

ORIGINAL ARTICLE

The effect of oxytocin nasal spray on social interaction deficits observed in young children with autism: a randomized clinical crossover trial

CJ Yatawara¹, SL Einfeld^{2,3}, IB Hickie², TA Davenport² and AJ Guastella¹

Interventions for autism are limited. The synthetic hormone oxytocin may provide a potential treatment to improve core social and behavioral difficulties in autism, but its efficacy has yet to be evaluated in young children who potentially may benefit to a greater extent. We investigated the efficacy, tolerability and safety of oxytocin treatment in young children with autism using a double-blind, randomized, placebo-controlled, crossover, clinical trial. Thirty-one children with autism received 12 International Units (IU) of oxytocin and placebo nasal spray morning and night (24 IU per day) for 5 weeks, with a 4-week washout period between each treatment. Compared with placebo, oxytocin led to significant improvements on the primary outcome of caregiver-rated social responsiveness. Overall, nasal spray was well tolerated, and the most common reported adverse events were thirst, urination and constipation. This study is the first clinical trial to support the potential of oxytocin as an early intervention for young children with autism to help improve social interaction deficits.

Molecular Psychiatry (2016) **21**, 1225–1231; doi:10.1038/mp.2015.162; published online 27 October 2015

INTRODUCTION

Autism represents a group of complex brain developmental disorders characterized by impairments in social interaction, social communication and stereotypical and repetitive behaviors.¹ The diagnosed incidence of autism is estimated to be 1 in 68 children.² However, effective interventions have remained limited. Some psychotropic drugs, such as risperidone, seem to alleviate behavioral problems but they are often associated with adverse events.³ There is also little evidence for effective pharmacotherapy to alleviate core social diagnostic features.⁴ Alternatively, behavioral interventions significantly improve impairments,⁵ but they are typically time-consuming and costly.^{6,7}

The hormone oxytocin has been identified as having an important role in social cognition and behavior.⁸ Oxytocin administration has been shown to enhance peer recognition, and bonding behavior in studies across numerous mammalian species.⁹ In neurotypical adult humans, intranasal administration of 24 International Units (IU) of oxytocin has been found to improve eye gaze,¹⁰ emotion recognition^{11,12} and trust.¹³ This has led to speculation for its potential application in the treatment of psychiatric disorders characterized by social difficulties.^{9,14} In adults with autism, initial studies evaluated effects of a single dose of oxytocin on acute symptoms. Intravenous administration was found to reduce repetitive behaviors¹⁵ and improve accuracy of recognizing emotion from speech.¹⁶ Intranasal administration has been the preferred route of administration owing to it being well tolerated and easy to use (for dosing regimens, absorption pathways and bioavailability discussion of nasal administration see review in Guastella *et al.*¹⁷). Intranasal administration of

oxytocin has been found to improve face processing, decisions in a social ball tossing game¹⁸ and emotion recognition in male adults.¹⁹ More recently, youth with autism (aged 12–19 years) have shown improved emotion recognition under oxytocin (18 IU).²⁰

Past research indicates that interventions for autism provided in the early years of life offer the best opportunity to improve long-term outcomes.^{14,21,22} Younger children appear to show greater response to intervention as observed by improved functioning and decreases in challenging behaviors.²¹ The present study investigated the efficacy, tolerability and safety of intranasal-administered oxytocin to improve social interaction deficits observed by caregivers of young children with autism.

MATERIALS AND METHODS

Study design

This study of intranasal-administered oxytocin in young children with autism was a double-blind, randomized, placebo-controlled, crossover, clinical trial. Participants were randomly allocated to study arms, where each one consisted of two consecutive treatment conditions. Treatment was administered in this study using the AB/BA model. Participants who were randomly allocated to the AB arm received oxytocin during phase 1 of treatment and then placebo during phase 2 of treatment. Those participants who were randomly allocated to the BA arm received placebo during phase 1 of treatment and then oxytocin during phase 2 of treatment. This consequently allowed for the response of a participant to oxytocin to be directly contrasted with their response to placebo, thus reducing the influence of any confounding covariates.

¹Autism Clinic for Translational Research, Brain and Mind Centre, Central Clinical School, Faculty of Medicine, The University of Sydney, Sydney, NSW, Australia; ²Brain and Mind Centre, Central Clinical School, Faculty of Medicine, The University of Sydney, Sydney, NSW, Australia and ³Centre for Disability Research and Policy, Brain and Mind Centre, University of Sydney, Sydney, NSW, Australia. Correspondence: Dr AJ Guastella, Autism Clinic for Translational Research, Brain and Mind Centre, The University of Sydney, Building F, 94 Mallett Street, Camperdown, Sydney, NSW 2050, Australia.

E-mail: adam.guastella@sydney.edu.au

Received 28 November 2014; revised 19 August 2015; accepted 17 September 2015; published online 27 October 2015

The study was conducted at the Brain and Mind Centre (BMC), The University of Sydney (Australia) between October 2010 and October 2012 with approval from the University of Sydney Human Ethics Committee (2012/2281). Informed consent from caregivers was obtained for each participant.

Participants

Inclusion criteria were children aged between 3 and 8 years of age who met the DSM-IV-TR (Diagnostic and Statistical Manual of Mental Disorders, 4th Edition, Text Revision) criteria for Autistic Disorder, Asperger's Disorder or Pervasive Developmental Disorder-Not Otherwise Specified (PDD-NOS). All children were recruited through advertisement and specialist networks within the Inner-Sydney area. To confirm eligibility, caregivers of participants initially completed a telephone screening assessment to determine whether their child had received a previous diagnosis associated with autism and were not likely to meet noted exclusion criteria. Participants then completed screening assessments conducted at the BMC, namely the Autism Diagnostic Observation Schedule (ADOS),²³ the caregiver-rated Social Responsiveness Scale (SRS-P)^{24,25} and the Developmental Behavior Checklist (DBC-P).^{26,27} The Leiter International Performance Scale-Revised²⁸ was also administered to participants who did not have a valid measure of intelligence within the past 5 years. All participants were stabilized on psychotropic medication for 8 weeks before commencement of the trial, and no changes to dose were made for the duration of the trial. The telephone screen and onsite assessments were followed by a medical interview with a pediatric psychiatrist with extensive research and clinical experience in autism (SLE). During the medical interview, a participant's autism diagnosis was confirmed using previous psychological reports and scores on the above assessments. Those participants who received a diagnosis of PDD-NOS did so because of the presence of autistic symptoms that did not reach the threshold for Autistic Disorder or Asperger's Disorder. In addition, participant's suitability for treatment based on exclusion criteria was assessed. Exclusion criteria included known sensitivity to preservatives in the nasal spray (in particular, E216, E218 and chlorobutanol hemihydrates).

Assessment schedule

Following informed consent by the caregiver, eligible children visited the BMC in week 1 for social interaction and behavioral assessments and phase 1 drug kit allocation. Participants returned to the BMC at week 5 for phase 1 post-test assessments. Following a 4-week washout period, participants returned at week 9 to complete phase 2 pretest assessments and drug kit allocation. Finally, phase 2 post-test assessments were completed at week 14.

Medication schedule

In phase 1, participants were randomly assigned drug kits containing either oxytocin or placebo (which included all of the same ingredients except oxytocin). All sprays contained sorbitol, benzyl alcohol glycerol and distilled water, within an amber 7 ml glass nasal spray with metered Pfeiffer pump spray bottle. In phase 2, participants received drug kits that contained the alternate nasal spray. Drug kits were manufactured and stratified by the compounding chemist. Nasal sprays were labeled with sequential numbers corresponding to order of entry into the trial and stratified by gender by an independent research assistant. Blocking was in sets of six (three active and three placebo) in a randomly generated order. All research staff conducting assessments, as well as caregivers and participants, were blind to condition allocation and unaware of randomization.

The drug kits for each treatment (oxytocin and placebo) contained two nasal spray bottles to be administered over the course of 5 weeks. For oxytocin-assigned participants, the first bottle (labeled week 1) contained a dose of 3 IU per spray, administered in a dose escalation schedule over 1 week, morning and night. Day 1 of the dose escalation week started at 3 IU and then increased every 2 days by 3 IU, until on day 7, the participant was receiving the full dose of 12 IU morning and 12 IU at night. The second bottle (labeled weeks 2–5) contained 6 IU per spray and was administered one spray per nostril, each morning and night (24 IU per day), over the following 4 weeks. Placebo assigned participants received identical bottles and instructions for delivery of these bottles over the dose escalation and treatment phase.

The first drug administration of each phase was conducted at the BMC. Instructions were provided to caregivers and training was consistent with our previous published guidelines.¹⁷ Children were monitored onsite for 40 min following the first nasal administration as this is the typical period of time employed in experimental studies to observe effects of oxytocin. Caregivers (and the child when appropriate) were provided with open-ended questions and then a caregiver-rated checklist was provided to parents. Follow-up of potential adverse events was by telephone during mid-condition for each phase (i.e. weeks 3 and 12) and at the completion of each phase (i.e. weeks 5 and 14).

Primary and secondary outcome measures

There were two primary outcome measures. The first was change in caregiver-rated social responsiveness on the SRS-P.^{23,24} Using a four-point Likert scale, the SRS-P assesses a child's social awareness, social information processing, capacity for reciprocal social communication, social anxiety avoidance, autistic preoccupations and traits, and total social impairment. Scores range from 0 to 195 (M = 50.0; s.d. = 10.0) and higher scores indicate greater impairment. The second was change in caregiver-rated severity of repetitive behavior on the Repetitive Behavior Scale-Revised (RBS-R-P: scores range from 20 to 60, M = 33.1, s.d. = 20.6, where higher scores indicate greater severity).²⁹ Secondary outcome measures included change in: experimenter-rated observations of reciprocal social interaction and communication on the ADOS²² (behaviors scored on an algorithm and higher scores indicate greater severity); caregiver-rated social and emotional difficulties on the DBC-P^{25,26} (measured on a three-point Likert scale where scores range from 0 to 192, M = 61.2, s.d. = 23.9, with high scores indicating greater severity);³⁰ experimenter-rated Clinical Global Impressions-Improvement scale;³¹ caregiver observations of perceived improvement, tolerability and adverse events; and caregiver stress on the Caregiver Strain Questionnaire (CSQ; scores range from 21 to 105, with greater scores indicating greater severity).³²

In addition to mid-condition telephone monitoring, adverse events were reported using a 12-item caregiver-rated checklist collected during the onsite monitoring of initial nasal spray administration and at post-test for phase 1 and 2. The checklist included thirst, frequency of daytime and nighttime urination, nausea, vomiting, diarrhea, constipation, skin rash, heart palpitations, headaches, shortness of breath and lightheadedness. Any serious adverse events were reported to the local institutional review board.

Statistical analyses

All statistical analyses used the IBM SPSS Statistics for Windows, Version 21.0 (IBM Corp., Armonk, NY, USA). For this study, power was calculated at 0.9 based on a moderate to large effect size, based on effect sizes from previous single dose data^{10,20} of $d=0.6$ and α -level of 0.05 for 31 participants with matched data. Owing to this sample size, it was decided to plug-in missing values using series mean substitution at the subscale level of all measures. From the total data analyzed, there was 4.1% missing data (Supplementary Table 1). Using this data set, total scores were then computed and examined to ensure violations were not met for linear model assumptions.

Baseline demographic and behavioral characteristics were explored using independent samples *t*-tests for continuous variables and Fisher's exact test for categorical variables. Repeated measures within general linear modeling were then used to evaluate participant response by time and condition.

The main disadvantage of a crossover study is the potential for carry-over between conditions, which confounds the estimate of effects. In this trial, carry-over between phases 1 and 2 was avoided with a washout period of 4 weeks. Taking advantage of the within-person design, this was confirmed by paired *t*-tests between phase 1 pretest and phase 2 pretest (Supplementary Table 2). Collapsed data was then analyzed for oxytocin and placebo over time (pre- vs post-test) and then oxytocin vs placebo regardless of time. Data were further analyzed using a difference-in-difference approach, which compared the average change over time in the primary and secondary outcome variables after oxytocin compared with the average change over time in these variables following placebo administration. Where appropriate, effect size (Cohen's *d*) has been reported, where 0.2 is indicative of a small effect, 0.5 a medium effect and 0.8 a large effect.³³

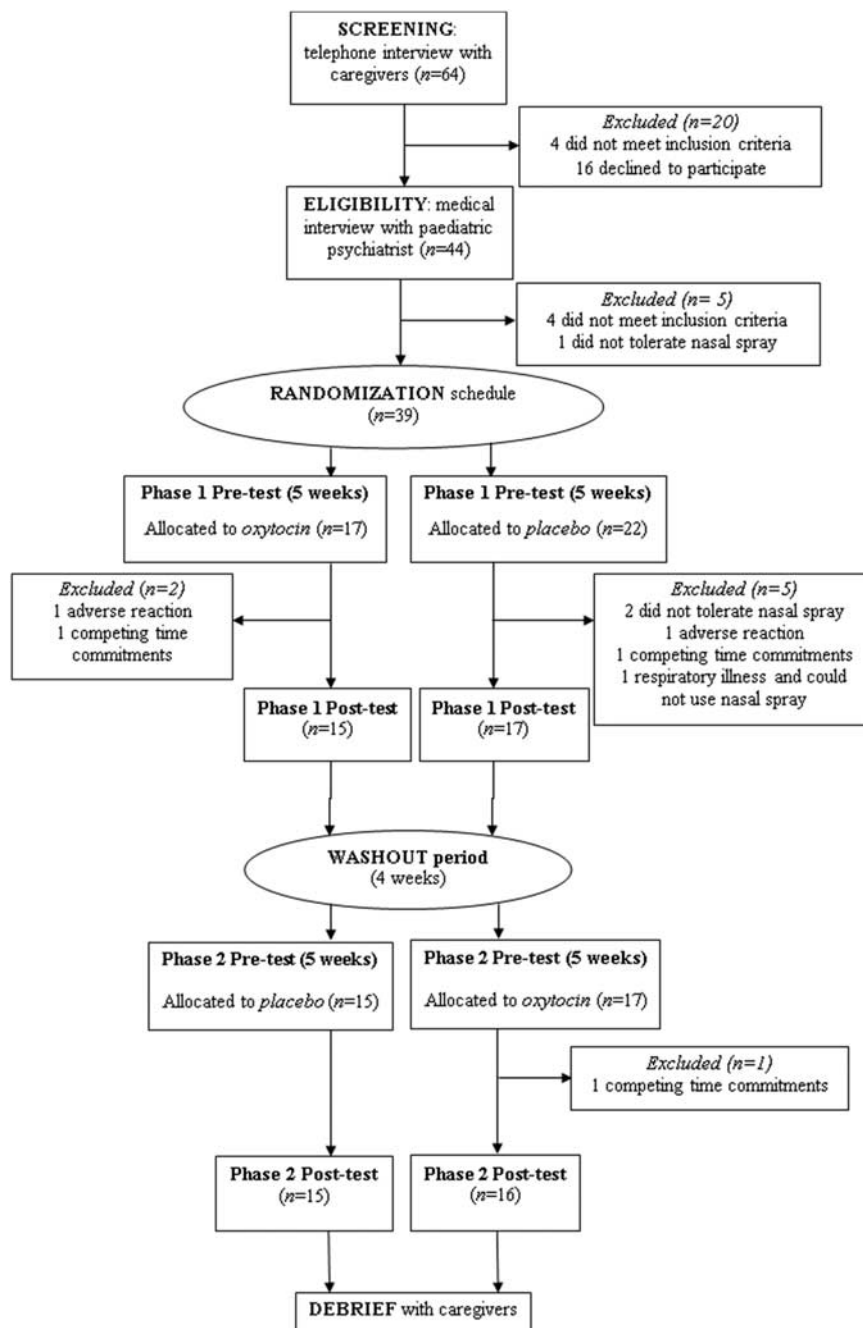


Figure 1. Consort diagram of study participants by randomization schedule. Parents of 64 children initially contacted the Brain and Mind Centre (BMC) to enquire about the study, of which 44 children were screened for eligibility. Thirty-nine children entered the randomization schedule, and 32 (82%) completed phase 1 and then crossed-over to phase 2. One participant was excluded before completion of phase 2 because of time commitments in following the protocol. The final number of participants included in the analyses was 31, including 15 randomized to 'oxytocin then placebo' and 16 to 'placebo then oxytocin'.

Clinical changes were also evaluated for individual participants using the Reliable Change Index (RCI),³⁴ which takes into consideration scale reliability of measures used. RCI is equal to an individual's score before intervention minus their score after an intervention, divided by the standard error of the difference of the measure.³⁴ If RCI is 1.96 or greater, then the difference is significant. If it is < 1.96, RCI is not significant.

Finally, experimenter-rated CGI-I scores and caregiver-rated adverse events and impressions of treatment allocation were evaluated using McNemar's χ^2 test for paired categorical data. For all analyses, the level of significance was set at $P < 0.05$.

RESULTS

Participants and baseline characteristics

The caregivers of 64 children initially contacted the BMC to enquire about the study, of which a total of 44 children were screened for eligibility. Thirty-nine of these children entered the randomization schedule and 32 (82%) completed phase 1 and then crossed-over to phase 2. One participant was excluded before completion of phase 2 because of competing time commitments. The final number of participants included in the

Table 1. Demographic and baseline measures by all study participants and by randomization schedule, $N=31$

Demographic characteristics	Total	Randomization schedule	
		Oxytocin then placebo	Placebo then oxytocin
<i>N</i>	31	15	16
Mean age in years (s.d., range, 95% CI)	6.2 (1.7, 3.0–8.9, 5.6–6.8)	5.7 (1.5, 3.1–8.0, 4.8–6.5)	6.7 (1.8, 3.0–8.9, 5.7–7.7)
Male, n (%)	27 (87.1)	14 (93.3)	13 (81.3)
<i>Diagnosis, n (%)</i>			
Autistic disorder	19 (61.3)	8 (53.3)	11 (68.8)
Pervasive developmental disorder-not otherwise specified	12 (38.7)	7 (46.7)	5 (31.3)
Mean non-verbal IQ (s.d., range, 95% CI)	83.6 (24.2, 42.0–124.0, 73.8–93.4)	76.4 (22.9, 42.0–121.0, 63.2–89.7)	91.9 (23.7, 45.0–124.0, 76.8–107.0)
<i>Medications, n (%)</i>			
Norepinephrine reuptake inhibitor (atomoxetine)	1 (12.5)	0	1 (14.2)
Melatonin (Circadin)	1 (12.5)	0	1 (14.2)
Anticonvulsant (Epilim, Lyrica)	2 (25)	0	2 (28.5)
Antipsychotic (risperidone)	2 (25)	1 (100)	1 (14.2)
Selective serotonin reuptake inhibitor (sertraline)	2 (25)	0	2 (28.5)
<i>Social interaction and behavioral measures, mean (s.d., range, 95% CI)</i>			
SRS-P	109.2 (22.6, 65.0–149.0, 101.0–117.5)	109.1 (21.7, 69.0–149.0, 97.0–121.1)	109.4 (24.0, 65.0–145.0, 96.6–122.2)
ADOS	14.8 (7.3, 2.0–35.0, 12.1–17.4)	14.1 (7.2, 5.0–32.0, 10.1–18.1)	15.4 (7.5, 2.0–35.0, 11.4–19.4)
DBC-P	59.3 (21.3, 17.0–107.0, 51.5–67.1)	59.3 (19.3, 31.0–107.0, 48.6–70.0)	59.4 (23.6, 17.0–98.0, 46.8–71.9)
RBS-R-P	23.5 (16.0, 2.0–62.0, 17.6–29.3)	27.1 (16.4, 11.0–62.0, 18.0–36.1)	20.1 (15.3, 2.0–57.0, 11.9–28.2)
CSQ	8.5 (2.4, 3.8–13.0, 7.7–9.4)	8.6 (2.2, 3.8–13.0, 7.4–9.9)	8.4 (2.6, 4.5–12.4, 7.1–9.8)

Abbreviations: ADOS, Autism Diagnostic Observation Schedule; 95% CI, 95% confidence interval; CSQ, Caregiver Strain Questionnaire; DBC-P, caregiver-rated Developmental Behavior Checklist; RBS-R-P, caregiver-rated Repetitive Behavior Scale-Revised; SRS-P, caregiver-rated Social Responsiveness Scale.

analysis was 31 (Figure 1), including 15 randomized to ‘oxytocin then placebo’ and 16 to ‘placebo then oxytocin’.

Baseline characteristics of participants completing the study were not significantly different from those who were excluded after commencement of the study ($n=8$) ($P>0.05$ for all demographic, social interaction and behavioral measures—Supplementary Table 3). Of those who completed, 16% (5/31) of participants were stabilized on psychotropic medication (treating attention deficit hyperactivity disorder, epilepsy, mood disorder, pain and sleep) before trial commencement. Mid-condition telephone interviews and post-test assessments indicate that participants adhered to the delivery of nasal spray morning and night at least 80% of the time, and 87% (27/31) of participants reported 90% and above adhered to the routine of delivery. Demographic and baseline measures for the 31 participants are shown in Table 1; importantly, the randomized groups shared similar baseline characteristics.

Confirming effectiveness of washout, there was no significant difference between phase 1 pretest scores and phase 2 pretest scores on any measure ($P>0.05$).

Statistical data

Repeated-measure analyses, specifying oxytocin/placebo as a between-subjects variable, found significant main effects for the first primary outcome measure (SRS-P, $F=13.8$, $d.f.=1$, $P<0.01$) (Figure 2). More specifically, when placebo was administered first (then oxytocin), significant linear relationships were observed (Supplementary Table 4). Significant main effects were also found for secondary measures of emotional and behavioral difficulties (DBC-P, $F=17.9$, $d.f.=1$, $P<0.001$). Here, when placebo was administered first (then oxytocin), a significant linear relationship was observed, and when oxytocin was administered first (then placebo), a significant quadratic relationship was observed (Supplementary Table 4). Compared with baseline, analyses

regarding the size of these differences yielded small to large effects when administered oxytocin first or second, but only small to medium effects when administered placebo first or second (Table 2).

For collapsed data, significant mean improvements were found for pre- vs post-test for both oxytocin and placebo on the RBS-R-P (oxytocin: 23.5 vs 17.7, $t=2.59$, $d.f.=30$, $P<0.05$; placebo: 23.5 vs 17.6, $t=2.45$, $d.f.=30$, $P<0.05$) and DBC-P (oxytocin: 59.3 vs 43.1, $t=5.05$, $d.f.=30$, $P<0.001$; placebo: 59.3 vs 47.5, $t=3.07$, $d.f.=30$, $P<0.01$). Importantly, oxytocin also resulted in a significant mean improvement on the SRS-P (109.1 vs 98.5, $t=4.37$, $d.f.=30$, $P<0.001$). A difference-in-difference approach found that participants achieved a significantly greater mean improvement on the SRS-P at post-test (as compared with pretest) when administered oxytocin vs placebo (Table 3). For the RBS and secondary outcome measures, no significant effects were found.

Clinical data

Figure 3 shows individual SRS-P data for participants after oxytocin (red triangles) and placebo (black triangles). Here, the solid diagonal line represents the ‘line of no change’ between pre- and post-test results, whereas the diagonal dotted lines represent the upper and lower RCI confidence limits. Inspection of the figure shows the majority of participants fall at or on the improvement side of the ‘line of no change’, including 90% (28/31) after oxytocin and 77% (24/31) after placebo. Importantly, no participant ‘deteriorated’ after oxytocin but 10% (3/31) did after placebo. RCI findings for secondary measures did not show any significant differences between treatments (Supplementary Table 5).

Clinical global improvement, caregiver burden and adverse events
Experimenter-rated impressions of clinical global improvement were significantly greater for oxytocin (72%, 21/29) compared with

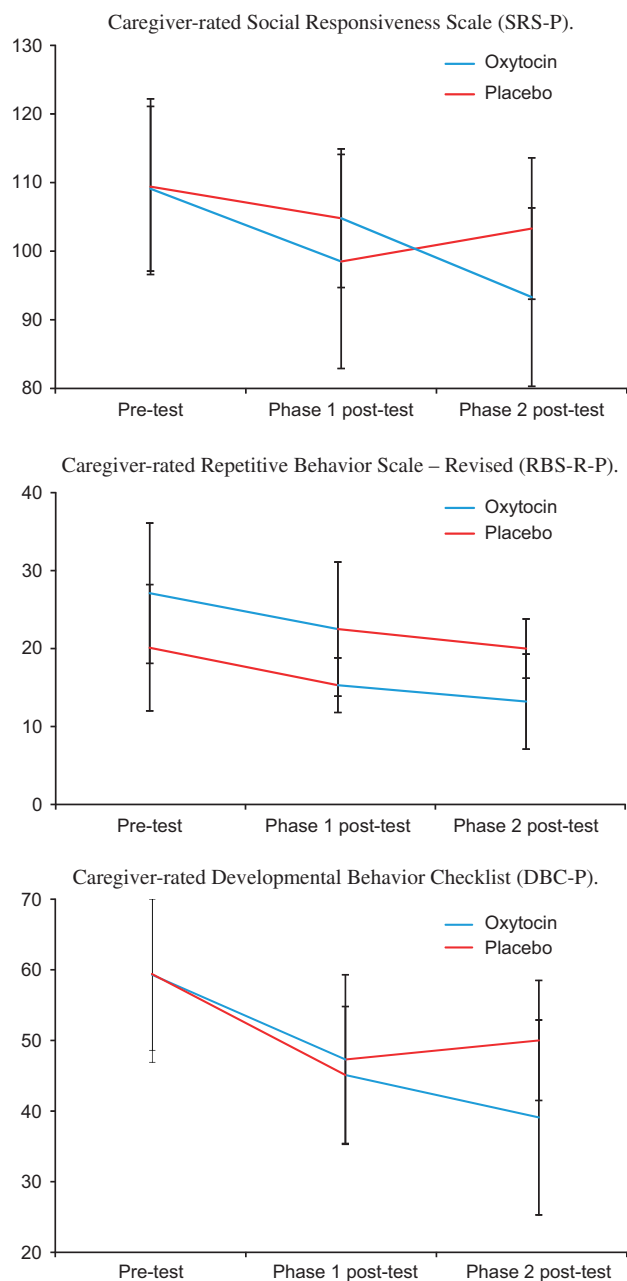


Figure 2. Plotting mean scores on the primary (caregiver-rated Social Responsiveness Scale (SRS-P), caregiver-rated Repetitive Behavior Scale-Revised (RBS-R-P)) and secondary (caregiver-rated Developmental Behavior Checklist (DBC-P)) outcome measures by time and condition, $N=31$.

placebo (41%, 12/29, $\chi^2 = 5.15$, $P < 0.05$). Analyses of the strain (or burden) experienced by caregivers found no significant differences, and caregiver impressions of whether (or not) their child received oxytocin did not differ at post-test 1 (16/31 vs 15/31, $\chi^2 = 0.82$, $P = 0.37$), but did differ significantly at post-test 2 (18/29 vs 11/29, $\chi^2 = 6.45$, $P < 0.05$). In terms of correct beliefs overall, however, only 25% (15/60) of caregivers rightly believed their child received oxytocin, whereas 32% (19/60) believed they did but instead received placebo.

Finally, caregiver reports indicated that participants may have been observed to experience slightly more adverse events when administered oxytocin as opposed to placebo (13/31 vs 7/31) but

Table 2. Effect sizes (Cohen's d) for social interaction and behavioral measures, $N=31$

Social interaction and behavioral measures	Administration of oxytocin		Administration of placebo	
	First	Second	First	Second
SRS-P	0.42	1.17	0.21	0.02
RBS-R-P	0.20	0.28	0.53	0.23
ADOS	0.09	0.21	0.11	0.01
DBC-P	0.58	0.79	0.68	0.11
RBS-R-P	0.29	0.67	0.41	0.03
CSQ	0.20	0.28	0.53	0.23

Abbreviations: ADOS, Autism Diagnostic Observation Schedule; CSQ, Caregiver Strain Questionnaire; DBC-P, caregiver-rated Developmental Behavior Checklist; RBS-R-P, caregiver-rated Repetitive Behavior Scale-Revised; SRS-P, caregiver-rated Social Responsiveness Scale.

this difference was not significant ($\chi^2 = 2.66$, $P = 0.10$). The most frequently reported adverse events were increased thirst as well as increased day/nighttime urination and constipation, and these adverse events were reported by twice as many participants during oxytocin (10 reports) than placebo (five reports). Two serious adverse reactions were reported for three participants, namely hyperactivity and aggression. These participants were immediately removed from the study and all adverse reactions ceased once the condition was discontinued. Two participants experienced these adverse reactions in week 1 of phase 1, whereas the third occurred during week 1 of phase 2. Two of the three participants were receiving oxytocin at the time, with one of these participants previously completing phase 1 under the placebo regimen.

DISCUSSION

To our knowledge, this preliminary study is the first double-blind, randomized, placebo-controlled, crossover, clinical trial investigating the efficacy, tolerability and safety of intranasal-administered oxytocin in young children with autism. A difference-in-difference approach found that a 5-week course of oxytocin resulted in statistically significant improvement on the primary outcome measure (SRS-P). These findings were further supported by improved experimenter-rated impressions of clinical global improvement when administered oxytocin as compared with placebo. There was, however, no influence of oxytocin on our second primary outcome, caregiver reports of the severity of repetitive behavior or our other secondary outcome measures. In general, oxytocin was found to be well tolerated and there were no significant differences in the report of adverse events between conditions.

Results additionally showed important moderators of 'treatment efficacy'. First, exploratory analysis indicated that order of condition likely impacted the degree of separation between oxytocin and placebo. When oxytocin was administered first and placebo second, quadratic patterns in outcome measures were noted. However, when placebo was administered first and oxytocin second, negative linear patterns were observed. One explanation for the latter is the presence of a placebo response—which occurs when a beneficial effect is produced simply because a person has an expectation that a treatment/condition will be helpful. Placebo effects are commonly observed in child treatment studies,³⁵ including autism,^{36,37} and may be compromising clinical outcome conclusions made by such trials as its observed effect on response can be similar in size to efficacious

Table 3. Difference-in-difference analyses of social interaction and behavioral measures, N = 31

Social interaction and behavioral measures	Mean (s.d., range, 95% CI) improved difference (pre- vs post-test)		Paired t-test (P-value)
	Oxytocin	Placebo	
SRS-P	13.5 (17.2, -13 to 59, 7.2 to 19.7)	5.2 (17.3, 28 to 43, -1.2 to 11.5)	2.32 (< 0.05)
ADOS	1.0 (4.8, -11 to 8, -0.8 to 2.7)	0.9 (4.9, -10 to 17, -0.9 to 2.7)	0.09 (0.93)
DBC-P	16.3 (17.9, -24 to 55, 9.7 to 22.8)	11.8 (21.5, -18 to 90, 4.0 to 19.7)	1.34 (0.19)
RBS-R-P	5.7 (12.3, -14 to 45, 1.2 to 10.3)	5.9 (13.3, -18 to 39, 1.0 to 10.8)	0.07 (0.95)
CSQ	-0.6 (2.4, -5.5 to 5.0, -1.5 to 0.3)	-0.9 (2.1, -6.5 to 2.2, -1.7 to -0.1)	0.75 (0.46)

Abbreviations: 95% CI, 95% confidence interval; ADOS, Autism Diagnostic Observation Schedule; CSQ, Caregiver Strain Questionnaire; DBC-P, caregiver-rated Developmental Behavior Checklist; d.f., degree of freedom 30; RBS-R-P, caregiver-rated Repetitive Behavior Scale-Revised; s.d., standard deviation; SRS-P, caregiver-rated Social Responsiveness Scale.

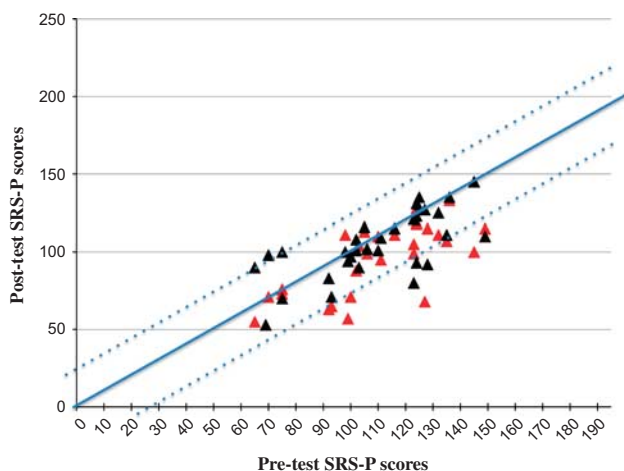


Figure 3. Caregiver-rated Social Responsiveness Scale (SRS-P) pre- and post-test scores after oxytocin (red triangles) and placebo (black triangles), N = 31. The solid diagonal line represents 'line of no change' and the dotted lines upper and lower Reliable Change Index (RCI) confidence limits. Test-retest reliability = 0.84; s.d. for the pretest = 22.6.

treatments. Our recent meta-analysis³⁷ showed a moderate placebo response effect size across autism pediatric pharmacological and dietary placebo-controlled trials, suggesting that the placebo response may account for 47% of observed improvement across trials. This interpretation may explain why earlier studies that used a simple between-subject design have shown strong effects of time but failed to show benefit of oxytocin over placebo.^{19,38,39} Consistent with this interpretation, our own previous study of intranasal-administered oxytocin to adolescents with autism³⁹ used a between-subjects design and found strong effects across both oxytocin and placebo treatment, but no overall benefit of oxytocin. In that study, we showed moderation of caregiver belief as to whether (or not) the adolescent received oxytocin. Future studies need to consider methods to control for placebo effects to improve detection of therapeutic responses, such as a phase of blinded placebo intervention before randomization to drug.

Recently, the safety of oxytocin treatment for children has been debated.^{14,40,41} This study showed no evidence of deterioration after oxytocin administration on any of the social interaction or behavioral measures. Minimal adverse events were reported for both oxytocin and placebo, although twice the number of these symptoms (namely increased urination, thirst and constipation) were recorded during oxytocin. In this sample size, the reports were not significant but they could reflect

oxytocin's influence on hypothalamic response.⁴² Three out of 39 participants also showed evidence of hyperactivity or aggression during dose escalation, with two of the three cases assigned to oxytocin. We cannot determine whether these effects were specifically associated with oxytocin or any other nasal spray ingredient and this finding should be the basis of at least a future population study. We also note that two participants who were allocated to oxytocin did not complete the study due to intolerance of the drug delivery procedure. Both of these children were non-verbal or showed minimal receptive language ability, which in turn posed a challenge for nasal spray delivery.

We note limitations of the current study, including a small sample size, the inclusions of participants on other psychotropic medications that were stabilized before drug assignment, and our reliance on caregiver reports as the main outcome measures. The development of sensitive observational and other markers of change for use in autism clinical trials remains an ongoing priority.¹⁴ Future studies will need to employ larger samples that include a broad representation of autism patients in the community.

In conclusion, among children with autism aged between 3 and 8 years, a 5-week course of oxytocin nasal spray improved caregiver-rated social responsiveness compared with placebo. Oxytocin treatment was found to be well tolerated and there were no significant differences in the report of adverse events between conditions. These findings require confirmation in larger studies with potential for development of a first medical treatment for social impairments in child autism.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGMENTS

We acknowledge an NHMRC Australian Fellowship (APP 511921) to IBH, an NHMRC Career Development Fellowship (APP 1061922) to AJG and a generous donation by Mr Geoff Stein toward the completion of this trial. These funders had no role in the design and conduct of the study; collection, management, analysis and interpretation of the data; preparation, review or approval of the manuscript; and decision to submit the manuscript for publication. We also thank ASPECT, Giant Steps and The Lizard Center in Sydney (Australia) for assistance with recruitment. This research was partially supported by NHMRC Grants (APP 623625; APP 1043664) and a BUPA Health Foundation Grant (APP 2012-00004) to AJG.

AUTHOR CONTRIBUTIONS

IBH is a Senior Principal Research Fellow of the Australian National Health and Medical Research Council (APP 1046899). He is a co-Director of the Brain and Mind Centre at The University of Sydney, which operates two early-intervention youth services under contract to headspace (Australia's National Youth Mental Health Foundation). He is a Commissioner of the Australian National Mental

Health Commission and was previously the Chief Executive Officer of beyondblue and a Director of headspace until January 2012. Previously, IBH has led a range of community-based and pharmaceutical industry-supported depression awareness and education and training programs. He has also led depression and other mental health research service evaluation or investigator-initiated research projects that have been supported by a variety of pharmaceutical partners. Current investigator-initiated studies are supported by Servier Australia (manufacturers of agomelatine) and Pfizer. He has received honoraria for his contributions to professional educational seminars related to depression, youth mental health and circadian-rhythms research and has recently received travel support (from Servier Australia) to attend scientific meetings related specifically to circadian rhythm disorders. AJG had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. CJY, SLE, TAD and AJG have no conflict of interest to declare.

REFERENCES

- 1 Association AP. *Diagnostic and Statistical Manual of Mental Disorders*, 4th edn. Text Revision. American Psychiatric Association: Washington DC, USA, 2000.
- 2 Autism Spectrum Disorders. Available at: <http://www.cdc.gov/autism/> (last accessed date 20th November 2014).
- 3 Sharma A, Shaw SR. Efficacy of risperidone in managing maladaptive behaviors for children with autistic spectrum disorder: a meta-analysis. *J Pediatr Health Care* 2012; **26**: 291–299.
- 4 Gibbs TT. Pharmacological treatment of autism. In: GJ Blatt (ed). *The Neurochemical Basis of Autism*. Springer: Boston, MA, USA, 2010, pp 245–267.
- 5 Reichow B, Barton EE, Boyd BA, Hume K. Early intensive behavioral intervention (EIBI) for young children with autism spectrum disorders (ASD). *Cochrane Database Syst Rev* 2012; **10**: CD009260.
- 6 Howlin P, Baron-Cohen S, Hadwin J. *Teaching Children with Autism to Mind-read: A Practical Guide*. Wiley: New York, NY, USA, 1999.
- 7 Howlin P, Udwin O. *Outcomes in Neurodevelopmental and Genetic Disorders*. Cambridge University Press: Cambridge, MA, USA, 2002.
- 8 Guastella AJ, MacLeod C. A critical review of the influence of oxytocin nasal spray on social cognition in humans: Evidence and future directions. *Horm Behav* 2012; **61**: 410–418.
- 9 Donaldson ZR, Young LJ. Oxytocin, vasopressin, and the neurogenetics of sociality. *Science* 2008; **322**: 900–904.
- 10 Guastella AJ, Mitchell PB, Dadds MR. Oxytocin increases gaze to the eye-region of human faces. *Biol Psychiatry* 2008; **63**: 3–5.
- 11 Schulze L, Lischke A, Greif J, Herpertz SC, Heinrichs M, Domes G. Oxytocin increases recognition of masked emotional faces. *Psychoneuroendocrinology* 2011; **36**: 1378–1382.
- 12 Lischke A, Berger C, Prehn K, Heinrichs M, Herpertz SC, Domes G. Intranasal oxytocin enhances emotion recognition from dynamic facial expressions and leaves eye-gaze unaffected. *Psychoneuroendocrinology* 2012; **37**: 475–481.
- 13 Kosfeld M, Heinrichs M, Zak PJ, Fischbacher U, Fehr E. Oxytocin increases trust in humans. *Nature* 2005; **435**: 673–676.
- 14 Guastella AJ, Hickie IB. Oxytocin treatment, circuitry and autism: a critical review of the literature placing oxytocin into the autism context. *Biol Psychiatry* 2015; pii: S0006-3223 (15) 00543-0.
- 15 Hollander E, Novotny S, Hanratty M, Yaffe R, Decaria CM. Oxytocin infusion reduces repetitive behaviours in adults with Autistic and Asperger's disorders. *Neuropsychopharmacology* 2003; **28**: 193–198.
- 16 Hollander E, Bartz J, Chaplin W, Phillips A, Sumner J, Soorya L *et al*. Oxytocin increases retention of social cognition in autism. *Biol Psychiatry* 2007; **61**: 498–503.
- 17 Guastella AJ, Hickie IB, McGuinness MM, Otis M, Woods EA, Disinger HM *et al*. Recommendations for the standardisation of oxytocin nasal administration and guidelines for its reporting in human research. *Psychoneuroendocrinology* 2013; **38**: 612–625.
- 18 Andari E, Duhamel J-R, Zalla T, Herbrecht E, Leboyer M, Sirigu A. Promoting social behavior with oxytocin in high-functioning autism spectrum disorders. *Horm Behav* 2010; **107**: 4389–4394.
- 19 Anagnostou E, Soorya L, Chaplin W, Bartz JA, Halpern D, Wasserman S *et al*. Intranasal oxytocin versus placebo in the treatment of adults with autism spectrum disorders: a randomized controlled trial. *Mol Autism* 2012; **3**: 16.
- 20 Guastella AJ, Einfeld SL, Gray KM, Rinehart NJ, Tonge BJ, Lambert TJ *et al*. Intranasal oxytocin improves emotion recognition for youth with autism spectrum disorders. *Biol Psychiatry* 2010; **67**: 692–694.
- 21 Rogers SJ. Brief Report: early intervention in autism. *J Autism Dev Disord* 1996; **26**: 243–246.
- 22 Harris SL, Handleman JS. Age and IQ at intake as predictors of placement for young children with autism: a four- to six-year follow-up. *J Autism Dev Disord* 2000; **30**: 137–142.
- 23 Lord C, Rutter M, Goode S, Heemsbergen J, Jordan H, Mawhood L *et al*. Autism diagnostic observation schedule: a standardized observation of communicative and social behavior. *J Autism Dev Disord* 1989; **19**: 185–212.
- 24 Constantino JN. *The Social Responsiveness Scale*. Western Psychological Services: Los Angeles, CA, USA, 2005.
- 25 Pine E, Luby J, Abbacchi A, Constantino JN. Quantitative assessment of autistic symptomatology in preschoolers. *Autism* 2006; **10**: 344–352.
- 26 Einfeld S, Tonge B. The Developmental Behavior Checklist: the development and validation of an instrument to assess behavioral and emotional disturbance in children and adolescents with mental retardation. *J Autism Dev Disord* 1995; **25**: 81–104.
- 27 Einfeld SL, Tonge BT. *Manual for the Developmental Behaviour Checklist (Second Edition)-Primary Carer Version (DBC-P-P) and Teacher Version (DBC-P-T)*. Monash University Centre for Developmental Psychiatry and Psychology and School of Psychiatry, University of New South Wales: Melbourne and Sydney, Australia, 2002.
- 28 Roid GH, Miller LJ. *Leiter International Performance Scale-Revised*. Stoelting: Wooddale, IL, USA, 1997.
- 29 Bodfish JW, Symons FJ, Parker DE, Lewis MH. Varieties of repetitive behavior in autism: comparisons to mental retardation. *J Autism Dev Disord* 2000; **30**: 237–243.
- 30 Brereton AV, Tonge BJ, Einfeld SL. Psychopathology in children and adolescents with autism compared to young people with intellectual disability. *J Autism Dev Disord* 2006; **36**: 863–870.
- 31 Guy W. *ECDEU Assessment Manual for Psychopharmacology*. US Department of Health, Education, and Welfare, Public Health Service, Alcohol, Drug Abuse, and Mental Health Administration: Rockville, MD, USA, 1976.
- 32 Brannan AM, Heflinger CA. The Caregiver Strain Questionnaire: measuring the impact on the family of living with a child with serious emotional disturbance. *J Emot Behav Disord* 1997; **5**: 212–223.
- 33 Cohen J. A power primer. *Psychol Bull* 1992; **112**: 155–159.
- 34 Jacobson NS, Truax P. Clinical significance: a statistical approach to defining meaningful change in psychotherapy research. *J Consult Clin Psychol* 1991; **59**: 12–19.
- 35 Birmaher B, Waterman GS, Ryan ND, Perel J, McNabb J, Balach L *et al*. Randomized, controlled trial of amitriptyline versus placebo for adolescents with 'treatment-resistant' major depression. *J Am Acad Child Adolesc Psychiatry* 1998; **37**: 527–535.
- 36 King BH, Dukes K, Donnelly CL, Sikich L, McCracken JT, Scahill L *et al*. Baseline factors predicting placebo response to treatment in children and adolescents with autism spectrum disorders: a multisite randomized clinical trial. *JAMA Pediatr* 2013; **167**: 1045–1052.
- 37 Masi A, Amit L, Glozier N, Hickie IB, Guastella AJ. Predictors of placebo response in pharmacological and dietary supplement treatment trials in pediatric autism spectrum disorder: a meta-analysis. *Transl Psychiatry* 2015; **5**: e640.
- 38 Dadds MR, MacDonald E, Cauchi A, Williams K, Levy F, Brennan J. Nasal oxytocin for social deficits in childhood autism: a randomized controlled trial. *J Autism Dev Disord* 2013; **44**: 521–531.
- 39 Guastella A, Gray K, Rinehart N, Alvares G, Tonge B, Hickie I *et al*. A randomized controlled trial of a course of intranasal oxytocin to treat youth diagnosed with autism. *J Child Psychol Psychiatry* 2015; **56**: 444–452.
- 40 Miller G. The promise and perils of oxytocin. *Science* 2013; **339**: 267–269.
- 41 Young LJ. When too much of a good thing is bad: chronic oxytocin, development, and social impairments. *Biol Psychiatry* 2013; **74**: 160–161.
- 42 Chou C, DiGiovanni SR, Mejia R, Nielsen S, Knepper M. Oxytocin as an antidiuretic hormone. I. Concentration dependence of action. *Am J Physiol* 1995; **269**: F70–F77.



This work is licensed under a Creative Commons Attribution-NonCommercial-NoDerivs 4.0 International License. The images or other third party material in this article are included in the article's Creative Commons license, unless indicated otherwise in the credit line; if the material is not included under the Creative Commons license, users will need to obtain permission from the license holder to reproduce the material. To view a copy of this license, visit <http://creativecommons.org/licenses/by-nc-nd/4.0/>

© The Author(s) 2016

Supplementary Information accompanies the paper on the Molecular Psychiatry website (<http://www.nature.com/mp>)