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Nasal oxytocin for social deficits in childhood autism: A randomized controlled trial.

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Running title: oxytocin and autism

Abstract:

Background: The last two decades have witnessed a surge in research investigating the application of oxytocin as a method of enhancing social behaviour in humans. Preliminary evidence suggests oxytocin may have potential as an intervention for autism.

Methods: We evaluated a 5-day 'live-in' intervention using a double-blind randomized control trial. 38 male youths (7–16 years old) with autism spectrum disorders were administered 24IU or 12IU (depending on weight) intranasal placebo or oxytocin once daily over four consecutive days. The oxytocin or placebo was administered during parent-child interaction training sessions. Parent and child behaviours were assessed using parent reports, clinician ratings, and independent observations, at multiple time points to measure side-effects; social interaction skills; repetitive behaviours; emotion recognition and diagnostic status.

Results: Compared to placebo, intranasal oxytocin did not significantly improve emotion recognition, social interaction skills, or general behavioral adjustment in male youths with autism spectrum disorders.

Conclusions: The results show that the benefits of nasal oxytocin for young individuals with autism spectrum disorders may be more circumscribed than suggested by previous studies, and suggest caution in recommended it as an intervention that is broadly effective.

Keywords: Autism, oxytocin, children, randomized controlled trial.

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Autism Spectrum Disorder (ASD) is characterised by core deficits in social communication and the presence of repetitive behaviours (Lord et al. 2012). In contrast to typical child development, children with autism show less interest in other people (Dawson et al. 2012). While the causes of autism remain unknown, an attentional preference or bias toward social stimuli is critical to

appropriate social development (Adolph et al. 2001). Impaired attention to critical social stimuli may compromise the developing neural circuitry that subserves higher social communication domains that are experience-dependent (Dawson 2008; Johnson et al. 2005; Marcus and Nelson 2001), possibly exacerbating or resulting in the core deficits observed in individuals with ASD.

Oxytocin is a powerful modulator of neural activity that is strongly linked with the formation of social bonds (Insel 2010). The last two decades have witnessed a surge in research investigating the application of oxytocin as a method of enhancing social behaviour in humans. In research involving healthy adults, intranasal oxytocin administration has shown a range of positive effects such as increasing levels of trust (Kosfeld et al. 2005), gaze to the eyes (Guastella et al. 2008), and accurate emotion processing (Simplicio et al. 2009; Ijzendoorn and Bakermans-Kranenburg 2012).

There is evidence that oxytocin systems may be disturbed in autism. Common polymorphisms, and epigenetic methylation of the promoter region of the oxytocin receptor gene are associated with risk for autism (Campbell et al. 2011; Lerer et al., 2008; Gregory et al. 2009). There is evidence that children with autism demonstrate lower plasma oxytocin levels (Modahl et al. 1998), and increased levels of oxytocin precursor peptides compared to controls (Green et al. 2001); although these results have not been found reliably in young people with ASD (Miller et al., 2013). Collectively, a growing but tentative body of evidence is accumulating that reduced oxytocinergic function may be a contributing factor to an endophenotype underlying social deficits in ASD.

Considering this evidence for the importance of oxytocin in social behaviour, the core deficits in ASD, and the possible reduced oxytocinergic function in ASD individuals, several researchers have proposed a role for synthetic oxytocin as a pharmacological treatment (Insel 2010; MacDonald and MacDonald 2010). So far, a handful of studies have examined the effects of nasal and intravenous synthetic oxytocin on individuals with ASD, and all have reported positive results, including a reduction in repetitive behaviours (Hollander et al. 2003), and increases in social memory (Hollander et al. 2007) and emotion processing

(Guastella et al. 2010; Andari et al. 2010). While these studies augur for a role of oxytocin in the treatment of autism; they are limited to small sample sizes (largest $n = 16$; Guastella et al., 2010), single dose administrations of oxytocin aiming to produce specific behavioural and/or cognitive effects, rather than broad ‘treatment’ studies. One recent study (Anagnostou et al., 2012) examined repeated administrations of intranasal oxytocin or placebo with $n = 19$ adults with autism (twice daily over 6 weeks). They found no improvements on primary measures of social function and repetitive behaviour; however the oxytocin group performed better on emotion recognition tasks and a quality of life measure. With regard to safety in young people, Tachibana et al. (2013) showed no adverse effects of long-term oxytocin use in a small sample of early adolescent boys with ASD.

Thus, there are preliminary data to suggest that nasal oxytocin may help with social functioning in autism; however, larger trials are needed. This study tested the role of oxytocin in potentiating the performance, development and generalisation of interpersonal social skills in young children with ASD. We conducted a randomised controlled trial of four consecutive daily administrations of oxytocin during parent-child interactions to address the following questions: 1) does oxytocin improve social communication skills, (i.e. eye contact, warmth, verbal content)? ; 2) does oxytocin reduce repetitive behaviours? ; 3) does oxytocin improve emotion recognition and 4) does oxytocin bring about generalised improvements beyond immediate effects?

Methods

Participants

Participants were $N = 54$ male children recruited through Royal Far West, Sydney Australia, between January 2010 and January 2012 (aged 7-16 years; $M=11.23$, $SD=2.6$). All met DSM-IV criteria for Autistic Disorder, Asperger’s Disorder or PDD-NOS (American Psychiatric Association 2000) using a multi-stage diagnostic procedure described below.

Inclusion criteria required a diagnosis of ASD, having English as the first language, and IQ=80 or above on a standardized intelligence test. Exclusion criteria were female gender, allergy to preservatives, major comorbid illness such as epilepsy or heart conditions. We limited this initial study to males as ASD is more common in males and there is evidence that oxytocin may impact differently on females (De Vries 2008).

The participant sample had a high level of comorbid disorders that are commonly associated with ASD (Simonoff et al. 2008). Twenty participants had comorbid Attention Deficit Hyperactivity Disorder; 13 had a diagnosis of Oppositional defiant disorder, and 6 had internalizing anxiety disorders. Seventeen participants were stabilized for over 8 weeks on psychotropic medication (Concerta n=8; Risperidol 2; Catapress 1; Ritalin 3; Dexamphetamine 3). Figure 1 shows recruitment and retention through the study and table 1 lists clinical variables and socio-demographics.

Child diagnoses was made by a specialist child psychiatrist using convergent information from existing referral diagnoses, the DISCAP-ASD diagnostic clinical interview, and observational assessment using the Childhood Autism Rating Scale (Schopler et al. 1988), the High Functioning Autism Spectrum Screening Questionnaire (ASSQ) (Ehlers et al. 1999), the OSU Autism Rating Scale-DSM IV (OSU Research Unit 2005a) and the OSU Autism Global Impression Scale (OSU Research Unit 2005b). To check the reliability, 20% of diagnoses were reviewed by a multi-disciplinary team who were ‘blind’ to the primary clinician’s diagnosis; inter-rater reliability kappa = 0.82 ($p < 0.001$).

IQ scores were gathered using the Wechsler Intelligence Scale (Wechsler 2005). All IQ tests were conducted by a trained clinician or had been done within the last 2 years. Participants also completed a medical review with a Pediatrician. Ethical approval was provided by the University of New South Wales Ethics Committee (09133) and The Research Institute at The Children’s Hospital Westmead (09/CHW/79). This trial was registered with

the Australian New Zealand Clinical Trials Registry (ACTRN12609000784213) and the Australian Government Therapeutic Goods Administration (TGA) 2009/009858(163).

Procedure

Participants were assessed twice prior to treatment: Initial (3-6 months before treatment) and Pretreatment (immediately before treatment), three times during treatment, immediately Post-treatment, and at 3-month follow-up. The treatment was delivered over five consecutive days and involved four key components; (1) nasal administration; (2) parent-child interaction training with the treating psychologist; (3) family interaction task (30 minutes); (4) outcome measures. Participant's and their parents stayed in Royal Far West accommodation for the duration of the treatment week. The attended only for the treatment study and had no other appointments during their stay. Following drug treatment randomization (oxytocin OT or placebo PL), participants were further randomized into two treatment orders: Group 1 (PL = 10; OT 10) completed the family interaction task at 9.30am each day (Tues-Thurs) and the parent-child interaction training at 11.30am each day (Mon-Thurs). Group 2 (PL=9; OT=9) completed the family interaction task at 11.30am each day (Tues-Thurs) and the parent-child interaction training at 9.30am each day (Mon-Thurs). Each group received the nasal administration at 11am (Mon/Wed) and 9am (Tues/Thurs). Table 1 supplementary information shows the complete treatment design. Regardless of order of delivery, each participant received two sessions where spray administrations (oxytocin or placebo) were prior to parent-child interaction training and two prior to the family interaction tasks; all observational data were collected for the latter.

Nasal Spray

Preparation: Sprays were prepared by the University of New South Wales pharmacist. Each puff per nostril contained 6 international units (IU) of oxytocin, mannitol, glycerine, methyl parraben, propyl, parraben and purified water (placebo contained all ingredients except the active oxytocin and mannitol). Each participant received a total of four doses (one daily over four consecutive days) 30-45 minutes before experimental procedures. Participants weighing 40+kg received 24IU (n=21, OT=10, PL=11), delivered as two puffs to each nostril, the standard dose usually given to adults (MacDonald et al. 2011) and participants under 40kgs received half the adult dose at 12IU (n=17, OT=9 PL=8), delivered as one puff to each nostril. Participants were instructed to abstain from alcohol and caffeine on the day of drug administration and food and drink (except water) two hours before drug administration.

Administration: Oxytocin administration was consistent with the recent recommendations of Guastella et al. (2012). The experimenter primed each nasal spray by pumping the spray until a fine mist was observed, thus removing any displaced air present in the tube. Then all participants were given clear instructions to hold one nostril closed and to breath in through the nose immediately following nasal administration to reduce any gravitational effects (this was practiced a couple of times prior to administration). The experimenter asked participants to slightly tilt head back and placed the spray approximately 50-100mm inside the nostril at a 30-45° angle. The nasal spray was then administered followed by three ‘sniff-like’ inhalations modeled by the experimenter. This process was completed once in each nostril for 12IU participants and twice in each nostril for 24IU participants, any issues with the procedures were recorded. There were two participants who received 3 doses instead of 4 due to feeling unwell on the day (cold/flu like symptoms). Both of these participants were receiving 12IU, one was placebo and the other oxytocin. There were only two individual puffs (6IU in each puff) where it appeared that a reduced amount of solution left the bottle

(different participants, both receiving OT). All participants were comfortable to receive the nasal spray, although a handful reported a strange smell and/or that they could feel some of it go down their throat.

Parent-Child Interaction Training

The key objective of this treatment study was to evaluate the effectiveness of nasal oxytocin for reducing social deficits in autism. A critical issue concerns the context in which the oxytocin should be administered. We evaluated the effects of administration in a therapeutic context that: 1) provided rich opportunities for positive affiliative behaviour, and 2) had some empirical support as being useful for promoting positive social behaviour for ASD. Thus, our Parent-Child Interaction Training was selected based on current knowledge of evidence-based psychological treatments for autism and specifically to complement the use of nasal oxytocin and consisted of two key intervention components, 1) teaching emotion recognition and 2) teaching key social interaction skills.

The emotion recognition training used the Mindreading (MR) program developed by Simon Baron-Cohen (Baron-Cohen 2007). The second part of this training program involved using positive video feedback to enhance the way in which both parent and child interact with each other. This approach is based on “Video Interactive Guidance” (VIG) (Kennedy 2011 for a comprehensive review of VIG) and involves using short video clips demonstrating the clients use of successful communication skill, such as, eye contact, positive body language and responding to others. Research has widely supported the use of video as a way of improving social and interpersonal skills in both adults and children with autism (Reichow and Volkmar 2010; Bellini and Akullian 2007; Kroeger et al. 2007).

The experimenter was a Child Psychologist with a Masters Degree and a Post Graduate Diploma in Autism. Furthermore the experimenter participated in formal training in VIG, which involved a 2 day initial training course, 7 hours of VIG supervision and 0.5

accreditation day. The experimenter conducted all of the intervention sessions with the parent and child. As we combined both emotion training and the use of video we created a comprehensive therapist manual to accompany the training program, this also promoted consistency across participants. During the final session a home task workbook was given to the child and parent which was to be completed prior to the three month follow-up visit.

Family Interaction Task

Parent-child dyads completed the Family Interaction Task at each assessment time-point. The specific tasks included: *Free Play (10 minutes)*, *Emotion Talk (10 minutes)* and *I-Love-You Task (2 minutes)*. In Free Play the parent and child are given a range of games/toys and asked to play together as they like for 10 minutes. In Emotion Talk the parent and child are asked to discuss a happy and sad time that they have shared together. This task aims to explore the child and parent's generally conversation skills, and more specifically their ability to discuss with each other emotion based conversational topics. The I-Love-You Task explores how the child responds to having his parent express positive emotion to the child (Dadds et al. 2012). The primary objective of the Family Interaction Task was to gather observational data. All of the Family Interaction Tasks were video-taped and later coded by a two trained video coders. The experimenter was not present during the Family Interaction Task and the observational data was extracted for the initial assessment, pre-treatment, time point 1-3, post-treatment and 3 month-post. The Family Observation Schedule-ASD (FOS-ASD) (MacDonald and Dadds 2010) was used as the coding instrument for scoring the parent-child interactions during the Family Interaction Tasks. This schedule was adapted from the FOS-6th Edition (Pasalich and Dadds 2009) for use with families of children with ASD. The theoretical underpinnings of this coding instrument are embedded in the behavioural principles of social learning theory (Patterson 1982); attachment theory (Bowlby et al. 1992) and inter-subjectivity (Trevarthen and Aitken 2001). The codes reflect the

behavioral and affective aspects of parent-child interactions which are required for successful social interactions and attachment formations. The FOS-ASD incorporates an amalgamated procedure for coding family interaction, bringing together the social learning micro-coding approach; tallying the frequency of behaviours occurring within discrete time-intervals, with the attachment macro-coding approach; globally scoring behaviours along a continuum.

There were two video coders, both blind to treatment conditions. Both coders were trained using the coding manual (MacDonald and Dadds 2010) and practice videos. Twenty percent of videos were coded independently by the two raters to check for inter-rater reliability; ICC = 0.801, $p < 0.001$ (95% CI = .49 - .92).

Outcome measures

Parent and child behaviours were assessed at multiple time points for: side-effects, social interaction skills, repetitive behaviours, emotion recognition and generalised effects (diagnostic change). Time points were Initial assessment; Pre-treatment; Time 1 (T1); Time 2 (T2); Time 3 (T3); Post-treatment; three-month post treatment. All observational data were collected during the family assessment tasks. Two of the assessment points (T1 & T3) were immediately (30-45 mins) after the child received oxytocin (or placebo). Time point 2 (T2) was used to assess the generalizability of oxytocin (or placebo) mid-treatment (after two parent-child interaction sessions and oxytocin or placebo administrations), but not after receiving oxytocin (or placebo) immediately beforehand.

Side-effects: Side-effects were monitored throughout the study. Parents completed a detailed physiological checklist at each time point (designed in consultation with a paediatrician and child psychiatrist and with reference to available information on oxytocin safety (Novartis, 2009). Participant blood pressure and heart rate was also recorded throughout the treatment, once at time point 1 and 2, before and after each nasal spray administration, and at time point 7

and 8. The parent and child were asked if they thought the child has received oxytocin or placebo each day following administration. The experimenter also recorded their own thoughts as to what each participant had received.

Social interaction: Social interaction was measured through parental questionnaires, video micro-coding and global coding of observations. Parental questionnaires included; the Social Skills Rating Scale (SSRS: Gresham and Elliot 1990). Video analysis of the family observation task took place at all 7 time points. A five-point likert scale (0-4) was used with a higher score indicating that more of the behaviour was occurring. Each video was coded for a global rating of social interaction which consisted of talk; warmth; responsiveness and eye contact. Each video was also micro-coded for positive body language, verbal content and asking questions over each 30 seconds of video time. At the end of each 30 second interval the video was stopped and parent and child were coded for eye contact (present=1 or absent=0). The coding manual is available from the first author.

Repetitive Behaviours: Repetitive behaviours were measured through micro-analysis of the family observation videos at all time points. The parents also completed the Social Reciprocity Scale which rated autistic mannerisms (Constantino and Gruber 2005) (both given at initial, pretreatment, posttreatment and 3 month posttreatment).

Emotion recognition: All participants completed the UNSW Facial Emotion task (Dadds et al. 2004). In this task the participant views sets of happy, sad, angry, fearful, disgusted, and neutral faces on a computer monitor (1-sec duration) and is asked to identify the emotion. This measure has established reliability and validity for measuring fear recognition in children (Dadds et al. 2008). Overall accuracy scores for each emotion were obtained.

Generalised effects/diagnostic status: At initial contact and then at three month post treatment, participants diagnosis was re-assessed using the OSU, CARS and the DISCAP-ASD as previously described.

Design and statistical analysis

The study was a randomized-controlled trial comparing oxytocin (OT) with placebo (PL) with participants and their parents, investigator team, outcome assessors, family interaction coders and data analyzers blind to treatment group. All statistical analyses were conducted using SPSS 20.0 (SPSS Inc, Chicago, IL, USA). A preliminary one-way ANOVA was conducted on demographic variables and pre-treatment diagnostic variables to ensure that the groups had been randomly assigned – there were no differences between groups. Drop-out after randomization was negligible (n=4) and equally distributed across groups; thus, missing data at the case level was trivial and treatment effects were evaluated using repeated measures ANOVAs. Missing values analysis of observational data indicated there was small amounts of missing-at-random data distributed across the data file; these were video-data and assessments lost due to equipment failure or participants missing a session. To correct for this Rueben's method of multiple imputation was used.

The treatment order effects (supplementary table 1) were not significant, $F(1,21) = 1.190$, $p=.288$, thus all further analysis was conducted on oxytocin versus placebo without reference to treatment order. All observational data were collected during the family assessment tasks. Two of the assessment points (T1 & T3) were immediately (30-45 mins) after the child received oxytocin (or placebo). Time point 2 (T2) was used to assess the generalizability of oxytocin (or placebo) mid-treatment (after two parent-child interaction sessions and oxytocin or placebo administrations), but not after receiving oxytocin (or placebo) immediately beforehand. Significant interaction effects were dismantled using

Tukey's HSD tests.

Results

Demographics and clinical sample

Figure 1 shows recruitment and retention through the study. Thirty-five participants completed time points 1-7; n=three participants failed to return for the three month follow-up. There were no significant baseline differences between groups in demographics or clinical variables (Table 1). There was a high level of co-morbid diagnosis which was equally distributed between treatment groups. Use of psychotropic medication was also equally distributed between groups, $\chi^2(33)=0.79$, $p=0.37$.

Safety, side effects and subjective awareness

Participants reported minimal side-effects throughout the study. There was a significant main effect for time, ($F(3.62, 115.87) = 7.96$, $p<0.0$), both groups reported decreased side effects from pretreatment to post. There were no significant group effect ($F(1, 32) = 1.48$, $p=0.23$), and no time x group interaction ($F(3.62, 115.87) = 0.59$, $p = 0.76$). Similarly, we found no significant main effects or interaction for group and time on systolic and diastolic blood pressure, and heart rate. Neither children nor parents were able to guess whether they had received oxytocin or placebo ($p>0.1$); however, guesses by the experimenter were borderline significant; that is, she was slightly above chance in guessing whether a participant had received oxytocin or placebo, $\chi^2(1) = 4.0$, $p=0.046$.

Social interaction skills

Micro-analysis of Verbal and Nonverbal communication;

Child eye contact: There was a significant main effect for time with child eye contact increasing from Initial to Time 3, ($F(6, 216) = 4.70, p < 0.001$). There was no main effect for group, ($F(1,36)=0.30, p = 0.59$), or time x group, ($F(6, 216)=1.58, p=0.16$) (Figure 2).

Parent eye contact: We found a significant main effect for time, ($F(6, 216) = 3.05, p = 0.01$), with parent eye contact increasing significantly from Pre-treatment to Time 3. There was no main effect for group, ($F(1,36) = 0.87, p = 0.35$) or time x group, ($F(6, 216) = 1.34, p = 0.24$) (Figure 3).

Positive non-verbal behaviours: There was a significant main effect for time on child positive non-verbal behaviours from Pretreatment-Posttreatment, ($F(6, 216)=7.51, p<0.001$); however, there was no main effect for group, ($F(1,36)=0.58, p = 0.45$) or time x group, ($F(6, 216) = 1.71, p=0.12$) (Figure 2).

Verbal Content: Micro analysis of child verbal content, asking questions and giving information, found no significant main effect for time, ($F(6, 216)=0.64, p=0.70$), group ($F(1, 36)=0.89, p = 0.35$) or time x group, ($F(6, 216) = 0.82, p=0.55$) (Figure 2).

Global ratings of social interaction

The video analysis of global ratings found a significant main effect on the quality of child social interaction skills over time, ($F(4.49, 161.72) = 3.105, p=0.01$), child social interaction skills increased from Pre-treatment to Post-treatment. There was no significant main effect for group, ($F(1,36)= 1.22, p=0.28$) or time x group, ($F(4.49, 161.72) = 1.41, p = 0.23$) (table 2). Analysis of SSRS scores, which looked at parental perceptions of social skills, found a significant main effect for time, ($F(3,99)=7.51, p<0.001$) from Pre-treatment-Posttreatment, but this was not maintained at three month post. We did not find a significant main effect for group, ($F(1,33)=0.003, p=0.96$) or time x group, ($F(3, 99)= 2.23, p = 0.090$) (figure 2).

Repetitive behaviours

Parental measures using the SRS showed a main effect over time with autistic mannerisms decreasing significantly, ($F(3,99) = 4.0, p=0.01$). There was no main effect for group, ($F(1, 33)= 0.69, p= 0.41$), however, a time x group effect was found with the placebo group decreasing in autistic mannerisms significantly more than oxytocin, ($F(3,99) = 2.98, p=0.04$) (Table 2). Micro analysis of repetitive behaviours from the family observation videos found no main effect of time, ($F(4.57, 164.48)= 0.41, p=0.87$), group, ($F(1, 36)= 0.06, p= 0.81$) or time x group, ($F(4.57, 164.48) = 1.35, p=0.24$) (Table 2).

Emotion recognition

For accuracy on the emotion recognition task, we found a significant main effect for time, ($F(2.20, 63.75)= 17.04, p<0.001$) between initial to pre-treatment and pre to post-treatment which could be attributed to practice effect. There was no main effect for group, ($F(1, 29)=.197, p= 0.660$) or time x group, ($F(2.20, 63.75)=0.25, p=0.80$) (Table 2).

Generalised Effects/Diagnostic change

Table 2 shows means and SDs for diagnostic ratings. On the autism global impression scale, there were a significant main effect for time, $F(2, 66) = 3.72, p = .03$, both groups experienced a decrease in diagnostic severity post-treatment. There was no significant group, $F(1, 33)=1.93, p=.17$ or time x group, $F(2, 66) = .30, p =.74$. Analysis of the DISCAP symptom severity also found no significant main effect for time, $F(1,33) = .02, p= .88$, group $F(1, 33)=.05, p= .82$ and time x group, $F(1, 33)= 1.79, p= .19$. The CARS assessment tool, administered at initial and then at three months post intervention found a main significant effect across time, $F(1, 33) = 8.47, p =.01$ in both placebo and oxytocin. There was no

significant main effect for group, $F(1, 33) = 1.71$, $p = .20$ or time $\times$ group, $F(1, 33) = .20$, $p = .66$.

Effects of medication and dosage

Given that the above analyses detected no positive benefits of oxytocin, we checked a number of possible issues. First, we repeated the ANOVAS with the sample split into participants on or off medication; the results were consistent with those reported above for both these groups and it was clear that the inclusion of participants on medication did not affect results. Second, we repeated the analyses comparing participants dosage levels (12IU compared with 24IU); again, there was no difference in the pattern of results and we concluded that the inclusion of participants who received the smaller dose of 12IU did not affect results.

Discussion

We tested treatment effects of daily administrations of intranasal oxytocin over a week period, on a range of measures in a sample of children with ASD. We found that, compared to placebo, intranasal oxytocin did not significantly improve emotion recognition, social interaction skills, or general behavioral adjustment in male youths with autism spectrum disorders. Given that previous studies have found evidence that nasal oxytocin has potential as a treatment for autism, these negative findings demand considerable interrogation.

Perhaps the most robust finding in literature has been that oxytocin enhances emotion recognition. Specifically, there have been several studies attesting to the positive impact of intranasal oxytocin on emotion recognition with healthy adults; however, a recent meta-analysis concluded that while oxytocin does enhance face recognition, the combined effect size is quite modest ($d = 0.21$) (Ijzendoorn and Bakermans-Kranenburg 2012) and perhaps decreasing as the initial powerful studies are subject to replication. Specific to autism, there

are three studies reporting positive effects of intranasal oxytocin on emotion recognition (Guastella et al. 2010; Andari et al. 2010; Anagnostou et al. 2012). Contrary to prior studies, our results found that intranasal oxytocin did not potentiate emotion recognition in youths with ASD. A notable difference here is that previous studies tested emotion recognition while participants were under the influence of oxytocin; our study looked at pre-post changes in emotion recognition following a multiple exposures to oxytocin combined with social interaction intervention.

We further found that intranasal oxytocin did not reduce repetitive behaviours and this was across parental report and independent observations. Our negative result is contrary to an earlier study involving intravenous infusion of oxytocin with a small sample of adult males with autism (Hollander et al. 2003). Our findings are consistent with Anagnostou et al. (2012) who also found no significant change in higher-order (ritualistic, sameness, compulsive and restricted subscales) repetitive behaviours follow 2 daily doses of intranasal oxytocin over 6 weeks using the same measure (Bodfish et al, 2000).

Animal studies have consistently linked oxytocin to social approach behaviours (Lim and Young 2006; Young 2002) and there are studies with adult human males demonstrating an increase in eye contact immediately following intranasal oxytocin administration (Gamer et al. 2010; Guastella et al. 2008). Specifically, there is evidence that oxytocin enhances attention to the eye region of computerised stimulus faces. This has never been tested however, in the real world during interactions with intimates. We found no effect of oxytocin on eye contact when the participants interacted with their mothers. Results were similarly negative for body language and verbal communication. We found that oxytocin did not significantly improve the quality of social interactions or any specific communication skills with a parent/carer giver.

Why did the oxytocin intervention not work in this study? Our first hypothesis was that mistakes had been made in the preparation, storage, or labelling of the oxytocin and placebo sprays. To check, we had nasal sprays independently tested for oxytocin content by the university chemistry department, and were able to reject this explanation. Second, it could be argued that our sample size was inadequate to detect oxytocin treatment effects. This is untenable; first, the current study is twice the size of the next largest existing study, and secondly, there is no evidence of even a trend favouring the oxytocin group over placebo on any measure.

Third, we examined whether the null effects were due to some of the sample using concurrent psychoactive medications. Repeating the tests with and without participants on medication it made no difference to our results. Similarly, we checked whether there were oxytocin effects for the sample using the common dose of 24IU versus 12IU given to the younger/smaller children. Again, we found no significant advantage for oxytocin in groups receiving higher versus lower doses.

Fourth, participant age ranged from 7-16 years and it is possible that this large age variation had an impact on results. When we repeated tests comparing age group we found no differences in result.

Our final hypothesis is that oxytocin may have limited or very specific benefits for autism. The studies prior to ours on autism have been characterised by small and generally older samples, using brief analogue measures while the participants had the oxytocin in their system. Both the current study and Anagnostou et al. (2012) tested whether repeated doses can lead to more robust generalised change, and both found no effects on social functioning and repetitive behaviors. The studies differ in that Anagnostou et al. (2012) did find some evidence for improvements in emotion recognition and a broad measure of quality of life.

It is likely that some of the differences in outcomes are associated with age and diagnostic characteristics of the specific cohorts being tested. Autism is a complex disorder and probably consists of several subgroups with differing phenotypes and etiologies. Given the recent findings on genetic variations of the oxytocin receptor system that characterise autism, it is possible if not likely that substantial proportions of people with ASD would not be able to benefit from exogenous oxytocin due to functional problems in the oxytocin receptor system (e.g., Gregory et al., 2009). These and the current data are consistent with a growing awareness that the therapeutic effects of oxytocin may be more specific and contextually grounded than previously thought (Bartz et al. 2012; Guastella and MacLeod 2012). This may suggest the need to more precisely target specific subsets of individuals with autism.

In summary, this study tested the generalized treatment effects of daily administrations of nasal oxytocin alongside a psychological intervention. These results show no benefit of oxytocin for young individuals with autism spectrum disorders, and suggest some caution in recommending nasal oxytocin as a general treatment for young people with autism. Next steps should focus on developing a fuller understanding of how best to deliver oxytocin, who responds to it and likely limitations as a therapeutic intervention.

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Figure 1: Consort diagram showing recruitment and retention of participants through the study.

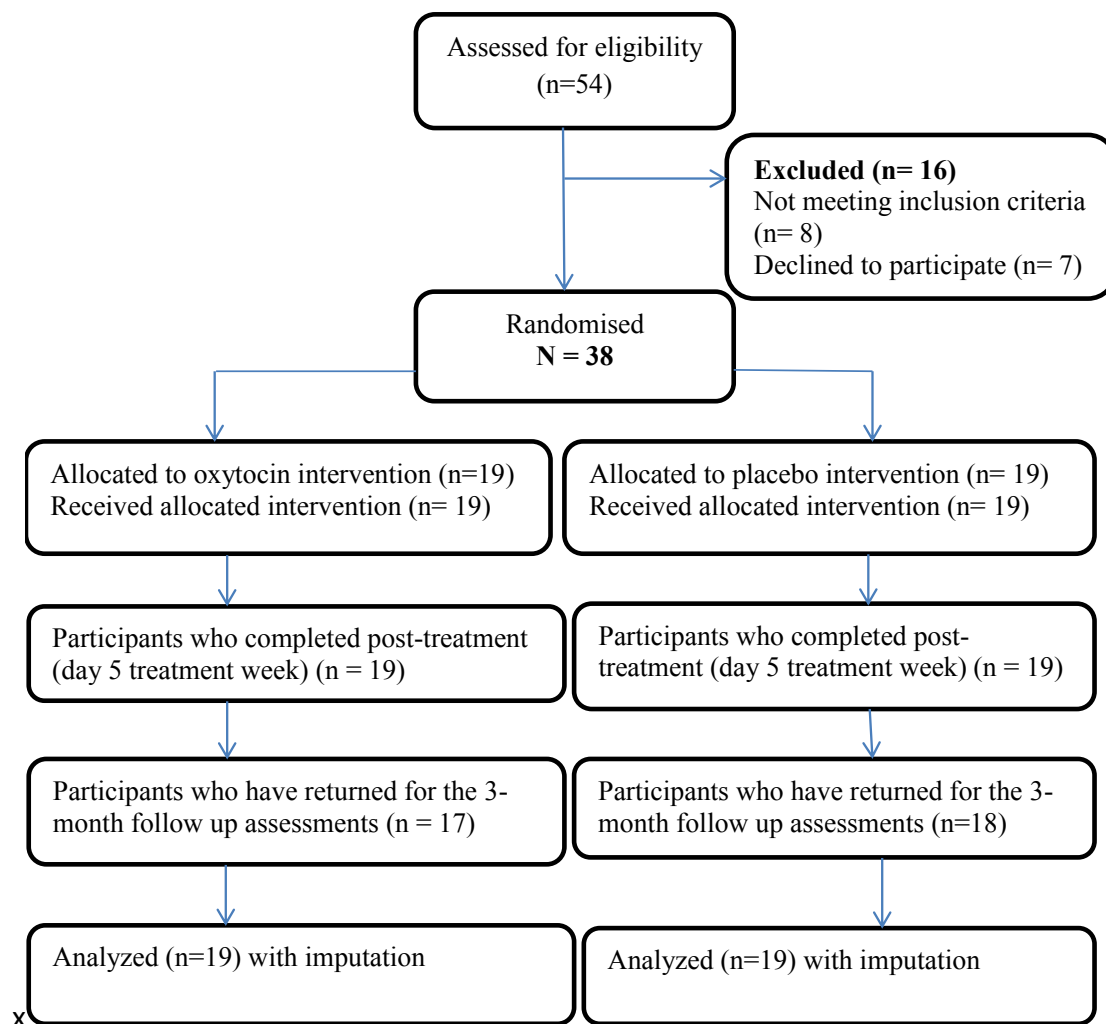


Figure 2: Oxytocin and placebo group means and *SE Means* for; (a) Mean parent and child eye contact; (b) Micro-analysis of child verbal content; (c) Micro-analysis of child positive non-verbal behaviours; (d) Global parent ratings on the Social Skills Rating Scale.

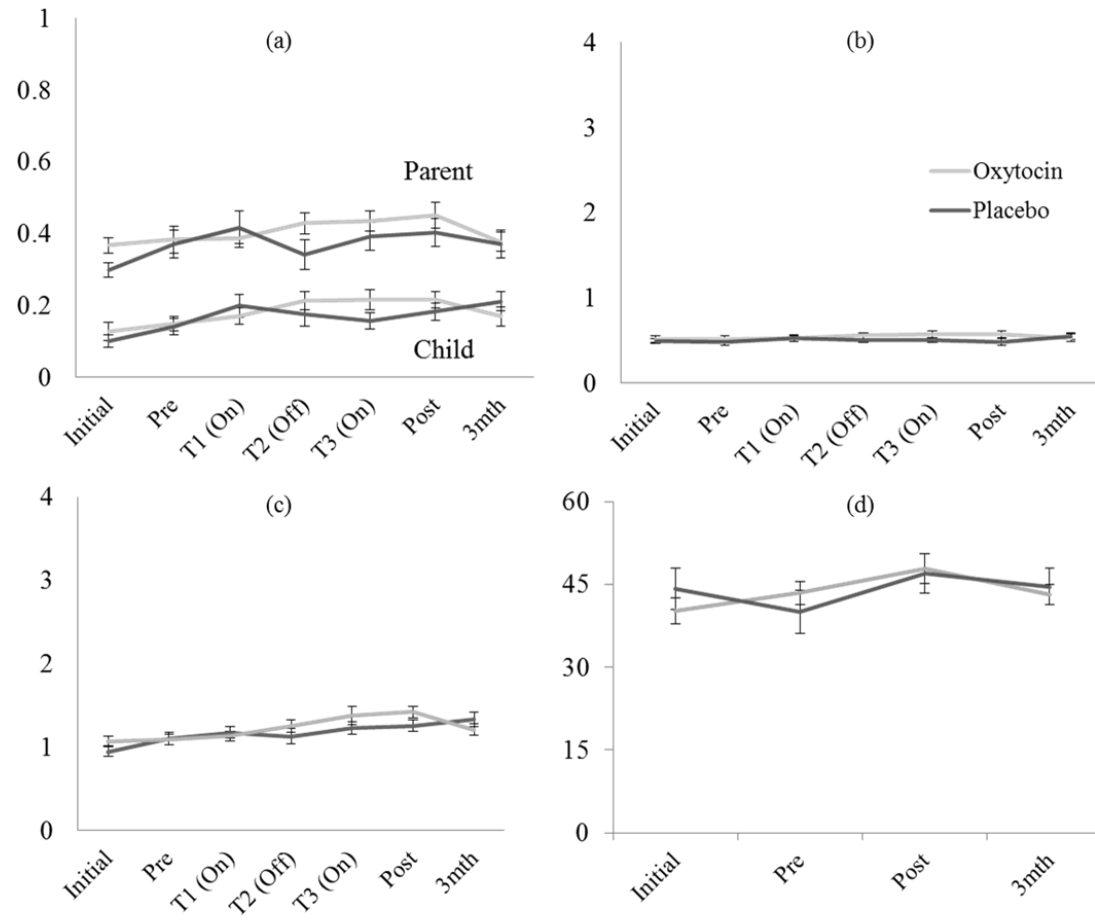


Table 1: Clinical variables and demographics of the sample split by oxytocin and placebo groups.

	Treatment Condition				
	Placebo		Oxytocin		
	M	SD	M	SD	<i>p</i>
<i>Clinical Variables</i>					
Age in Years	10.74	2.38	11.79	2.82	.22
Initial DISCAP Severity	3.63	.87	3.42	.96	.29
Number of Diagnoses	2.05	.97	2.11	.94	.86
ADHD Diagnosis	n = 9		n=10		1.0
ODD Diagnosis	n =7		n=5		.73
Anxiety Diagnosis	n = 2		n = 4		.66
CARS Total Score	33.84	3.70	32.26	3.25	.17
Child's Weight (Kilograms)	43.77	17.25	47.13	23.35	.53
WISC-IV - Full Scale IQ	88.64	7.98	90.47	11.70	.66
OSU-Autism Global Impression Scale	4.14	1.09	4.13	1.08	.98
<i>Demographics</i>					
Mothers Education Level	3.69	1.31	4.14	1.23	.51
Fathers Education Level	3.61	1.44	3.50	1.34	.97
SEIFA Rank of Residential Postcode	4.72	1.67	6.32	1.60	.81

Table 2: Means and SDs scores over time for oxytocin and placebo groups.

	Oxytocin		Placebo	
	<i>Mean</i>	<i>SD</i>	<i>Mean</i>	<i>SD</i>
<i>SRS Autistic Mannerisms</i>				
Initial	17.13	5.57	20.56	6.70
Pre	15.60	5.74	18.83	7.37
Post	17.20	6.57	16.61	8.44
3mth	16.47	5.94	15.78	7.85
<i>Video Observation of Repetitive Behaviours</i>				
Initial	.24	.26	.20	.25
Pre	.15	.22	.27	.19
Time 1 (On)	.21	.24	.23	.22
Time 2 (Off)	.21	.25	.29	.26
Time 3 (On)	.22	.25	.22	.18
Post	.27	.26	.18	.20
3mth	.27	.24	.25	.24
<i>Video Observation of Social Interaction Skills</i>				
Initial	1.63	.50	1.69	.38
Pre	1.78	.50	1.64	.45
Time 1 (On)	1.76	.43	1.65	.50
Time 2 (Off)	1.80	.40	1.63	.43
Time 3 (On)	1.97	.55	1.74	.53
Post	1.99	.52	1.67	.47
3mth	1.92	.51	1.96	.49
<i>Facial Emotion Recognition Task</i>				
Initial	.79	.11	.77	.14
Pre	.82	.13	.80	.14
Post	.86	.08	.86	.09
3mth	.87	.08	.86	.07
<i>OSU Autism Global Impression Scale</i>				
Initial	3.76	.70	4.17	.79
Pre	3.47	.64	3.72	.89
3mth	3.60	.63	3.78	.94
<i>CARS Total Impairment Scale</i>				
Initial	31.63	3.26	33.56	3.59
3mth	30.07	2.78	31.06	5.20
<i>DISCAP Axis 1 Severity</i>				
Initial	3.20	.86	3.61	.85
3mth	3.47	.64	3.39	1.09