**Beyond the hype and hope: a critical review of intranasal oxytocin research in
autism spectrum disorder**

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Abstract

Extensive research efforts in the last decade have been expended into understanding whether intranasal oxytocin may be an effective therapeutic in treating social communication impairments in individuals with autism spectrum disorder (ASD). After hyped early findings, subsequent clinical trials of longer-term administration have yielded more conservative and mixed evidence. In this review, we evaluate the evidence from randomised controlled trials, case reports, and open-label studies of oxytocin administration in individuals with ASD to highlight a number of critical considerations for future research in this area, including choice of outcome measures, dosing and device issues, and participant selection. Despite these, there remains significant potential for oxytocin to ameliorate aspects of the persistent and debilitating social impairments in individuals with ASD. Given the considerable media hype around new treatments for ASD, as well as the needs of eager families, there is an urgent need for researchers to prioritise considering such factors when conducting well-designed and controlled studies to further advance this field.

1 Autism spectrum disorder (ASD) is a group of complex and heterogeneous
2 neurodevelopmental conditions characterised by qualitative differences in social
3 interaction and communication, as well as restricted range of interests and/or
4 stereotypic behaviours. Aetiological factors in ASD are multifactorial, likely including
5 genetic and environmental contributions. Despite conservative population prevalence
6 estimates exceeding 1% in almost all surveyed countries,¹ there remains a
7 significant lack of progress in identifying and implementing effective treatments that
8 target core social impairments across the broad spectrum of individuals.

9 A potential novel pharmaceutical target that has emerged in the last decade is
10 the neuropeptide and hormone oxytocin. Converging basic and clinical research
11 implicates intranasally-administered oxytocin as a potential treatment to modulate
12 social communication behaviours via the neural systems supporting these complex
13 interactions. The initial hype and promise generated from preclinical models ² and a
14 prominent paper in *Nature* ³ spurred a flurry of clinical studies, all focused on the
15 treatment implications for social impairments across a variety of disorders,
16 particularly ASD and schizophrenia. However, variable efficacy at the clinical trial
17 end, combined with modest effect sizes in healthy populations, and failed
18 replications, has tempered enthusiasm about the potential of this hormone for
19 reducing ASD symptomatology.⁴

20 This pattern of enthusiasm towards oxytocin, and subsequent drop in
21 expectations, parallels the so-called 'Hype Cycle' of innovation, a framework
22 developed to understand the progression of new technologies for optimal periods of
23 investment.⁵ Applied to the early interest in oxytocin as a therapeutic (see Figure 1),
24 many early adopters accepted initial claims of efficacy and proceeded to design
25 clinical trials. The ensuing 'trough' of negative studies and contrary effects have

appeared to result in a period of disillusionment surrounding the oxytocin's therapeutic potential (for example, see ⁴). In the current review, we examine the theoretical rationale for oxytocin as a novel treatment for social-communication difficulties, provide a comprehensive examination of published studies of oxytocin in individuals with ASD, and suggest a number of critical factors for consideration in the design of future research. We suggest that in this current period of 'enlightenment', researchers should accelerate systematic investigations of potential therapeutic mechanisms of action and strive to address the critical factors outlined in this review that are urgently needed to move this field forward.

<Insert Figure 1 around here>

From obstetric applications to off-label use

Synthesis, storage, and sites of action

Oxytocin, and the closely related peptide vasopressin, are neurohypophyseal hormones with well-characterised physiological functions. Oxytocin promotes uterine contractions and lactation during childbirth, while vasopressin plays a key role in the regulation of water retention by the kidneys.⁶ Synthesis of oxytocin and vasopressin occurs mainly via the supraoptic and paraventricular accessory magnocellular nuclei of the hypothalamus. These nuclei project to the posterior pituitary, or neurohypophysis, which stores and releases oxytocin peripherally into the bloodstream.⁷ Parvocellular, and some magnocellular, neurons in the paraventricular nuclei synthesize oxytocin and vasopressin and project to various regions within the central nervous system. A single oxytocin receptor and vasopressin receptor subtypes (V1a and V1b) are centrally expressed and distributed widely throughout

the brain (two other vasopressin receptors are found peripherally). Centrally, particularly high expressions of these receptors are found in regions underlying the control of many social behaviours, such as the nucleus accumbens, ventral tegmental area, amygdala, and hippocampus.⁸ Although oxytocin and vasopressin exert a range of neuropeptide specific physiological functions, there may exist a potential for cross-reactivity at either receptor site given similarities in structure, suggesting that increased activity of one peptide may also exert some influence over expressivity of the other.⁹

Endogenous oxytocin

Animal models, in particular those in rodents, have been critical in characterising the complex roles of endogenous oxytocin and vasopressin in pair bonding, parental care, and formation of social memories.¹⁰ In addition to these well described roles, peripheral and central levels of oxytocin are involved in reducing responses to physical and social stressors.¹¹ Endogenous oxytocin functions as an anxiolytic, increasing release of the inhibitory neurotransmitter γ -Aminobutyric acid (GABA) in the central amygdala¹² and attenuating hypothalamic-pituitary-adrenal axis activity¹¹ in response to fearful stimuli.

Variations in endogenous oxytocin levels in human plasma are associated with positive social behaviour and stress responses. For example, elevations in peripheral oxytocin are associated with increased positive social interaction with a partner.¹³ Lactating women, in whom endogenous oxytocin is increased, show attenuated cortisol responses to psychosocial stressors.^{14,15} Reduced plasma oxytocin levels have also been linked to a number of neuropsychiatric disorders, including ASD, depression, and schizophrenia (see review in ¹⁶).

1 Together, these findings suggest an ‘oxytocin deficit’ hypothesis, which
2 proposes that lower concentrations of peripherally circulating oxytocin may be
3 causally related to social impairments, particularly those characteristic of individuals
4 with ASD. Evidence in favour of this hypothesis has often been used to support trials
5 of oxytocin administration to increase such levels in individuals with a supposed
6 deficit. However, inconsistent evidence in individuals with ASD does not appear to
7 support this hypothesis. Several studies have reported lower oxytocin levels in
8 ASD,¹⁷⁻¹⁹ while others have not exhibited any differences,^{20,21} and one report of
9 increased oxytocin levels in ASD.²²

10 These conflicting findings may relate to methodological debates surrounding
11 the relationship between central and peripheral measurements of oxytocin, and the
12 most reliable assay(s) for measuring oxytocin levels.²³ For example, some studies
13 employ a dilution method²⁴ or do not follow manufacturer-recommended extraction of
14 plasma samples²⁵ before oxytocin assay. This may result in physiologically
15 implausible levels of oxytocin, rendering conclusions about differences between
16 groups, or associations with clinical measures, unverifiable. The comparative control
17 group in these studies also differs, with some studies comparing matched blood
18 samples from typically developing samples^{17,19-22} and others using existing
19 normative data.¹⁸ Lastly, there exists considerable debate regarding whether
20 peripheral levels of oxytocin reflect central expression in humans, with mixed
21 evidence reported to date.^{26,27} Thus, differences in plasma levels of oxytocin in ASD,
22 and the extent to which this relates to central levels of symptomatology, remains a
23 significant question of interest.

Exogenous manipulation via intranasal administration

The Greek origin of the word oxytocin is 'quick birth', given because of the well-known physiological effects in stimulating or facilitating uterine contractions during birth. Since the identification of the nine amino acid sequence and subsequent synthesis of the hormone over five decades ago,²⁸ intravenous oxytocin (*Pitocin*, *Syntocinon*) has been widely adopted in clinical obstetrics for the induction of labour,²⁹ management of postpartum haemorrhage,³⁰ and to facilitate lactation via nasal spray administration,³¹ although conflicting data exists for this latter indication.³²

Easy administration, combined with a minimal side effect profile, made investigations of intranasal oxytocin (most commonly, *Syntocinon*) for the study of psychological and behavioural phenomena an attractive area of research. Indeed, subsequent to Kosfeld, Heinrichs and colleagues³ first influential paper reporting that acute intranasal oxytocin increased trusting behaviour in a neuroeconomic paradigm, a wealth of single-dose administration studies emerged arguing that oxytocin modulates aspects of social cognition, including emotion recognition, stress responsivity, and approach-related social behaviours (reviewed in ¹⁶). This led to our proposal that oxytocin influences detection and appraisal of social information at both automatic and strategic levels of processing mediated by different nose-to-brain delivery pathways.³³ Converging functional imaging evidence also suggests oxytocin increases activity in brain regions associated with social cognition and modulates function connectivity between these regions (reviewed in ^{34,35}), indicating a role in modulating not just social behaviours but the neural and biological responses underlying social interaction.

Clinical trials of oxytocin nasal spray in neurodevelopmental disorders

Subsequent investigations into the effects of oxytocin in individuals with ASD have progressed rapidly, due in part to the surge of oxytocin-related publications in the last decade, as well as the urgent need to identify effective therapeutics for ASD to mitigate core social communication deficits. To date, there have been 15 randomised placebo-controlled trials, one case report, and two open-label investigations reporting on a variety of different clinical, cognitive, behavioural and physiological outcomes in individuals with ASD ranging from single-dose challenge studies (see Table 1) to various reports of repeated daily administrations (Table 2).

Acute effects of oxytocin administration

The first study of oxytocin in ASD reported, across two publications, the effects of intravenous oxytocin (*Pitocin*) compared to placebo in a crossover design.^{36,37} The authors examined 15 adults with ASD over the course of a four-hour administration period, finding that oxytocin infusion compared to placebo decreased the number and severity of repetitive behaviour symptoms and increased social cognition.

Specific to the latter finding, an order effect was demonstrated, with individuals receiving placebo in the first session significantly improving comprehension of affective speech under oxytocin, whilst those who received oxytocin first retained better performance under placebo infusion. The authors argued that the group of individuals with ASD who received oxytocin first appeared to retain their knowledge from the first visit, implying a carryover effect after infusion had ceased. It was also noted that this carryover effect was not observed for repetitive behaviours, suggesting that this may be a specific effect to social cognition.

1 The first report of intranasal oxytocin use in ASD was a study of adolescent
2 males with ASD.³⁸ The primary outcome, the Reading the Mind in the Eyes Test
3 (RMET), is a widely used task in the study of social cognition in ASD.³⁹ Oxytocin
4 administration was found to improve accuracy of classifying emotions, particularly
5 with less challenging items, and was the first report to indicate feasibility and efficacy
6 for intranasal oxytocin for improving social cognition in younger individuals with ASD.

7 Subsequent studies demonstrated effects of intranasal oxytocin on
8 physiological and experimental outcomes. Andari and colleagues first demonstrated
9 oxytocin significantly increased eye gaze to social regions of faces in adults with
10 ASD compared to placebo and typically developing control groups,¹⁸ replicating
11 findings in typically developing adults.⁴⁰ Andari and colleagues also found that
12 oxytocin increased the willingness to engage in a virtual 'ball-tossing' social
13 interaction game, enhancing feelings of trust and preference in other players
14 perceived as more cooperative. Others have shown effects of oxytocin on
15 physiological responses, in particular, skin conductance, to auditory stimuli after
16 oxytocin administration in male adults with ASD compared to placebo and typically
17 developing controls.⁴¹ In healthy individuals, a well-replicated finding has been in the
18 modulation of eye gaze to static facial image after oxytocin administration.^{18,40,42,43} In
19 individuals with ASD, Auyeung and colleagues further showed that oxytocin
20 modulated changes in eye gaze during a real-time social interaction.⁴⁴ Compared to
21 a typically developing control group, adult males with ASD fixated significantly less
22 on eye regions under the placebo condition; after oxytocin administration, both
23 groups spent significantly more time fixating at eye regions. Within the ASD group,
24 this beneficial effect of oxytocin was particularly prominent for those individuals who
25 spent less time looking at the eye region under placebo. These cumulative

experimental findings from single-dose studies suggest that the acute effects of oxytocin in individuals with ASD, mostly male adults with average-to-high cognitive functioning, extend to a number of physiological measures of social cognition, particularly in the modulation of eye gaze.

Neurophysiological effects have been investigated in four studies of adults with ASD,⁴⁵⁻⁴⁸ and one in children.⁴⁹ Significant changes in activation after oxytocin administration have been reported in areas involved in social information processing, including the amygdala, medial prefrontal cortex, and anterior insula, as well as in broader areas involved in reward processing in the child study.⁴⁹ Across all studies, participant samples were relatively homogenous, restricted to higher-functioning males (that is, a lack of comorbid intellectual disability) and consistent sample sizes (ranging between 14-33 participants). It is clear that the generalisability of neural effects of oxytocin is yet to be determined, particularly in females with ASD and across a wider range of symptom severity and cognitive functioning levels.

Effects of repeated administration

The first investigation of repeated administration of oxytocin employed a randomised between-subjects design over a six week administration period.⁵⁰ Compared to a placebo group (n=9), individuals in the oxytocin group (n=10) did not significantly improve on measures of social cognition, caregiver ratings of repetitive behaviours, and global clinical ratings of improvement. However, secondary outcome analyses suggested the oxytocin group showed improvements on the RMET, caregiver reported quality of life, and lower-order repetitive behaviours (stereotypy and self-injury). Whilst not adequately powered to demonstrate statistically significant

1 clinical improvements, this study provided the first evidence for the safety and
2 tolerability of oxytocin in adults with ASD after extended administration.

3 Following this, Dadds and colleagues reported on the effects of four
4 consecutive daily doses of oxytocin, compared to placebo, in male children with
5 ASD.⁵¹ Doses were administered during either parent-child interaction training or a
6 family interaction task. Oxytocin was administered in the morning at different times
7 on alternating days, with two doses stratified based on weight. No significant
8 differential improvements were observed on changes in parent or experimenter
9 reports of symptoms, or on observed behaviours during interactions. However, it
10 remains unclear whether a lack of effect in this study may be due to the complicated
11 study design. Of note, analyses had to account for randomisation of drug, two
12 dosages, randomisation of parent-child interaction treatment, and time of
13 administration (early or mid-morning). Additionally, the parent-child interaction
14 training described in this study had not been shown to be effective independent of
15 drug administration, although many of the intervention components have empirical
16 support.⁵² When combining an experimental intervention within a therapeutic
17 context, it is imperative to test that the therapy is independently validated for efficacy
18 (for example, ⁵³). Given the considerable number of factors involved, the study may
19 not have been adequately designed to detect any subtle changes due to oxytocin
20 manipulation.

21 Another study of adolescent males with ASD observed no significant benefits
22 of oxytocin after an eight week administration period, compared to placebo.⁵⁴ No
23 changes were observed on primary outcomes of social behaviours and ratings of
24 clinical improvement, or secondary outcomes examining social cognition, repetitive
25 and other ASD-related behaviours. However, exploratory analyses revealed that

1 parents who believed their child had received oxytocin reported significant
2 improvements in their child's behaviour over time irrespective of actual treatment
3 assignment. This unexpected finding highlighted the significant potential for
4 expectancy biases to mask treatment efficacy within clinical trials of children with
5 ASD. While this study was the first in this area to recruit a wider range of
6 participants, including individuals with ASD and intellectual disability, the potential
7 efficacy of oxytocin may have been masked by the greater amount of diagnostic
8 heterogeneity within this sample.

9 Watanabe and colleagues reported the first evidence of significant
10 improvements in diagnostic symptoms after a six-week administration period in high-
11 functioning adults with ASD.⁵⁵ In a crossover design, 20 adults with ASD (18
12 completed) with average-to-high intelligence were assessed on clinical outcomes of
13 ASD severity, as well as experimental and neuroimaging measures previously
14 shown to be modulated by oxytocin administration.^{41,46,55} Significant improvements at
15 the end of oxytocin treatment were specifically noted on the reciprocity subscale of
16 the Autism Diagnostic Observation Schedule (ADOS), as well as greater resting
17 state functional connectivity between the anterior cingulate cortex and the
18 dorsomedial prefrontal cortex, and increased fixations to eye regions of faces.
19 Although the primary outcome in this trial, the ADOS, has been utilised in some
20 clinical trials to measure change in clinical symptoms (for example, ⁵⁶), this measure
21 was not originally designed for use in clinical trials as a sensitive or reliable measure
22 of change in individuals with ASD. In particular, increased social reciprocity observed
23 in this trial could also be confounded by increased familiarity with the task demands
24 or administrator of the ADOS.

Very recently, Yatawara and colleagues reported positive effects of oxytocin administration on social communication in the first study of very young children with ASD, aged between 3 to 8 years.⁵⁷ Similar to Watanabe et al., these authors employed a crossover trial, with a five week administration period of either oxytocin or placebo, switched to the alternate drug after a one month washout period. The authors observed significant improvements in positive social behaviours, as measured by caregiver ratings on the Social Responsiveness Scale, with minimal to moderate side effects and good tolerability. In particular, Yatawara et al. observed significant order effects, implying that families whose child had been randomised to receiving placebo first exhibited greater improvements compared to baseline than children in the alternate condition. Although both of these recent studies recruited modest sample sizes only, the critical design difference of a crossover randomised trials appears to have ameliorated potential placebo effects and resulted in significant improvements in social communicative behaviours.

Oxytocin in other neurodevelopmental disorders

Three studies have reported on the effects of oxytocin administration in neurodevelopmental disorders that can co-occur with ASD or ASD-like symptoms. The first study was conducted in Fragile X syndrome (FXS), a rare genetic disorder caused by a disruption in expression of the FMR1 gene. Currently, this mutation is the most common known cause of inherited intellectual disability and can cause behaviours characteristic of ASD, including social impairments, sensory difficulties, and stereotypic behaviours.⁵⁸ Compared to placebo, oxytocin administered to eight adolescent and adult males with FXS significantly increased overall eye gaze during a brief social interaction.⁵⁹ Interestingly, lower doses of oxytocin in this sample

appeared to show a greater effect than the higher administered dose, consistent with a recent study in healthy adults.⁶⁰

Prader-Willi syndrome (PWS) is a rare neurodevelopmental disorder caused by a deletion or lack of expression of paternally inherited imprinted genes (known as maternal uniparental disomy; when both copies, or part thereof, the chromosome are maternally inherited) on chromosome 15q11-q13. Tauber and colleagues observed significant improvements in trusting behaviours, reductions in sad mood, and less disruptive behaviour in the two days following a single dose of oxytocin in 24 adults with PWS.⁶¹ A second study of PWS tested effects of twice-daily oxytocin administration over a two month period, in a crossover placebo-controlled design in 30 adolescents and adults with PWS, aged between 12 and 29 years.⁶² However, there were no significant changes in developmental or emotional behaviours, particularly on hyperphagia and pica, and obsessionality, but an increase in temper outbursts was also noted.

Registered studies

Despite the number of studies supporting use of intranasal oxytocin in modulating ASD-relevant social behaviours, social cognition, and neuroimaging markers, the translation of evidence to appropriately pre-registered randomised placebo-controlled trials has been much more limited. This appears to be particularly apparent in single dose crossover challenge studies where pre-registration is often not mandated by journals. Thus, it is difficult to determine the extent to which publication bias may be influencing interpretations of efficacy in this field, although several meta-analyses have not shown evidence of publication bias in studies of healthy individuals administered oxytocin.^{35,63} There is also a time lag between when

1 studies are registered, completed and then subsequently published. Given the early
2 stage of research in this area, we attempted to ascertain the current status of
3 research, both previously conducted, and those currently underway. We searched
4 the five most well used clinical trial registries worldwide (clinicaltrials.gov; EU Clinical
5 Trials Register; Australia and New Zealand Clinical Trials Registry; Iranian Registry
6 of Clinical Trials; Japan Primary Registries Network) in November 2015.

7 Searches for the words “oxytocin” AND “autism” revealed 57 registered trials
8 across registries, with no date restrictions. After excluding studies of neurotypical
9 individuals or individuals with high autistic traits, as well as studies measuring
10 endogenous levels of oxytocin, 46 studies remained. Of these 46 studies, 20 were
11 reported as ‘completed’ (either updated online as completed, n=7, and/or could be
12 attributed to a published study n=13). The remainder were described as either
13 actively recruiting or had been recently registered online prior to recruitment
14 commencing (n=22), or withdrawn/unknown (n=3); see Figure 2. Of the completed
15 trials, only 65% (n=13) could be linked to publications or could be retrospectively
16 associated with a clinical trial registration based on protocol records. Completed and
17 published trials were registered between 2006 and 2012, with an increased number
18 of new registered trials over the last 3 years. Registration numbers have been linked
19 to published studies, where known, in Tables 1 and 2. Based on this search, the
20 conversion from pre-registration to a completed published outcome exhibits a lag of
21 about three years.

22
23 <Insert Figure 2 around here>

Critical considerations in oxytocin research in ASD

Despite the initial promise from single-dose administration studies, subsequent clinical trials have overall failed to deliver upon these expectations. Many trials have found little evidence to support benefit on primary or secondary outcomes, and any benefits observed are associated with a small effect size or have limited clinical benefit. Although laudable attempts to determine the clinical efficacy of oxytocin as a therapeutic, there exists a critical need to address key methodological issues within this field to guide the interpretation and design of further trials.

Outcome measures to determine efficacy

A major consideration in neuropsychiatric clinical trials is the overreliance on reported symptom and clinical observational outcomes. Parental or caregiver reports of observed symptom improvement, or researcher observations of behaviour, are vulnerable to subjective biases and placebo effects, particularly given the extensive media hype generated in this field of research. Placebo effects are formed as a result of the expectations of trial outcomes and have been the subject of interest in not just oxytocin research,⁶⁴ but in ASD and related conditions.⁶⁵ Although placebo responses in ASD have been well described,⁶⁶ little research has been dedicated to identifying the factors that influence placebo responses that may then lead to better designed clinical trials. For example, expectation biases of randomization to oxytocin were found to significantly moderate parental reports of ASD symptom improvement.⁵⁴ Additionally, many of the outcome measures used by these studies were not designed to be sensitive to short-term and subtle changes in behavior. For example, it is debatable whether measurable change could be expected when administering the Social Responsiveness Scale before and after a five day

treatment⁵¹ nor the ADOS when administered three times over a three month period.⁵⁵

Given these issues, many have advocated for the discovery and use of more objective markers as primary outcomes to determine clinical change. For example, changes on measures of social cognition, such as performance on emotion recognition tasks, has already been used as a complement to more traditional measures of parental and clinician reports (for example, ⁵⁰) due to the hypothesis that changes in understanding of social information should be related to changes in actual social behaviours. Biological markers may also provide evidence for individually targeted approaches through the identification of treatment responders based on underlying changes in biological substrates related to the treatment. Some measures that have already been tested in oxytocin clinical research include (1) neural activity, using resting-state or task-dependent functional magnetic resonance imaging or other neuroimaging measures; (2) physiological changes in heart rate, respiration, skin conductance, or even eye fixations as proxy markers for neural changes; or (3) changes in endogenous levels of oxytocin, vasopressin or cortisol, as measured by plasma, salivary, or even cerebrospinal fluid levels. Whilst a promising approach, there is currently not enough summary evidence to indicate that these reliably index change due to oxytocin administration, nor whether they correlate with behavioural outcomes. Changes in amygdala activation, and other limbic areas, after oxytocin administration has been replicated in a number of studies,³⁵ but only limited studies have been reported in ASD and oxytocin to date. Furthermore, the logistical difficulties of magnetic resonance imaging in individuals with sensory difficulties bias recruitment towards relatively high functioning individuals with ASD. Clinically, however, the critical dependent measure for any

ASD intervention trial is change in a clinical outcome – most prominently, behaviour and cognition - and associations between these more objective measures to clinical change appear to be variable. Thus, the search for objective biomarkers to quantify changes in response after oxytocin administration need to be complemented by measures of clinical efficacy.

Dosage

A second issue to be addressed by future research is the most appropriate dosage of oxytocin. Most studies to date have employed a dose of 24 International Units (IU) in healthy adults, with variables doses employed for children and for repeated administration designs. Both published case studies⁶⁷ and anecdotal evidence⁶⁸ suggest off-label prescriptions of oxytocin are already a common practice by treating clinicians, despite concerns about pediatric use outside of a clinical trial setting.⁶⁹ To date, there has yet to be a dose-response study demonstrating that this current dosage is the most appropriate for children and/or adults with ASD to exhibit reliable changes in social, behavioural, or cognitive outcomes, whilst minimizing side-effects. Safety outcomes have been recently reported from a modified maximum-tolerated-dose study in ASD,⁷⁰ confirming the mild to moderate side effects reported in adult studies,⁷¹ however the open-label design limits interpretations about these reports.

The critical question for researchers is how much oxytocin is required to reach the central nervous system to induce behavioural and cognitive effects.⁷² Guided by other pharmaceutical trials, four randomized placebo-controlled trials of children and adolescents have employed dosages stratified by weight or age.^{38,49,51,54} However, these dosage regimes were based on best-guess estimates, rather than guided by dose-response evidence. Although oxytocin is stable in plasma,⁷³ the distribution

1 half-life of oxytocin is approximately 3 minutes, with an elimination half-life of
2 approximately 20 minutes.⁷⁴ This raises the question of how intranasal
3 administration may promote long-lasting behavioural effects. One potential
4 explanation may be that oxytocin, despite exhibiting very limited penetration across
5 the blood-brain barrier,⁷⁵ could enter the central nervous system through intracellular
6 junctions of the olfactory epithelia⁷⁶ to mimic endogenous oxytocin release⁷⁷ or could
7 indirectly influence behaviour via oxytocin receptors or cross-reactivity at
8 vasopressin receptors activated at high concentrations. Studies that have examined
9 CSF levels of oxytocin after intranasal administration have shown small CSF
10 increases in animal models at around 40 minutes to 1 hour post-administration,^{78,79}
11 although mixed evidence has been observed in humans.⁸⁰ Although current dosing
12 regimens (mostly 24 IU per dose) deliver a supraphysiologic concentrations of
13 oxytocin into peripheral circulation,⁷² recent investigations into dose-dependent
14 effects of oxytocin on social cognition have demonstrated superior effects at lower (8
15 IU) doses.⁶⁰ There is an urgent need for dose-response studies, with controls groups
16 employing receptor antagonists, to determine appropriate dosages for reliable
17 behavioural effects.

Route of administration

20 Surprisingly little methodological consideration has been devoted to intranasal
21 administration methods.⁸¹ Unlike oral and intravenous administered treatments,
22 intranasal administration requires careful consideration due to nasal cavity
23 physiology. There are at least three potential pathways by which intranasal
24 administration of oxytocin may enter central and peripheral circulation to exert
25 behavioural effects.³³ Intranasally administered oxytocin needs to bypass the narrow

nasal valve to be absorbed into the respiratory and olfactory epithelia, located in the upper posterior region of the nasal cavity, for transport to the central nervous system (CNS). Thus, oxytocin via a nasal spray device needs to prioritize deposition to the respiratory and olfactory epithelia for optimal transport to the CNS. Conventional pump actuated devices may deliver only a suboptimal amount of spray to this region,⁸² with newer evidence supportive of a novel “Breath Powered” nasal spray device.⁸³ Initial evidence suggests that lower dose (8IU) oxytocin delivered via this device is effective in modulating aspects of emotional behaviour.⁶⁰ However, research is yet to examine these devices in children or individuals with ASD. Observed variability in behavioural effects of oxytocin may therefore be influenced by variability in oxytocin deposition onto the olfactory epithelium for optimal drug absorption.³³ Standardized nasal spray protocols⁸⁴ and greater understanding of optimized delivery devices may provide more clarity around these issues.

Gender and individual differences

Amongst the publications presented in Tables 1 and 2, only 3-7% of reported individuals were female. Cited reasons for this exclusion were potential unknown effects of oxytocin in females or confounding effects due to variable hormonal cycles. Oxytocin is naturally female-specific and steroid-dependent when acting peripherally during childbirth and lactation. Centrally, the release of oxytocin and its expression are influenced by estrogen in animal models,⁸⁵ with neuroimaging studies confirming diverging sex effects after oxytocin administration in humans.³⁵ Across animal and human models, it is apparent that sex plays an important role in the interplay of oxytocin’s effects within the brain; the current low recruitment rate of females in ASD

clinical trials places significant limitations on the generalisability and interpretation of findings to date

An additional limiting factor is the exclusion of individuals with a range of behavioural symptoms. Complicated study designs and significant time commitments often preclude many families from participating in randomized controlled trials. For individuals with greater needs, such as a high-level of therapy commitments or additional physical or intellectual disabilities, clinical trials are often challenging to commit to. Studies that provide greater consideration for individual differences across the spectrum also require larger sample sizes to statistically account for such individual variability within groups. However, variations in cognitive capacity, verbal ability, and extent of sensory profiles will provide greater insight into treatment responder subgroups based on behavioural or cognitive symptom profiles.

Future directions and conclusions

Despite the considerable promise and hype around use of oxytocin as a new-generation therapeutic to address core social impairments in ASD, the clinical evidence supporting its efficacy is small. Only a small number of individuals with ASD have actually been reported being administered oxytocin or placebo (a total of 390 individuals with ASD across 21 publications, ranging from randomised trials to open-label investigations and case-studies), with very little investigation in females and almost all studies exclusively recruiting higher-functioning individuals. In considering the oxytocin Hype Cycle (Figure 1), with the initial explosion of interest, the resultant trough of disillusionment, and subsequent plateau of productivity, we have suggested that in this current period of enlightenment, the improvement of research standards is of critical importance to advance this field. In particular, we

1 have highlighted a number of critical considerations for this field to move forward,
2 including the choice of outcome measures, dose and nasal spray device, and types
3 of participants recruited. In addition to these, recent reviews have also highlighted
4 important issues relating to statistical power,⁸⁶ potential publication bias and post-
5 hoc analyses,⁷² and understanding more precise pharmacokinetics.⁸⁷ Despite these
6 limitations, there are still many unanswered questions to be addressed that will
7 provide further insight into the potential treatment utility of oxytocin in the context of
8 ASD.

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Table 1. Summary of studies investigating the effects of a single-dose of oxytocin, compared to placebo, in individuals with ASD

Study	Study Design	Trial Registration Number	Sample analysed	Oxytocin Treatment	Outcome Measures	Effects of oxytocin administration
Hollander et al. ³⁶	Randomised, double-blind, crossover	-	15 adults (14 male) Age: 19-56 years	10 U/ml Pitocin within 1L saline, infused at 10ml/hour, titrated up to 700ml/hour by the fourth hour	Repetitive symptoms	Reductions over time in self-reported repetitive behaviours
Hollander et al. ³⁷					Social cognition	Improvements in comprehension of affective speech; improvements maintained for those who received oxytocin first
Guastella et al. ³⁸	Randomised, double-blind, crossover (one-week)	ACTRN126090003 68235*	16 male adolescents Age: 12-19 years	18 (<16 years) or 24 IU	Social cognition	Improved emotion recognition, particularly for easier items
Andari et al. ¹⁸	Randomised, double-blind, crossover (one-week)	2006-006126-25^	13 adults (11 male) Asperger's disorder or high-functioning autism Age: 17-39 years	24 IU	Social interaction during a game, eye gaze, and plasma oxytocin levels	Changes in behaviour during social ball-tossing game and enhanced visual scanning of eye regions of faces. Plasma oxytocin levels significantly increased
Domes et al. ⁴⁵	Randomised, crossover (one-week)	2010-022511-18^	14 male adults Asperger syndrome Mean age: 24 years	24 IU	Functional activation during face processing	Increased activation in the right amygdala to faces relative to houses
Domes et al. ⁸⁸					Functional activation during emotion recognition	Improved emotion recognition, associated with left amygdala activity
Gordon et al. ⁴⁹	Randomised, crossover (between 3-78 days)	-	17 children and adolescents (14 male) ASD	12 (< 12 years), 18 (12-15 years), or 24 (16 years +) IU	Functional activation during face and vehicle processing	Increased activation during processing of faces relative to vehicles in areas associated with

			Age: 8-16 years				reward and social cognition
Lin et al. ⁴¹	Randomised, single-blind, crossover (one-week)	UMIN000005809 ^{^^}	16 male adults Asperger's syndrome, high-functioning autism, or PDD-NOS Age: 19-51 years	24 IU	Autonomic responses to sounds		Increased skin conductance to human sounds in both neurotypical and ASD groups; change in skin conductance significantly correlated to measures of social functioning and autistic traits in ASD group
Watanabe et al. ⁴⁶	Randomised, crossover (one-week)	UMIN000002241 ^{^^}	33 male adults High-functioning autism Age: above 20 years	24 IU	Functional activation during a social cognition task		Increased nonverbal judgements and increased activity of the medial prefrontal cortex (including the anterior cingulate cortex and dorsomedial prefrontal cortex) and functional connectivity between regions
Aoki et al. ⁴⁷		UMIN000004393 ^{^^}	(2 individuals excluded from Aoki study)		N-acetylaspartate (NAA) using magnetic resonance spectroscopy		No significant effect on NAA; increased functional activation in the mPFC associated with differences in NAA and predicted changes in nonverbal judgements
Aoki et al. ⁴⁸	Randomised, double-blind, crossover (one-week)	UMIN000002241 ^{^^} UMIN000004393 ^{^^}	20 male adults High functioning autism Age: 22-41 years	24 IU	Functional activation during a false-belief task		Increased social-emotional judgements during false-belief task and increased activity in the right anterior insula

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Auyueng et al. ⁴⁴	Randomised, double-blind, crossover (one-week)	-	32 male adults Autism or Asperger Syndrome Age: 18-56 years	24 IU	Eye-gaze during a social interaction	Increase gaze to eye regions in both control and autism groups; in ASD group, moderation of effects by individual differences in fixations under placebo
Althaus, Groen ⁸⁹	Randomised, double-blind crossover (one week)	-	32 male adults High-functioning ASD (ID > 80)	24 IU	Cardiac and cortical responses to affective images	No significant differences between ASD and controls; moderator analyses indicated individual differences
Total number of individuals with ASD:		208	Percentage administered oxytocin:		100% (208)	
Percentage of males:		97% (202)	Percentage of adults:		84% (175)	

* Australian and New Zealand Clinical Trials Registry

^ EU Clinical Trials Register

^^ Japan Primary Registries Network

Table 2. Summary of studies investigating the effects of repeated oxytocin administration in ASD

Study	Study Design	Trial registration number	Sample	Treatment	Outcome Measures	Effects of oxytocin administration
Kosaka et al. ⁹⁰	Case study, open-label Duration: two months	-	Female Age: 16 years	4 IU, twice daily	Clinical improvement	Improvements in social functioning and global improvement
Anagnostou et al. ⁵⁰	Randomised, double-blind, placebo-controlled, parallel design Duration: six weeks	NCT00490802**	19 adults (16 male) High-functioning autism or Asperger's disorder Mean age: 33.2 years	24 IU, twice-daily Oxytocin: 10, Placebo: 9	Clinical improvement, repetitive behaviours, social cognition	No improvements in primary outcomes; improvements on secondary outcomes (RMET and quality of life)
Tachibana et al. ⁶⁷	Single-arm, open-label dose-titration design Duration: seven months	UMIN000003812^^	8 males Age: 10-14 years	8 IU, 16 IU, 24 IU for two months each, with 1-2 weeks of placebo prior to each dose increase. Twice-daily	Clinical improvement	Improvement in ADOS-G scores over time; no significant change in parent ratings. No significant changes in plasma oxytocin. Urinary oxytocin significantly increased post-administration
Dadds et al. ⁵¹	Randomised, placebo-controlled. Duration: four consecutive days	ACTRN12609000784213*	38 males Autistic disorder, Asperger's disorder or PDD-NOS. Age: 7-16 years	12 IU (< 40kg) or 24 IU Oxytocin: 19, Placebo: 19 Once-daily	Social interaction skills, repetitive behaviours, emotion recognition	No significant effects on primary or secondary outcomes
Guastella et al. ⁵⁴	Randomised, double-blind, placebo-controlled Duration: eight weeks	ACTRN12609000513213*	50 male youths Autistic or Asperger's disorder Age: 12-18 years	18 IU (< 16 years) or 24 IU Oxytocin: 26, Placebo: 24 Twice-daily	Social behaviour and overall clinical improvement	No significant effects on primary outcomes. Parental expectations of being randomised to oxytocin moderated symptom improvement

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Anagnostou, Soorya ⁷⁰	Open-label, modified maximum-tolerated-dose design Duration: 12 weeks	NCT01256060**	15 children and adolescents (11 male) High-functioning autism or Asperger's Disorder Age: 10-17 years, mean age 13.8	4 doses (0.2, 0.26, 0.33, 0.4 IU/kg/dose); 0.4 IU/kg/dose the maximum tolerated dose	Safety	No serious adverse events or clinical alterations. Global improvements noted in some participants.
Watanabe et al. (2015)	Randomised, placebo-controlled, crossover Duration: 12 weeks	UMIN000007122^^	20 (18 completed) high-functioning males (IQ > 80) Age: 24-43	24 IU, twice daily Oxytocin: 9 Placebo: 9	Change in ADOS scores Secondary: behavioural task, functional imaging	Significant improvement in social reciprocity, measured by ADOS
Yatawara et al. ⁵⁷	Randomised, placebo-controlled, crossover Duration: 5 weeks	ACTRN12611000061932*	31 children (27 male), Age: 3-8 years	12 IU, twice daily; dose-escalation period in first week	Social behaviours and clinical improvement	Significant improvements in social behaviours and clinician ratings of improvement, particularly in patients receiving oxytocin in second phase of crossover
Total number of individuals with ASD:			182	Percentage administered oxytocin:		65% (119)
Percentage of males: 93% (170)				Percentage of adults:		21% (39)

* Australian and New Zealand Clinical Trials Registry

^^ Japan Primary Registries Network

** ClinicalTrials.gov

1 **Figure List**

2

3 *Figure 1. Oxytocin ‘hype cycle’*

4 *Note.* Numbers in parentheses ^{3,4,33,36-38,40,51,53-55,57,72,91-100} refer to references

5

6 *Figure 2. Number of studies registered on clinical trial registries by current study*

7 *status.*

8 *Note.* ‘Active studies’ refers to studies that were listed as currently recruiting, open

9 for recruitment, and active but not recruiting. Two studies were withdrawn for

10 unknown reasons, one study had not been updated and its status could not be

11 confirmed. Year of registration refers to the year the study was first listed online.

12

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1 Figure 2