PROTOCOL

Title: Single Dose Intranasal Oxytocin (IN-OT) Versus Placebo in Autism: Examining Cognitive Effects

Running Title: IN-OT in ASD

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Principal Investigator: Suma Jacob, M.D., Ph.D.
University Status: Associate Professor
Department: Psychiatry
College: Medicine

Purpose

There are more than 1 million people living with autism spectrum disorder (ASD) according to the most recent report from the US Centers for Disease Control and Prevention (*1*). Unfortunately, current treatments targeting the core ASD symptom domains are insufficient because there is a gap in the knowledge of understanding the underlying mechanisms. Aside from doing treatment studies, our goal is to study how a particular drug changes a core symptom. This will lead to developing better outcome measures and ways to test new drugs that influence the same neurochemical pathways. Single-dose oxytocin (OT) administration in ASD facilitates retention of social cognition increases emotion recognition measured by reading the mind from the eyes task (RMET) (*2*), and promotes social behavior in games (*3*). Only one study focused on repetitive behaviors, and showed less severity, frequency, and number of repetitive behaviors during OT infusion versus placebo in a small sample (*4*). No studies with intranasal OT (IN-OT) to date have focused on mechanisms of how OT may change restrictive/rigid behaviors in ASD, which is a unique goal of this preliminary study.

Lay Summary

Autism spectrum disorder (ASD) is a group of severe, life-long developmental disorders. Oxytocin (OT) is a neurohormone involved in both repetitive/rigid and social behaviors. This study is focusing on how a single dose of intranasal OT (IN-OT) affects cognitive rigidity and social perception tasks. Taking OT as a spray through the nose increases social and decreases repetitive behavior in some adults with ASD, and we are exploring if it helps children with ASD similarly. However, it is unclear whether every person with ASD has an abnormal OT level, and if OT affects restrictive or social behavior differently. Consequently, we aim to study whether OT treatment can be effective in treating subgroups with specific features of ASD. We will use approaches utilizing both behavioral and physiological responses to clarify the role of OT in ASD. We will develop a deeper understanding of the range of social and rigid behaviors and use that information to identify persons with ASD who would benefit from OT treatment. Potential subjects will be asked if they want to participate in two sessions in our clinical laboratory where they will get either single dose IN-OT or placebo. After receiving the substance, they will be asked to do a handful of tasks while we monitor heart rate, eye movements, and collect baseline and post intranasal blood, urine and saliva. The levels of hormones, metabolites and peptides related to or interacting with OT will be measures in the collected samples of blood plasma, urine and saliva. Additionally DNA will be extracted from the blood samples to study genes related to OT and ASD if the participant consents to blood collection for genetic analysis..

Background

The goal of this study is to understand how oxytocin (OT) relates to restricted social interests and cognitive inflexibility in autism. Impairments in social functioning and cognitive inflexibility represent two of the most agreed upon impairments in autism that affect quality of life. There is an unmet need in understanding the neural mechanisms that underlie these social communication and cognitive flexibility deficits, as well as treatments to alleviate specific symptom domains in autism.

OT & behavior: OT is involved in modulating social and repetitive behavior. Intranasal OT (IN-OT) administration in healthy human subjects promotes prosocial, trust behavior (*5-7*), increases gaze toward the eye region of faces (*8*), improves facial memory (*9*), enhances salience of social cues (*10*), and improves performance on the reading the mind in the eyes (RMET) task (*11*). Animal studies knocking out OT receptor (OTR) or OT regulators report that these mice have decreased social memory or recognition (*12*). Recently, OTR knockout mice also showed decreased cognitive flexibility and a resistance to change a learned pattern of behavior, comparable to restricted/repetitive interests (*13*). Both social deficits and behavioral rigidity were ameliorated by OT administration (*13*).

OT dysregulation & ASD: Children with autism spectrum disorder (ASD) have lower average levels of blood OT compared to typically developing children (*14*). Single-dose OT administration in ASD facilitates retention of social cognition (*15*) and promotes social behavior in games (*3*). It heightens skill recognizing and complex psychological states measured by the RMET (proposed as a primary outcome measure in this study (*2*). Only one study has focused on the effects of OT on repetitive behaviors, and it showed less severity, frequency, and number of repetitive behaviors during the OT infusion versus placebo in a small sample (*4*). No studies with IN-OT have focused on mechanisms of restrictive/repetitive behaviors in ASD. The question remains as to whether all persons with ASD have significant disruptions in the OT system or whether OT disruption affects rigid/repetitive behavior or social behavior differentially. Given increased prosocial behaviors with IN-OT in healthy, typical individuals, we expect that many children or adults with ASD will benefit from IN-OT, but will have variable responses within each symptom domain based on individual differences (e.g. genetic and/or plasma level). Another question concerns whether such relationships are observed in subclinical expression of ASD related traits among relatives, believed to represent genetic liability. Demonstration of such relationships would provide powerful evidence that disruption in the OT system pays a role in the etiology of ASD related features.

Neural circuits, neuropeptides, social & restrictive/repetitive behaviors: One of the longer-term goals, to determine neural mechanisms by which IN-OT may improve social behaviors and behavioral flexibility, is in preclinical study. In a complementary manner, molecular studies of both OT and AVP in individuals with ASD may inform an understanding of the relationship among OT, neural processes and behavior. There is growing evidence that although social/communicative and restrictive/repetitive behaviors are core to the diagnosis of ASD, they may be independent groups of traits with distinct neural pathways (*16*). OTR and the receptors for vasopressin (AVP), a evolutionarily related nonapeptides, are found differentially in these functional pathways (*17*). Consequently, OT's action on its own receptor and cross-talk with AVP receptors may influence both flexibility/rigidity and social learning, but through different pathways. In this study, we will determine whether a single treatment with OT is effective in alleviating behavioral inflexibility impairments for the first time while attempting to replicate reported social perception improvements in the same subjects.

Significance

Autism is a developmental disorder characterized by qualitative impairments in social functioning, communication, and repetitive behaviors and restricted interests (APA, 2000). The prevalence of autism is reported to be on the increase, with the most recent figures suggesting that 1 in 88 individuals in the US are affected by some form of autism (*1*). Autism is a disorder with high caregiver burden. Up to 50% of mothers and 21% of fathers of children with autism have elevated depression scores (*18*)**.** It severely impacts both affected individuals and their families. The financial burden on these families is also quite substantial. The cost of disability related to autism in the US alone is estimated to be $30 billion a year (*19*)

In 2003, the Interagency Autism Coordinating committee, IACC, convened an expert panel which created the IACC Autism Research Matrix; a road map for autism research. A major goal in the road map is to establish "Efficacious drug treatments that target core symptoms". This was identified as a high risk long term goal (7-10 years), which positions this study within the guidelines of the matrix. Now, the IACC has released a blueprint for autism research (2009) (*20*). The document specifically states that: "Medications to improve some of the symptoms associated with autism have been studied. However, thus far, no medication has been shown in controlled trials to enhance social behavior or communication….biological and pharmacological treatments that have been investigated in small studies and may warrant fuller attention include …oxytocin". Thus, this project could lead to a new treatment for alleviating core deficits in a major neurodevelopmental disorder.

Project Plan

We will collect pilot data and develop pilot research in order to develop into a federally funded proposal. A unique aim of this study is to test hypotheses about alterations in specific neurocognitive processes as potential contributors to cognitive rigidity which is the opposite of cognitive flexibility. Alterations in behavioral flexibility will be assessed using probabilistic reversal learning (PRL) paradigms. In PRL, a subject must learn to choose between two stimuli. The "correct" choice is reinforced on a majority of trials (80%), and the other choice reinforced on 20% of trials. Subsequently, the contingencies are reversed between choices. Our recent work found that individuals with autism are impaired on PRL. We also showed that reversal learning errors are related to clinical measures of repetitive behaviors. Furthermore, individuals with autism also exhibit social deficits. Thus, it is critical to expand on the center's focus to build a more comprehensive understanding of autism, as well as to develop the most efficacious and rationale treatments.

As an essential first step to achieving these longer-term goals, we have the following specific aims. Our goal is to recruit 60 individuals with autism who have already demonstrated an ability to perform the reversal learning paradigm.

Aim 1: Examine whether acute IN-OT treatment vs. placebo improves social cognition and replicates other ASD studies to date.

Aim 2: Determine whether acute IN-OT treatment vs. placebo improves cognitive flexibility as a novel drug target or future treatment outcome measure.

Preliminary human data with this Protocol: Preliminary PRL and genetic data has been collected at the University of Illinois at Chicago (UIC) on over 300 individuals. Also at UIC, the same protocol for the Single Dose Intranasal Oxytocin (IN-OT) Versus Placebo in Autism study was completed while Dr. Jacob, the PI, moved to the University of MN. Eighteen subjects safely completed this protocol between May and October of 2012 (UIC IRB Protocol #: 2011-1082; Title: Single Dose Intranasal Oxytocin (IN-OT) Versus Placebo in Autism: Examining Cognitive Effects). Subjects tolerated the drug and protocol well and all safety data was sent to the FDA, IND #114259 (UIC IND).

Task Data: Rigidity was examined using computer paradigms that were designed to closely approximate 2-choice T-maze and 4-arm radial maze tasks, which are widely used in studies of reversal learning in rodents (*13*, *21*). It was found that children with ASD demonstrate impairments in flexibly shifting from preferred behavioral patterns to new adaptive response strategies. The ASD group required more trials to successfully complete their reversal phase (t(79)=-2.08, p=0.04) and made significantly more regressive errors (p=0.019). In addition, the number of regressive errors were correlated with total score on the RBS-R (p=0.003) (Figure 1).

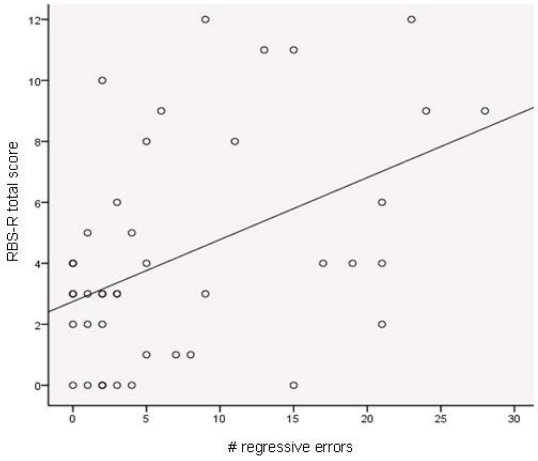

Figure 1. Repetitive Behavior Scales-Revised (RBS-R). r^2=0.45, *p=0.003*

Physiology Data: Autonomic Nervous System (ANS) measures as physiological indicators of emotional state were also studied. Preliminary results of reactivity to stressful challenges show that children with ASD have a higher heart rate (HR; F(1, 51)=5.53, p=0.023)) and lower respiratory sinus arrhythmia (RSA; F(1,51)=4.25, p=0.045) than controls, indicating a tendency for decreased flexibility during novel or stressful situations. Only one study has examined ANS with IN-OT (20 IU) in 40 adults and reported that it increased RSA but less in individuals who reported high levels of loneliness (*22*). Given that children with ASD may be less able to self-report emotional state information, ANS measures may also help us assess baseline anxiety or social aloofness and see how they correlate to response outcomes with intranasal challenge (IN-C).

Eye Gaze Tracking: During facial/video recognitional task the movement of the eyes will be tracked. This measurement allows us to correlate the gaze area and time spent gazing with the subject's ability to interpret emotions. An earlier study showed that children with less severe ASD symptoms and ASD children with increased gaze to the eye region were able to more accurately recognize emotions (*23*).

Methods

We will contact families through the Autism Spectrum and Neurodevelopmental Disorders (AS/NDD) clinic. In addition, potential participants will be recruited from the Fairview Health System list after IRB approval has been obtained. Fairview will be asked

to provide the names and addresses of the parent/caregiver of children with Autism ages 5-17 and adults with Autism (or their legal guardians) ages 18-40. Study staff will send the standard letter from Fairview and a study specific recruitment flyer in the mail, and a consent form will be signed when the families come into the clinic face-to-face. They can voluntarily call if they are interested in participating. If they call and are interested, study staff will schedule an appointment for the first evaluation.

We will also advertise at ASD Intervention Service Providers, Autism Matters and Fraser Child and Family Center. Fraser is a nationally renowned Minnesota nonprofit serving children and adults with special needs through comprehensive education, healthcare and housing services. Fraser is a leading provider of autism services. Autism Matters is a center-based applied behavior analysis (ABA) therapy center run by licensed speech-language pathologists that has recently expanded to two locations. At the intervention centers, this study will be advertised with a flyer. A copy of the proposed flyer is enclosed with this application. If a parent/caregiver calls to say they want to participate, study staff will schedule an appointment for the first evaluation.

We also will recruit potential participants using the Interactive Autism Network (IAN), an online environment that disseminates information on autism to families and facilitates research. Through IAN researchers can apply to receive help with subject recruitment. If our study qualifies, IAN queries its research database based on the study's recruitment criteria, identifies participants who qualify, and sends potential participants the study's IRB-approved recruitment flyer via email. Those interested in participating will contact the study for further information.

Individuals will come to the lab to participate in a randomized, placebo-controlled, crossover single dose challenge study to examine the effects of IN-OT challenge on social as well as cognitive flexibility outcomes. After collecting baseline data, the IN-C will be given on two visits separated by approximately two weeks. Vital signs will be taken and subjects will be monitored for adverse events (AEs). Blood, urine and saliva will be collected before and after IN-C at both study visits. We will measure plasma, urine and saliva for levels of hormones, metabolites and peptides related to or interacting with OT. Additionally DNA will be extracted from the blood samples to study genes related to OT and ASD if the subject consents to genetic analysis. Subject assessments will be done by an evaluator who is blinded to content of IN-C. All ASD studies published to date report 24-48 IU of OT to be well-tolerated and intranasal-placebo>intranasal-oxytocin side effects. Subjects will be monitored for side effects. Safety evaluations will be done by a blinded MD investigator.

OT acquisition & dosing schedule: IN-OT is currently not manufactured in the U.S. but has been obtained from Victoria Apotheke (Zurich, Switzerland). They are also providing the drug for Dr. Jacob's current 12-week intranasal trial in autism. We will use a weight-adjusted dose based on the 40 IU dose, block design based on 0.6 IU/kg/dose. The randomized IN-OT versus IN-placebo session will be separated by approximately two weeks. Placebo will be identical to the OT formulation with the exception of the active compound. It is prepared under aseptic conditions with carrier ingredients identical to OT nasal spray.

Aim 1: Social tasks: ASD subjects exhibit impairments in processing both social visual and auditory cues. The purpose of Aim 1 is to determine whether a single dose of IN-OT reduces deficits in processing social and auditory recognition tasks, especially, tasks that

require assessment of an emotional state to make a decision. The data collected will indicate the degree of impairment in the subject's ability to recognize social visual and auditory cues.

Autonomic Nervous System (ANS) and Emotional Regulation: Because ANS measures can serve as physiological indicators of emotional state, we hypothesize that autistic subjects will have altered ANS functioning during tests that require more emotional regulation. Also, given that children with ASD may be less able to report emotional state information, ANS measures may help us assess baseline anxiety or social withdrawal and see how they correlate to response outcomes with IN-OT vs. placebo. Recently, it has been shown that ASD children focus more on non-eye areas of the face than those in the control group when fear was presented. This effect remained even when age and IQ (verbal, non-verbal, and composite) were used as covariates (cite). HR and RSA, used to assess reactivity to stressful challenges, showed that during a two minute baseline period, children with ASD had a higher HR and lower RSA indicating a tendency for decreased flexibility in stress reactivity. Only one study has examined ANS with IN-OT (20 IU) in 40 adults and reported that it increased RSA but less in individuals who reported high levels of loneliness (*22*).

Aim 2: Cognitive Flexibility task: The PRL task will assess individuals' ability to learn associations between stimuli and subsequent reward or punishment, as well as adaptive control of behavior following changes in reinforcement contingencies. To examine cognitive flexibility, we have been using computer paradigms that were designed to closely approximate the 2-choice T-maze and 4-arm radial maze tasks widely used in studies of reversal learning with colleagues at UIC (*24*). We found that individuals with ASD demonstrate impairments in flexibly, shifting from preferred behavioral patterns to new adaptive response strategies. The participant' cognitive flexibility, as well as, social abilities will be assessed by answering questions and performing tasks such as those in Table 1.

Taken together, the integrative approach to systematically investigating the effects of IN-OT on social functioning and cognitive flexibility in ASD subjects is a critical first step for determining whether OT may be an effective treatment for these deficits in ASD, as well as for the longer-term goal of investigating what may be the neural mechanisms by which OT affects social and cognitive functioning.

Design

The treatment intervention will be a double-blind placebo-controlled cross-over challenge study of IN-OT versus placebo separated by a drug-free period of approximately two weeks. Enrollment will be up to 30 subjects. The primary outcome measuring changes in cognitive flexibility will include regressive errors on our PRL task. In addition, to examine changes in social cognition RMET will be utilized. We will test cognitive flexibility along with social tasks via computer. One of the goals of this initial study is to choose the best outcome measures for future trials. Additionally, the standardized clinical measurements, Repetitive Behavior Scale-Revised (RBS-R), State-Trait Anxiety Inventory for Children (STAI; or Adults based on age and developmental abilities) and ABC-CV stereotypy, will be included.

Table 1: Study Outcome Measures for Aim 1 and Aim2

Social Cognition Tasks	
Primary Social Outcome	Reading the Mind in the Eyes Task (RMET)
Extended Social Battery	Includes computerized & photo tasks such as Diagnostic Analysis of Nonverbal Accuracy (DANVA2; has auditory emotion ID component) (25), an updated version of Lets Face IT! computer task (26)
Cognitive Rigidity Tasks	
Primary Rigidity Outcome	Probabilistic Reversal Learning (PRL) Task (27)
Extended Rigidity Battery	Includes Rapid Automatized Naming (RAN) (28), Stop Signal Task
Brain-Body Physiology Measures	
Autonomic Nervous System (ANS)	Data will be collected continuously during the session to examine if specific tasks require more emotion regulation. Blood pressure will be collected twice during the session, once pre challenge and once post challenge.
Eye Tracking with Face Attribution Tasks	Eye tracking paired with Dynamic Affect Recognition Evaluation (DARE) (23) and facial/video recognitional task images.

Inclusion Criteria

Participants will be between 5 and 40 years of age. All subjects will have a diagnosis of autistic disorder or ASD that was confirmed by administration of the Autism Diagnostic Observation Schedule-WPS (ADOS-WPS) (30). Eligible participants must be able to perform the cognitive learning tasks and must have an IQ score of ≥70..

Exclusion Criteria

Although we acknowledge that concomitant medications, or other types of intervention, may potentially bias study results, participants will be allowed to stay on concomitant medications and non-pharmacologic treatments, provided that no changes are made within 3 months prior to baseline and that no changes are made during the study. Individuals that are on antipsychotic drugs will be excluded from participation. All subjects must lack a significant medical history. Subjects with any condition, including alcohol and drug abuse, which might interfere with the conduct of the study, confound interpretation of the study results, or endanger their own well-being will be excluded. This includes, but is not limited to impairment of renal function, evidence or history of malignancy or any significant hematological, endocrine, respiratory, hepatic, cardiovascular or gastrointestinal disease. All female subjects of childbearing capacity will have a urine pregnancy test (a positive test will exclude the subject from participation). A pregnancy test will be conducted at both visits prior to drug administration. Uterine contractions may occur in women and are more likely to occur in pregnant women, especially towards the end of pregnancy. As a result, we exclude pregnant female patients, sexually active female patients on hormonal birth control sexually active females who do not use two types of non-hormonal birth control. In

addition, we will exclude any nursing females. Sexually active male participants must agree to use an effective form of contraception for the duration of the study.

All interested potential subjects will be contacted via phone. If they meet eligibility criteria, two sessions that are approximately two weeks apart and approximately the same time of day will be scheduled. At their first visit, we will review the study and undergo informed consent procedures. Overall study procedures per visit are estimated to take approximately 2-3 hours per session, and are detailed in Table 2.

Table 2: Schedule of Procedures

Procedure	Screening Visit	Study Visit 1	Study Visit 2
Obtain consent	X	X	
Diagnostic Assessments	X		
IQ Test	X		
Medical history review and past medications	X	X	
Vitals (blood pressure, pulse, height, weight)	X	X	X
Review current medications	X	X	X
Blood draw		X	X
Urine collection		X	X
Pregnancy Test		X	X
Saliva Collection		X	X
Receive study drug or placebo		X	X
ANS measures/EKG		X	X
Behavioral outcomes		X	X
Eye-tracking		X	X
Monitor side effects		X	X
Post follow up phone call		X	X

Participants, caretakers, and investigators involved with clinical ratings and research assessments will be blinded to IN-OT versus placebo assignment throughout the study.

ANS measures: We will use a system with ultra miniature recorders enabling us to record EKG waveforms in children and adults without the need for belt mounted recorder or lengthy wires. ANS data will be collected and analyzed with current software at baseline

and throughout the two IN-C sessions, but data analysis will focus on resting intervals between tasks similar to other ASD studies (cite).

Eye-tracking: A computerized system will be utilized to collect and quantify the eye-gaze data. This system has previously been successful in our research group for collecting data from ASD subjects. It is composed of a camera, a video head movement unit, a control computer, and a stimuli presentation computer. The eye-tracking system uses algorithms to locate and track corneal reflection and bright-pupil location and collects the coordinates of the separation between these two using an optical camera. The system then transposes the coordinates to corresponding locations on the monitor showing the stimuli being viewed. Data will be collected during the DARE task and facial/video recognitional task.

Blood work for biomarkers: During the two blinded, randomized IN-C sessions, blood will be drawn at baseline and 60 minutes post IN-C administration. The timing is according to differences reported in plasma OT after IN-OT in ASD (3). The Jacob lab has extensive experience for optimizing OT collection methods. See plasma level time course from two control subjects previously obtained by Dr. Jacob and a collaborator (figure 2).

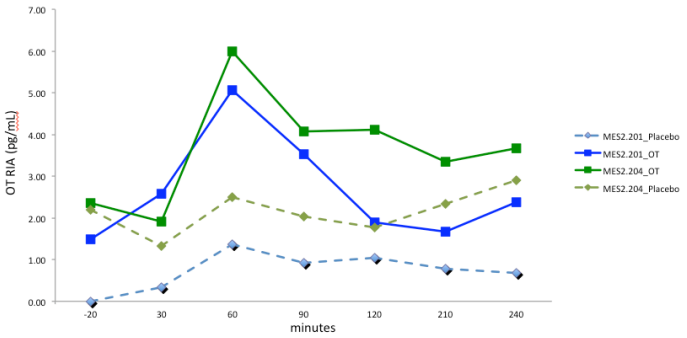

Figure 2. Plasma OT Time Course changes within Subjects with IN-OT vs. Placebo

Anticipated results & interpretation: This study will be essential in allowing us to examine mechanisms of OT modulation of social or rigid behaviors in ASD. Our primary analyses will focus on IN-OT versus placebo differences in RMET for social perception and the PRL task as a measure of rigidity. Additional social cognition and rigidity tasks will be explored to assess appropriate measures for future studies investigating mechanism of OT change or treatment outcomes. Our ultimate goal is to see if IN-OT can be used to augment social or behavioral learning therapies and compare the risk and benefits of paired IN-OT treatment versus chronic administration (currently being conducted at UMN) in targeting specific subdomains within ASD across development.

Sample composition: Sample heterogeneity, for instance with respect to symptom severity, might affect treatment response. Restricting the sample to prescreened subjects who can perform cognitive tasks reduces heterogeneity.

Concomitant medication and treatments: Although we acknowledge that concomitant medications, or other types of intervention, may potentially bias study results, participants will be allowed to stay on concomitant medications (except antipsychotic drugs) and non-pharmacologic treatments, provided that no changes are made within 3 months prior to baseline and that no changes are made during the study. Information from the treatment history form can be used in ad hoc analyses to highlight potential confounding factors.

Intervention attrition and missing data: An intent-to-treat analysis will be used including every randomized participant who has baseline data. We will make every effort to minimize dropouts in order to preserve the integrity of the study; however, we recognize that missing data in pre-post designs are inevitable. For missing data, multiple imputation methods will be considered. Logistic regression will be used to obtain adjusted estimates of treatment effects for the primary measures. We will document reasons for dropouts and we will compare the characteristics of the subjects who drop out to those who complete.

Data management & Sources of Material

The sources of research material obtained from each participant will be in the form of information gathered specifically for research purposes. This will include data from diagnostic testing, cognitive and neuropsychological data, clinician and evaluator ratings (e.g. safety measures), and blood, urine and saliva samples. Additionally, DNA will be extracted from the blood samples to study genes related to OT and ASD if the participant consents to the collection. Data and records will be rigorously protected. To protect participants' confidentiality, only authorized persons will have the right to review research records. Confidentiality of those records will be protected to the extent permitted by law. For all data, the identity of study participants will be kept confidential, such that all participants will be assigned a unique identifying number under which all obtained information will be coded. Research records will de-identified or be kept in secure, encrypted, password-protected databases and will be accessible only by key study personnel and will not be released without the subject's consent unless required by law (e.g. suspected abuse) or a court order. All biological samples will be labeled using unique numerical identifiers that can only be linked to subject identity by a key stored in the locked archive. When the results of this research are published or presented at scientific meetings, the identity of subjects will not be disclosed.

Potential Risks & Safety Monitoring and Assessment

This study may present greater than minimal risk to subjects. For the screening, behavioral testing and evaluations, and blood draw, the risks to the subjects are minimal, sometimes involving test anxiety, apprehension and fatigue, discomfort, and breach of confidentiality. For the administration of IN-OT, the risk to subjects may be greater than minimal. We will take numerous safety precautions to ensure the safety and well-being of participants.

Intranasal oxytocin: For the OT component of the study, the primary risks are related to potential side effects. Side effects of IN-OT may include nasal irritation, runny nose, or tearing of the eyes. Additional rare side effects reported in single cases and of unknown relationship with the medication include allergic dermatitis, unusual bleeding, difficulty passing urine, sudden weight gain, convulsions, nausea, drowsiness, headache and rarely confusion, anxiety, sad mood, fast or irregular heartbeat (palpitations) and unusual swelling. Large doses of intravenous OT decrease both systolic and diastolic blood pressure through a transient relaxation of vascular smooth muscle. Any OT-induced decrease in blood pressure is followed by a mild but sustained increase. IN-OT, which will be used in the present study, has not been found to substantially affect blood pressure.

Medication: Exogenous OT is metabolized by chymotrypsin in the GI tract and thus cannot be administered orally. It can be administered in either an intravenous or an

intranasal form. Currently, the only form of OT that is available in the US is the intravenous form. Although this formulation has been found to produce positive effects on social cognition in ASD (*11*), it is invasive to administer and the extent to which this formulation crosses the blood-brain barrier (BBB) is unknown. One alternative is IN-OT, which is absorbed through the highly permeable nasal mucosa and has been shown to pass the BBB (*25*). IN-OT remains on the market outside the US and Canada (e.g., Syntocinon®, NOVARTIS, Switzerland). The applicant currently holds INDs for studying oxytocin in ASD children and adults (IND#109833; #114259). The effect on smooth muscle after intranasal administration of 10-20 IU lasts approximately 20 min, which reflects its duration of action in the periphery and outside the BBB (*26*). Mean CSF concentrations of peptides like OT begin to rise within 10 minutes of intranasal administration and last for up to 90 minutes after intranasal administration (*25*). OT is distributed throughout the extracellular fluid and most is rapidly destroyed in the liver and kidneys. Only small amounts are excreted unchanged in the urine. IN-OT will be accessed in the same ways as our chronic OT treatment studies, and will be obtained the same way for this project.

Safety: A recent literature review summarizing the **safety data of 1,529 individuals** exposed to IN-OT reported that it produces no reliable side effects and is not associated with adverse outcomes in doses of 18-48 IU in the short term (*31*). The majority of the reviewed studies were single dose studies; four multi-dose studies (7 days to 13 weeks) were also included. In our Toronto collaborator's 6-week feasibility study using 24 IU IN-OT (morning & noon) in adults with ASD, IN-OT was well tolerated and no serious adverse effects were reported (*32*). Three participants discontinued early, 2 in the placebo group and one in the active group. Early terminations in the placebo group were due to 1) worsening tics, and 2) panic attack in response to the nasal spray use. In the active group, one participant with stable epilepsy disorder was noted to have starring spells by his wife. The events were not observed by a physician or at any visit; the participant terminated for safety reasons. There were no significant differences between IN-OT and placebo in any of the blood work and no abnormal EKGs. In addition, the same protocol was performed at UIC (UIC IRB Protocol #: 2011-1082) using a weight-adjusted dose based on the 40IU dose, block design based on 0.6IU/kg/dose. In this pilot 18 subjects safely completed the study, 4 AEs were reported. Three of the AEs were mild and possibly related to the study intervention. The mild AEs reported were a bloody nose, fatigue reported by the mother, and pain in the roof of the mouth. The fourth AE was moderate in severity and also possibly related to the intervention of the study. This subject reported a headache. Finally, previous research that investigated the role of IN-OT in enhancing lactation in women has reported no safety concerns (*33*, *34*).

Definition of Adverse Event (AE)
An AE is defined as any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product whether or not the event has a causal relationship with the treatment. An AE may be any unfavorable and unintended sign, symptom, or disease temporally associated with the use of a study medication, whether or not considered causally associated with the use of the study medication.

The following guidelines will be used to characterize AEs.
Attribution is classified as follows:

- Definite: AE is clearly related to the study drug.
- Probable: AE is likely to be related to the study drug.

- Possible: AE may be related to the study drug.
- Unlikely: AE is doubtfully related to the study drug.
- Unrelated: AE is clearly not related to the study drug.

Severity of AEs is classified as follows:

- Mild: Transient discomfort that does not interfere with the participant's normal functioning.
- Moderate: Produces limited impairment of function and can require therapeutic intervention, but unlikely to produce sequelae.
- Severe: Results in marked impairment of function and can lead to temporary inability to resume usual life pattern. The AE may produce sequelae that require prolonged therapeutic intervention.

Definition of a Serious Adverse Event (SAE)
An SAE is defined as any event that meets the following criteria:

- It results in death or is life threatening (i.e., presents an immediate risk of death from the event as it occurred). This criterion is not intended to include an AE that, had it occurred in a more severe form, might have caused death.
- It results in persistent or substantial disability or incapacitation. (This criterion is not intended to include events of relatively minor medical significance, such as uncomplicated headache, diarrhea, or sprained ankle.)
- It results in inpatient hospitalization.
- It results in prolongation of an existing hospitalization.
- It is a congenital anomaly or birth defect.
- It requires medical or surgical intervention to prevent any of the above outcomes.

Medical and scientific judgment should be exercised in determining whether an AE is serious when considering important medical events that may not be immediately life threatening or result in death or hospitalization, but may jeopardize the patient or may require intervention to prevent any of the other outcomes listed. Examples of such medical events that may also be considered serious include, but are not limited to, intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias, or convulsions that do not result in hospitalization.
Hospitalization for elective treatment of a pre-existing condition that did not worsen from baseline does not meet the definition of an SAE.
Social or convenience admission to a hospital or prolongation of a hospitalization for social or convenience reasons not associated with the occurrence of an AE does not meet the definition of an SAE.

Recording, Evaluating and Reporting Adverse Events and Serious Adverse Events
The investigator will attempt to establish a diagnosis of the event on the basis of signs, symptoms, or other clinical information. In such cases, the diagnosis, not the individual signs or symptoms, should be documented as the AE or SAE. Any adverse events--whether due to assessment, imaging, medication or other aspects of any project--will be reported to the principal investigator or the co-investigator of each study. Unanticipated (non-serious) events will be reported to the IRB within 30 days of the principal investigator becoming aware of the event, via submission of an Adverse Event Report. Adverse events will be documented and reviewed by the principal investigator within 24 hours of the principal investigator becoming aware of the event. Serious events will be reported the IRB and FDA within 48 hours of the principal investigator becoming aware

of the event. Notification will be by phone, email, or fax. A completed Adverse Event Report will be submitted within 10 days of initial IRB notification. All deaths will be reported to the IRB and FDA within 48 hours of the principal investigator becoming aware of the event. All adverse events will be compiled, and reported in summary form to the IRB, on an annual basis and at the conclusion of the study.

Dosage: The question of appropriate dosage remains. The majority of studies of IN-OT in ASD have used 24 IU per dose to document behavioral and biological (fMRI) effects (2). The range reported across neuropsychiatric disorders including ASD is 18-48 IU per dose (35). At this point, we plan to use a weight-adjusted dose based on the 40 IU dose, i.e. 0.6 IU/kg/dose with the assistance of University of Minnesota Medical Center, Fairview, Investigational Drug Services Pharmacy. We have had to make such a weight adjustment for our current international multidose study in adolescents with ASD. In the case that regulatory agencies raise an issue with the chosen dosage, we will follow their guidelines. Participants will be asked to take the dose of IN-OT or placebo in the form of intranasal spray and then begin the tasks.

Side effects observed during our current multidose IN-OT feasibility trial in Toronto, Canada and our intravenous OT pilot study at UIC in autism were mild in nature. Because very few studies have reported on the extended use of IN-OT, there may be other unknown side effects. The PIs and study physicians (or a covering clinician) will be available at all times to study participants in the event of a clinical emergency. Both availability and contact information in the case of an emergency will be clearly communicated orally and in writing to study participants and their families.

Psychological stress and anxiety: Fatigue, anxiety, discomfort or stress are potential adverse effects associated with the content or length of clinical and medical evaluations, behavioral testing and administration of rating scales. The evaluations will be conducted by investigators and staff with extensive experience and expertise who are sensitive to the clinical state of participating individuals. Experienced clinicians will always be available to help in efforts to reduce any discomforts. These effects may also be encountered during other study procedures (e.g., blood draw) and we will attempt to minimize them by familiarizing participants with the personnel, setting and closely monitoring them during the study. Well-trained and supervised personnel administer tests, and participants are debriefed after each session. Parents will be present either in the same room or a nearby room. Research staff working on the project all have experience working with children and adolescents and will therefore do everything possible to accommodate the children and adolescents who come to our centers as part of this study. Participants will be allowed to take breaks and stop at any time to minimize fatigue and discomfort. Each visit will be organized to involve breaks for the family and to allow the child to rest between procedures. There may be discomfort associated with participation, but in our experience subjects who are well informed on the purpose of the study and who are accompanied throughout the procedures by a member of the research team tolerate this discomfort well and without complications. The research team is trained to provide appropriate responses, both clinically and legally, to any reports of abuse or harm.

Blood draw: Risks to the study subjects might stem from blood drawing, which can result in temporary bruising or, very rarely, infection. There is the possibility of some discomfort from the withdrawal of blood. A topical anesthetic will be offered to the participant to minimize this discomfort, if necessary. The amount of blood removed is

minimal and generally will not cause significant hemodynamic problems. The venipuncture is performed by nurses and staff who have received special training and are skilled at drawing blood from children and adolescents.

Breach of Confidentiality: Because this study involves clinical evaluation there is the risk that, if this information became public, both participants and their family members could have adverse consequences. We will make every effort to protect the individual's confidentiality; confidentiality is carefully guarded to address issues of privacy and insurability. However, the greatest risk to participants is the rare possibility of an inadvertent breach of confidentiality that might adversely affect a participant's future employability, insurability, or family and personal relations. This loss of confidentiality is unlikely because the investigators will not disclose information to anyone outside of the immediate research group working on this project. However, there is no absolute legal protection against discrimination based on such information. To minimize the risk, each subject is assigned a study number immediately upon recruitment, all research records will be kept confidential and all research information will be associated with codes and not personal identifiers. Although a list linking the study number to the subject's name is maintained for future studies and contact, the name does not appear on any other document or laboratory specimen. The families are informed of this link and are assured that only relevant study staff has access to that list. The purpose of the list linking study ID number to the name is to record who we have already recruited so that we do not accidentally re-approach any family.

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