design	two visits, 1 week apart	social ball game and a face perception task (static emotional faces) while recording eye gaze	IN-OXT increased gaze time on the face, namely the eyes. In the ball game, patients exhibited stronger interactions with most cooperative partner and reported enhanced feelings of trust
4	Quintana et al., 2017	n= 17 ASD	18-35	8 IU, 24 IU, and placebo 40 mins before tasks	randomized, placebo-controlled, double-blind, three-period crossover design	minimum 24 hour washout	emotion sensitivity, Reading the Mind in the Eyes Test (RMET), emotional dot probe and emotional face-morphing	8IU and 24 IU treatment increased overt emotion salience. Found no significant effects for RMET performance or emotional dot probe and face-morphing
5	Gordon et al. 2016	n= 20 ASD	7-19	16-19y (24 IU) 12-15y (8 IU) 7-11y (12IU) and placebo 45 mins before	fMRI and a randomized, double blind, placebo-controlled crossover design	2 visits separated by a minimum of 72 hours	fMRI during a biological motion perception paradigm, a vocal affect perception paradigm, and an emotion recognition paradigm	OT increases activity in brain regions important for perceiving social-emotional information. OT enhances connectivity between nodes of the brain's reward and socioemotional processing systems, preferentially for social stimuli
6	Kosaka et al. 2016	n= 60 ASD	>15	32 IU, 16 IU, or placebo	single-center phase 2, pilot, randomized, double-blind, placebo-controlled, parallel-group, clinical trial	double-blind (12 weeks), open-label (12 weeks), follow-up (8 weeks)	single-nucleotide polymorphisms (SNPs) in the oxytocin receptor gene (OXTR) and Clinical Global Impression-Improvement (CGI-I) scores	Only scores in the high-dose group were significantly higher than in the placebo group (>21 IU per day oxytocin was more effective than ⩽21 IU per day). In addition, an SNP in OXTR (rs6791619) predicted CGI-I scores for ⩽21 IU of IN-OXT per day
7	Guastella et al. 2015	n= 48 ASD	12 - 18	18IU or 24 IU	double-blind, randomized, controlled trial	twice daily for 8 weeks	SRS, Clinical Global Impression-Improvements subscale (CGI-I)	Participants who received oxytocin showed no benefit following treatment on any of the outcome measures.
8	Strathearn et al. 2018	n= 16 ASD n=16 HC	M = 12.5	>16(24 IU) 12-15(16.8 IU) 8-11 (12 IU) or placebo 30 mins before task	randomized, double-blind, placebo-controlled cross over study	two visits, 1-2 week intervals	Eye tracker, systemizing picture task to test gaze preferences using real life images of people, animals, scenes and objects	ASD preferred to fixate on more highly systemized pictures. INOXT eliminated this preference : showed a similar response to control subjects on placebo. Controls increased visual preference for more systemized images after IN-OXT.
9	Andari et al. 2016	n = 20 ASD	M = 26.3	24 IU or placebo 30 mins before scan	randomized, placebo-controlled, double-blind, between-subjects design	single visit	Interactive ball game, face matching task, fMRI	IN-OXT enhanced brain activity of early visual areas in response to faces, modulated BOLD activity of amygdala and hippocampus, enhanced activity of mid-orbitofrontal cortex in response to a fair partner, and insula region in response to an unfair partner

10	Kanat et al. 2017	n= 29 ASD n= 31 HC	M = 38.2	24 IU or placebo 45 mins before task	randomized, double-blind placebo-controlled, cross-over design	two visits, 14 days apart	dot probe task for attentional preference to social stimuli as compared to a simultaneously presented non-social distractor stimulus	Under placebo, ASD individuals paid less attention to faces presented for 500 ms than did controls. IN-OXT increased the allocation of attention toward faces in ASD to the level observed in controls.
11	Althaus et al. 2016	n= 31 ASD n= 30 HC	18 - 34 M = 22.67	24 IU or placebo 30 min before scan	randomized, placebo-controlled, double-blind design	two visits, 1 week apart	IAPS task: 414 series of social scenes and non-social scenes, plasma OXT analysis, EEG and ECG recording	No effects of IN-OXT on orienting responses. No difference in their treatment response. Found a significant interaction of OXT plasma levels with the OXT treatment effect on responses to pictures with humans
12	Domes et al. 2013	n= 14 ASD n= 14 HC	M = 24.3	24 IU or placebo 45 mins before scan	randomized, placebo-controlled, within-subject, cross-over fMRI design.	two visits, 1 week apart	FMRI during a face discrimination task	Under placebo, ASD group showed decreased activity in the right amygdala, fusiform gyrus, and inferior occipital gyrus during face processing. After IN-OXT, right amygdala activity to facial stimuli increased in the ASD group.
13	Gordon et al. 2013	n= 17 ASD	8 - 16.5	>16y = 24 IU, 12-15y = 18 IU, 7-11y = 12 IU 45 mins before scan	randomized, double-blind, cross-over functional MRI (fMRI) study	two consecutive visits	fMRI during modified Reading the Mind in the Eyes Test (RMET) and Social Responsiveness Scale (SRS)	OXT enhanced activity in social brain areas during social judgments while reducing activity during nonsocial judgments. Effects on brain activity differed as a function of severity only when subjects were making social judgments. Performance on RMET did not differ for OXT vs. Placebo visits
14	Tachibana et al. 2013	n= 8 ASD	10-14	8 IU, 16 IU, 24 IU and placebo	singled-armed, open-label, long term study	stepwise increase dosage every 2 months (8 IU, 16 IU, 24 IU) with a placebo period (1-2 weeks)	Autism Diagnostic Observation Schedule – Generic (ADOS-G), Child Behavior Checklist (CBCL), and the Aberrant Behavior Checklist (ABC)	Participants showed improved scores on the communication and social interaction domains of the ADOS-G. CBCL and ABC scores showed marginal improvement. Caregivers reported positive effects of the OXT therapy, especially on the quality of reciprocal communication
15	Yamasue et al. 2018	n= 103 ASD	18-48	48 IU or placebo	randomized, parallel-group, multicenter, placebo-controlled, double-blind trial	n = 51 assigned to IN-OXT group and n = 52 assigned to placebo	ADOS Module 4, Gaze Fixation Time using GazeFinder, STAI, Centre for Epidemiologic Studies Depression Scale, CGI and Global Assessment of Functioning (GAF) scores	ADOS reciprocity scores were significantly reduced for IN-OXT groups. Changes in plasma OXT were correlated with the changes in ADOS reciprocity with marginal significance. Participants of the OXT-group fixated on the eye region of a talking face for a greater percentage of time than placebo. Gaze-tracking did not correlate with the changes in ADOS reciprocity.
16	Greene et al. 2018	n= 28 ASD	M = 13.4	24 IU or placebo	placebo-controlled double-blind study	two visits, (mean = 15 days, range = 3-46 days)	fMRI task during social and nonsocial incentive delay tasks	For social reward anticipation and outcomes, there were no significant increases in brain activation for OXT group. Significant increases in brain activation were observed for nonsocial incentive salience stimuli

17	Hirosawa et al. 2017	n= 10 ASD	23 - 41 M = 30.3	24 IU	open-label, single-arm, nonrandomized and uncontrolled manner	IN-OXT administration every day for 10 weeks	AQ, EQ, RBS-R, STAI, SDS, WHOQoL scales. MRI and PET examinations to measure binding of brain serotonin (11C-DASB)	Each scale indicated positive trends but no significant improvement. After long-term OT administration (8–10 weeks), significant elevation in 11C-DASB binding potential was observed in the left middle frontal gyrus. No significant correlation between changes in 11C-DASB BPND and changes in clinical variables
18	Kruppa et al. 2018	n= 15 ASD n = 24 control	M = 21.9	20 IU 45 mins before scan	single-center, double-blind, placebo-controlled cross-over trial	two consecutive visits	fMRI during probabilistic social reinforcement learning task	IN-OXT significantly increased percentage of correct trials for social learning targets and social feedback. Significant brain activation was observed within the striatum, including NAcc and putamen for social feedback.
19	Watanabe et al. 2015	n= 18 ASD	M = 32.2	24 IU x 2 (48IU/day) and placebo	randomized, double-blind, placebo-controlled, crossover trial	6 weeks on placebo, 6 weeks on IN-OXT daily administration	CARS2 and ADOS (15 min after morning IN-OXT) and fMRI while observing 80 original monochrome emotional movies (40 min after IN-OXT) evaluated other scales such as AQ and SRS	6-week OXT administration could improve social reciprocity (ADOS). No significant effects found in other clinical outcomes. Improvement of social reciprocity deficits was accompanied by enhancement of functional coordination between ACC and dmPFC and this correlated with the OXT-induced decrease in ADOS reciprocity score
20	Yatawara et al. 2016	n= 31 ASD	M = 6.2	24 IU and placebo	double-blind, randomized, placebo-controlled, crossover, clinical trial	phase 1 (5 weeks) crossover (4-week washout) phase 2 (5 weeks)	SRS-P, RBS-R-P, reciprocal social interaction and communication (ADOS), CGI-I, Caregiver Strain Questionnaire (CSQ)	OXT led to significant improvements on SRS and RBS-R-P scales. Effects were also found for secondary measures of emotional and behavioral difficulties. Larger effects seen for group where IN-OXT was administered first.
21	Dadds et al. 2014	n= 38 ASD	7-16 M = 11.2	12IU or 24 IU or placebo	double-blind randomized control trial	once daily for 4 consecutive days	Family interaction task: Free Play, Emotion Talk, and I-Love-You Task scored using Family Observation Schedule-ASD (FOS-ASD)	IN-OXT did not significantly improve emotion recognition, social interaction skills, or general behavioral adjustment. There was a significant increase in child-parent eye contact and non-verbal positive behaviors
22	Aoki et al. 2015	n= 31 ASD	M = 20	24 IU and placebo	randomized, double-blind, placebo-controlled, within-subject cross-over clinical trial	two visits, 1 week apart	fMRI while observing emotional movies, measured N-acetylaspartate (NAA) levels, a marker for neuronal energy demand, in the vmPFC	Differences in NAA levels between groups were associated with OXT-induced fMRI signal changes in the vmPFC. NAA differences in vmPFC triggered increases in the task-dependent fMRI signals and led to improvements in socio-communication difficulties
23	Anagnostou et al. 2012	n= 19 ASD	M = 33.2	24 IU or placebo	randomized, double-blind, placebo-controlled, parallel design trial	IN-OXT twice daily for 6 weeks	Diagnostic Analysis of Nonverbal Accuracy, RBS-R-P, SRS, RMET, Yale Brown Obsessive Compulsive Scale, WHOQoL scale	No significant changes in the nonverbal accuracy and repetitive behavior scales. Improvements after 6 weeks we observed in RMET and quality of life questionnaire.
24	Munesue et al. 2016	n= 29 ASD	15-40	16 IU and placebo	pilot, randomized, double-blind, and placebo-controlled crossover study	8 weeks placebo 8 weeks IN-OXT daily cross-over without washout	20-min play session and interview every 2 weeks, CARS, CGI-I, Global Assessment of Functioning (GAF), Interaction Rating Scale Advanced (IRSA), ABC	Outcome measures revealed no difference between groups. Social interactions in the play sessions significantly increased in the OXT group. Significant correlations between the plasma OXT concentration and subscale scores for irritability on the ABC.