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Integrated Oxytocin and Nonverbal, emotion recognition, and theory of mind training for children with Autism Spectrum Disorder (ION-ASD)

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Statement of Compliance

This study will be carried out in accordance with Good Clinical Practice (GCP) as required by the following (use applicable regulations depending on study location and sponsor requirements; samples follow):

- United States (US) Code of Federal Regulations (CFR) applicable to clinical studies (45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 56, and 21 CFR Part 312)
- ICH E6; 62 Federal Register 25691 (May 9, 1997)
- NIH Clinical Terms of Award

All key personnel (all individuals responsible for the design and conduct of this study) have completed Human Subjects Protection Training.

Contents

1. Background and Significance

Neuroscience research is rapidly unfolding cognitive, behavioral, and affective processes that sustain the striking human capacity for social engagement. Converging evidence indicate a critical role for social cognition in conditions defined by social disability such as autism spectrum disorder (ASD), schizophrenia, and social anxiety disorder (SAD)[1,2]. Mental mechanisms supporting social cognition include social perception, emotion perception, mental state attribution, and attributional style/bias[3]. In ASD, impairments in emotion perception and mentalizing are increasingly understood as central to the phenotype[4]. A recent study reported atypical neurophysiological responses on mentalizing tasks in adolescents with ASD who showed no differences to controls on behavioral theory of mind tasks[5]. The data support a fundamental dysfunction in social cognitive networks and a possible reliance on compensatory strategies (e.g. verbal mediation) during behavioral tasks in older individuals with ASD.

Socialization interventions in school-aged and older individuals with ASD

Empirical support is growing for psychosocial interventions in older, verbal youth with ASD including peer-mediated interventions and social skills training (SST) groups[6-9]. Available interventions increase social contact in a disorder associated with greater levels of disenfranchisement than predicted by IQ and educational attainment[10]. SST groups in particular are a widely used treatment modality in this subset of individuals with ASD. Similar to recent approaches to pharmacological management of ASD[11], the majority of available SST curricula represent adaptations of approaches in other pediatric conditions[12]. Adaptations vary in level of specificity to ASD with most curricula focused on downstream skills deficits presenting across social-emotional learning disorders (e.g. friendship, calling a friend).

Critical methodological issues persist in social skills research in ASD. Issues include relative efficacy compared to other active treatments, variability in treatment response, and treatment durability[13-15]. A handful of cognitive behavioral interventions (CBI) target social cognitive impairments in school-aged youth with ASD. Data from pilot studies and our randomized comparative trial[16] indicate weak to minimal effects on emotion recognition and theory of mind outcomes within targeted CBI-SST interventions[9,17-19]. We contend that available interventions provide effective models to modify social behavior (e.g. social competency) and environmental supports (e.g. parent training, peer mediated interventions) (Figure 1). However, social cognition represents an under-treated social learning domain and critical treatment target for CBI-SST. The lack of efficacious computerized and pharmacological treatments[11] underscores the importance of developing innovative strategies to improve the efficacy and durability of available interventions for core social deficits in older, verbal youth with ASD.

Neurobiological substrates of ASD.

Core deficits in ASD are characteristically heterogeneous. Genomics research points to both inherited and de novo mutations in the pathogenesis of the heterogeneous ASD phenotype[20]. In addition, evidence is accumulating for shared liabilities and biological mechanisms underlying ASD, neurodevelopmental disorders and psychiatric disorders. Specifically, mechanisms supporting neurodevelopment, synaptic plasticity, and learning and memory appear to be common pathways to neuropsychiatric disability[21].

Oxytocin and Arginine vasopressin (AVP)

Oxytocin (OXT) is a nine-amino-acid peptide which is synthesized in the paraventricular and supraoptic nuclei of the hypothalamus and projected to the posterior pituitary, limbic areas including the hippocampus, amygdala, striatum, hypothalamus, nucleus accumbens, and other sites such as the locus coeruleus[22]. OXT is closely related to AVP, differing by only 2/9 amino acids. OXT/AVP are fairly large molecule compounds which have not been shown to penetrate the blood-brain-barrier (BBB). These sister peptides are associated with related but distinct behaviors. Both are produced in the hypothalamus and project to the pituitary. OXT is critical to social attention, relaxation, maternal care/bonding[23], maternal aggression, and parasympathetic responses; AVP plays a role in social recognition[24], vigilance, territorial aggression, threat responses, and sympathetic responses. OXT in particular is implicated in developmental processes. Peripheral OXT in early development has been linked to lasting consequences in adult social behavior in animal studies[25].

OXT has a single receptor, located in the uterus, bone, heart, and brain. The oxytocin receptor (OXTR) increases triphosphoinositol (IP3) and is coupled with Gq protein receptors. AVP has three receptor subtypes. V1a and V1b also increase IP3. V1a is located in vascular and brain regions, also coupled to Gq and V1b is located in the anterior pituitary and brain regions. The third receptor, V2, increases cyclic adenosine monophophate (cAMP) and is located in the kidney. The pharmacodynamics of OXT/AVP are well known. OXT/AVP are degraded by protease and have a half-life in plasma of approximately two minutes, and in CSF of approximately 20 minutes.

Intranasal oxytocin and the treatment of social cognition deficits in psychiatric disorder

In human studies, single-dose intranasal OXT administration is associated with enhanced emotion recognition/mentalizing[26], face identity[27], and increased attention to the eye region[28]. In ASD, studies find reduced plasma OXT levels[29], absence of normal developmental increase in oxytocin blood levels with age[30,31] and variations in the OXT receptor (OXTR)[32,33]. The greatest excitement has surrounded replicated findings of improved attention to the eye region, mentalizing abilities, and partner preference from single-dose OXT challenge studies in ASD[34-36]. The effects of intranasal oxytocin (INOXT) and its ability to cross the BBB have yet to be determined. INOXT has been shown to affect functional responses in several brain regions including the medial prefrontal cortex, anterior insula, orbitofrontal cortex, amygdala, and ventral striatum.

The convergence of evidence on the impact of OXT/AVP on social behaviors has fueled several investigations of the therapeutic potential of these compounds in neuropsychiatric conditions characterized by social impairment. The vast majority of human studies have been conducted on OXT compounds. Pilot data from oxytocin monotherapy trials indicate daily, intranasal OXT is safe and feasible in adult[37,38] and pediatric populations[37,39] delivered over a period of 6 weeks[37,40] to seven months[38]. The applicant and colleagues recently published on a dose-finding, safety trial of intranasal OXT in 15 adolescents with ASD[40]. Four doses were tested every two weeks for 12 weeks. Follow-up testing was conducted at 16 and 24 weeks. Mild to moderate side effects were reported including irritability, headache, upper respiratory infections, and decreased appetite. In this small open-label trial, significant pre-post changes were found on measures of social cognition, social behavior, anxiety and repetitive behaviors with carry-over effects found at 3-month follow-up on social cognition, repetitive behavior, and functional communication skills. Overall, preliminary findings from multi-dose OXT studies support medium to small effects but require replication in well-powered, controlled trials[41].

Enthusiasm for OXT's therapeutic potential is tempered by the largely unknown mechanisms underlying its effects on social processes. Emerging data suggest OXT triggers its own release through hypothalamic neurons and shows sensitivity/plasticity to both physiological and environmental inputs[41]. Leading theories also posit a role for OXT in altering reward processing for social stimuli in autism[42,43] and role for its anxiolytic properties in several psychiatric disorders[40,44,45]. The parameters governing OXT's influence on social processes also appear to be specific to species, developmental period, and sex[45,46]. In the ASD literature, exploratory analyses in a single-dose challenge study found an association between Wing's qualitative "active but odd" subtype and enhanced social behavior in adults with ASD[36]. In a recent study in adults with schizophrenia, pre-treatment OXT dosing prior to a 6-week social cognitive intervention enhanced high-level social cognitive processes but provided no measurable symptom relief[47]. Taken together, results support the continued investigation of the therapeutic potential of OXT but point to a need for systematic research to untangle its contextualized effects in complex neuropsychiatric conditions.

Synthetic Oxytocin Characteristics and Side Effects

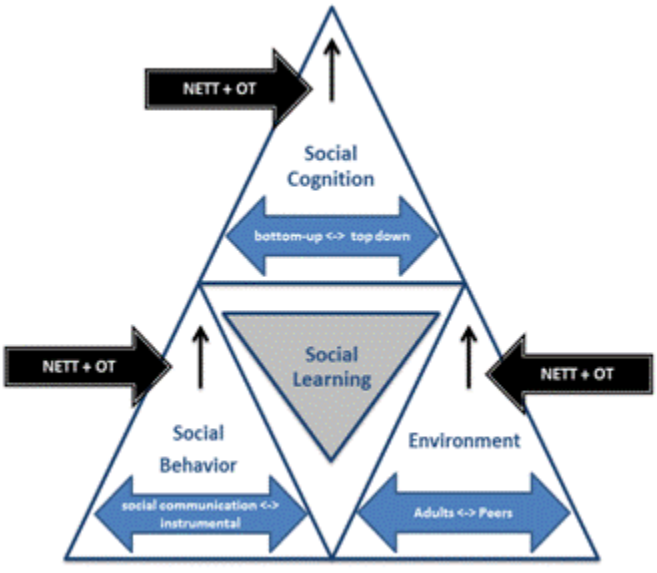
Synthetic OXT (Pitocin®/Syntocinon®) is a 9 residue cyclic peptide; the hormone is prepared synthetically to avoid possible contamination with vasopressin and other small polypeptides with biologic activity. There have been multiple studies of IN-OXT in lactating and non-lactating women which provide data regarding the safety of both the peptide and administration route[47,48,49]. These studies have systematically reported none or only minimal side effects. In addition, there have now been at least a dozen published studies using IN-OXT in healthy adults; here too, minimal side effects have been reported. Finally, data from our 6-week pilot treatment

study[40] indicate that the medication was very well tolerated compared to placebo (see pilot studies section). Side effects of IN-OXT may include nasal irritation, runny nose, or tearing of the eyes, as well as an allergic reaction. Additional rare side effects reported in single cases and of unknown relationship with the medication include unusual bleeding, convulsions, nausea, drowsiness, headache, anxiety, and sad mood. Uterine contractions may occur in women and are more likely to occur in pregnant women, especially at the end of pregnancy. Estrogen mediates Pitocin®'s effect on uterine muscle. Large doses of intravenous OXT decrease both systolic and diastolic blood pressure through a transient relaxation of vascular smooth muscle. Any OXT-induced decrease in blood pressure is followed by a mild but sustained increase. IN-OXT, which will be used in the present study, has not been found to substantially affect blood pressure. The recent pilot data in pediatric ASD did not find effects on blood pressure. However, the proposed trial will continue to monitor blood pressure given the great need for safety data on this high-profile compound.

Rationale for Intranasal Administration

OXT is metabolized by chymotrypsin in the GI tract and therefore is not administered orally. Currently, the only form of OXT that is available in Canada and the US is the intravenous form and although this formulation has been found to produce behavioral effects, it is invasive to administer and the extent to which this formulation crosses the blood-brain barrier (BBB) is not known. However, several recent studies support the impact, direct or indirect, of peripherally administered OXT on centrally mediated processes. Ring et al.[48] investigated anxiolytic effects of centrally and peripherally administered OXT in male mice. Peripheral administration was found to produce behavioral effects but required substantially larger doses to produce similar effects as centrally administered OXT. These researchers argue, that "the anxiolytic-like effects of peripherally administered OXT can be accounted for by the passage of relatively small, but sufficient, amounts of the peptide across the BBB into the CNS" 50.

IN-OXT is a widely used alternative to IV administration. IN-OXT is absorbed through the highly permeable nasal mucosa and has been shown to pass the BBB51; it is also easy to self-administer. Withdrawal from the US and Canadian market by the manufacturer of the IN-OXT (Syntocinon®, Novartis) in 1997 and 1992 respectively, was not related to any safety issues but was at the request of the manufacturer, for poor market profits. Specifically the FDA has stated in prior exemptions for this compound "Intranasal oxytocin has previously been approved for marketing in the United States from March 20, 1962 until its withdrawal from the market on August 7, 1997. The Federal Register notice issued on that date [Federal Register, Vol. 62, No. 152, Docket No. 97N-0326, pp. 42575 –42577], clearly states that this withdrawal was at the request of the manufacturer, because the drug was no longer being marketed. No safety reasons were cited in connection with the withdrawal." The intranasal form of OXT remains on the market outside Canada (e.g. Switzerland), and Pitocin® administered via intravenous infusion is still available in Canada and the US.



The proposed study is a proof-of-concept trial designed to investigate the integration of IN-OXT into a social cognitive skills training program for youth with ASD. The complexity and heterogeneity of neurodevelopmental disabilities such as ASD suggests interventions targeting multiple domains are more likely to have a therapeutic impact. The proposed research plan will build upon the findings from our team's pilot data on effects of targeted social skills groups as well as IN-OXT in individuals with ASD.

We hypothesize that targeted OXT administration during a cognitive behavioral therapy-based social cognitive skills group will hit multiple domains contributing to social learning and: 1) enhance social cognitive processes,

Figure 1. Hypothesized mechanisms of social learning through impact on key social learning domains

2) improve social communication behaviors, and 3) positively impact environmental contingencies (e.g. parent-training) promoting pro-social behavior in youth with ASD (Figure 1).

The research program stresses behavioral science data and developments in the biological sciences to inform our understanding of the optimal modality (augmentation, not monotherapy) and mechanism (enhancement of extinction learning) underlying IN-OXT and social skills treatment effects on social behaviors. If data are promising, future studies will test critical treatment components for social learning and inform novel treatment development pathways for core symptom impairments in youth with ASD.

1.1. Preliminary studies

Randomized controlled, comparative trial of social skills groups for youth with ASD

The proposed trial builds upon an NIH-funded, randomized comparative trial of a social cognitive skills therapy group[16]. The study was designed to address several methodological limitations in SST research including questions regarding specificity of treatment effects and treatment durability[13]; and investigate behavioral and neural mechanisms underlying treatment response[19]. The NIMH trial built upon open-label pilot data collected in outpatient therapy clinics and imaging data from the co-PI, A. Ting Wang, indicating effortful, top-down processing strategies, such as those used in CBI, normalized mentalizing networks in youth with ASD[49]. Method: The study enrolled a well-characterized, ethnically diverse sample of verbal, 8-11 year old youth with ASD (n=69) into an RCT of NETT compared to a facilitated play group. NETT is a 12-session CBI with concurrent child and parent training groups. The facilitated play group served as a control for time, therapist training, peer exposure, and parent engagement.

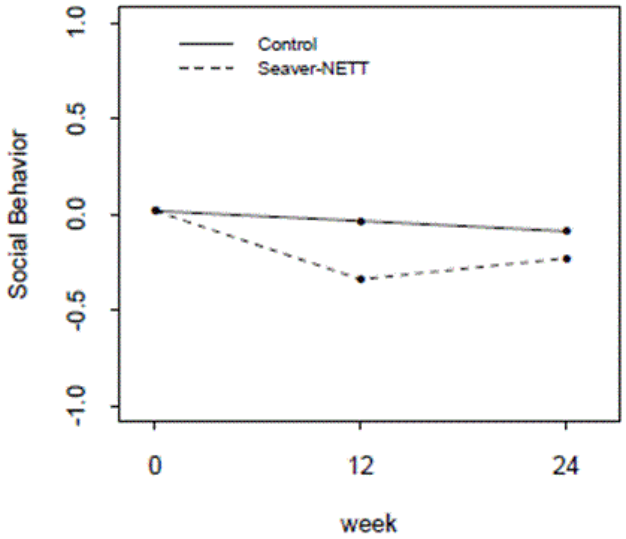


Figure 2: Improvements in social behavior from Seaver-NETT

Measurement: Outcomes collected at baseline, endpoint, and 3-month follow-up included 1) social cognition, neuropsychological assessments conducted by blinded raters, 2) social behavior composite, parent/caregiver ratings on standardized assessments and 3) neural outcomes, on fMRI tasks of eye gaze and interpretation of irony. In addition, experimental measures of peer interactions in familiar and unfamiliar settings were also piloted.

Data analysis. Principal components analysis was conducted on assessments to yield composite scores for social cognition and social behavior. Mixed model analyses were conducted on individuals with two or more time-points (i.e., completers). Variables examined for moderation effects were: VIQ, autism symptoms, comorbid mental health conditions including hyperactivity and anxiety (i.e., BASC-3).

Results: NETT participants improved on the social behavior composite capturing nonverbal communication, social relations, and empathic responding skills at endpoint compared to controls (B=-.31, SE=.14, p=.04). No group differences were found on the social cognition composite (B= 0.00, SE=.13, p=.98). Outcomes at a three-month follow-up interval were not significant suggesting a potential waning effect of the CBI intervention over time (B=-.14, SE=.19, p=.47). Moderator analyses indicated higher verbal IQs were associated with social behavior improvements in NETT but not controls (B=-.02, SE=.01, p=.03). Results also show improvements on low-intensity (i.e. more ambiguous) facial emotion identification items in NETT but not controls (estimate =1.1, p<.01, Cohen's d = .56). Poor quality data and compliance issues limited the imaging sample to approximately

half of the behavioral data. In this subset of participants, NETT increases activation in mentalizing regions including the medial prefrontal cortex and temporal pole junction during an averted eye gaze task (Figure 2)[50].

Summary: Our findings demonstrate short-term, specific treatment effects of targeted CBI-based SST curricula of NETT on core social-communication behaviors in youth with ASD. The limited effects on social cognitive processes are supported by two recent Cochrane reviews[14,51].

Intranasal oxytocin for the treatment of children and adolescents with autism spectrum disorder

ClinicalTrials.gov lists 22 clinical studies of intranasal oxytocin (IN-OXT) in ASD which are actively or recently completed recruitment. Of the handful of published findings in this area, the vast majority include small single-dose challenge studies in adults with ASD. Studies suggest a medium effect size on measures of social cognition and social impairment[41]. Overall, IN-OXT was well-tolerated and had a side effect profile including restlessness, increased energy, and increased irritability. The PI and collaborators published on a modified maximum dose-finding and safety study of a 12-week oxytocin daily monotherapy in verbal, youth with ASD[52]. The highest dose evaluated was .4 IU/kg/dose and was well-tolerated. Several measures of social behavior, repetitive behavior, and social cognition demonstrated change at endpoint (12 weeks) as well as at a 3-month follow-up post-discontinuation of IN-OXT[40].

2. Study Aims

2.1. Primary aim

To determine safety, tolerability, and efficacy of integrating OXT dosing within a CBI-SST curriculum for social cognitive impairments in ASD. Eighty youth, ages 8-11 with ASD will be randomized into 1) Integrated Oxytocin and NETT- ASD(ION-ASD) or 2) control condition (facilitated play group) to evaluate: (A) safety and tolerability of targeted, intra-nasal OXT dosing within a 12-session, CBI-SST group curriculum, and (B) group differences on dual domains associated with social learning: social behavior and social cognition. Our working hypothesis, supported by pilot data, is that ION-ASD will be associated with greater differential improvement on dual targets of social behavior and social cognition relative to the control condition.

2.2. Secondary aim

To evaluate the sensitivity of candidate tasks to refine treatment targets and identify predictors of treatment response from ION-ASD. The long-term goal of this aim is to support development of a brain-based behavioral toolbox for use in future intervention research on social learning in youth with ASD. The goals in this proposal will investigate 1) putative treatment targets associated with change following ION-ASD and 2) pre-treatment factors predictive of response to ION-ASD. We predict treatment response in ION-ASD, as defined by a clinical global impression improvement score of <2 (improved to very much improved, and 25% improvement on the Social Skills Improvement System (SSIS), will be associated with improvement on candidate tasks of social-emotional perception, attention to eye region, mental state attributions, and affiliation.

2.3. Exploratory aim

To assess maintenance of treatment effects from ION-ASD. Effects on social-communication behaviors and social cognition will be evaluated at 1- and 3- month post-treatment to evaluate the relationship between changes in domains underlying social learning and durability of treatment effects. This exploratory aim probes evidence indicating persistent effects of oxytocin in pilot studies of multi-dose OXT treatment in adolescents with ASD[40] and translational data indicating long-term developmental effects from peripheral oxytocin administration during early development[35].

At the conclusion of this study, we expect to develop a safe, feasible, and socially valid multi-modal treatment protocol for core social impairments in youth with ASD. The treatment protocol seeks to enhance the automaticity of social learning processes required to negotiate interpersonal interactions. Data will inform selection of outcomes tapping into hypothesized mechanisms and predictors of treatment effects, as well as advance combined psychosocial and pharmacologic treatment approaches to ASD.

3. Study Overview

3.1. Study Design

This is a two-phase, 3 year, randomized, single-blind, parallel group, contact controlled pilot trial of OXT augmentation of CBI groups in youth with ASD. Phase 1 (n=10) is an initial development trial to refine manuals, procedures, and outcome evaluations. Phase 2 (n=70) will evaluate feasibility, safety, and preliminary efficacy of ION-ASD relative to the contact control (i.e. social play group). All participants are expected to be outpatients and will participate in a 12-session group therapy, with a follow-up study visit at 24 weeks. Evaluations will be conducted at four time points: Screening, Baseline, Endpoint (week 12), and Follow-up (1-month & 3-months post-treatment).

Each phase will randomly assign in a 1:1 ratio to either ION-ASD or control in blocks of 10. Efficacy outcomes will be evaluated by both caregiver report and blinded independent evaluators (IE); safety assessments will be conducted by unblinded treating medical clinicians (Medical TC). Caregivers and participants will be instructed to conceal group assignment from IEs. IEs, participants, and caregivers will also be instructed on reporting strategies should group assignment be revealed. Corrective action plans in these scenarios will be employed if necessary and include changing IE raters and flagging data. IEs will be asked to “guess” patient conditions at the end of the trial to evaluate integrity of the blind.

Medical TCs will monitor side-effects using weekly ratings with the participant and caregiver. Clinical IEs will evaluate global functioning and social communication skills. Neurocognitive IEs will conduct blinded neurocognitive assessments (see Appendix A: Time & Events Schedule).

3.2. Study Recruitment/Participants

RUMC is located in close proximity to public transportation and serves families within the city of Chicago and surrounding suburbs. Approximately, 1000 patients with ASD were seen across clinics at RUMC primarily within the departments of psychiatry and pediatrics. We anticipate local interest and the larger clinical pool at RUMC will facilitate similar or better recruitment rates, enabling enrollment of 90 participants within 3 ½ years. Experience in the R21 suggested weekend groups facilitated recruitment and consequently will be offered in this pilot trial.

3.3. Inclusion/Exclusion Criteria

Inclusion Criteria

1. Male or female outpatients, 8-11 years of age inclusive. Given the length of time to commence social skills groups, screening will be permitted starting ages 7 years, 6 months with randomization only occurring in children ages over 8 year inclusive.
2. Meet Diagnostic and Statistical Manual of Mental Disorders, 5th Edition for Autism Spectrum Disorder. DSM-V criteria will be established by a clinician with expertise with individuals with ASD. Best estimate Diagnosis will be reached using DSM-V criteria, the Autism Diagnostic Observation Schedule (ADOS-2) and the Autism Diagnostic Interview (ADI-R), or Autism Screening Interview.
3. Mean score of 9 or less on mentalizing items of Strange Stories Test (Highest possible score = 12, items 21-25, 27) and/or parent reported impairments in perspective taking and theory of mind.
4. Have a Clinician’s Global Impression–Severity (CGI-S) score ≥ 4 (moderately ill) at Baseline.
5. Verbal, Performance and/or Full-Scale IQ ≥ 80.
6. If already receiving stable concomitant medications, have continuous participation during the preceding 30 days prior to Screening, and not electively initiate new or modify ongoing medications for the duration of the study. For serotonergic agents, 6 months on a stable dose is required.
7. If already receiving stable non-pharmacologic educational, behavioral, and/or dietary interventions, have continuous participation during the preceding 3 months prior to screening/baseline, and not electively initiate new or modify ongoing interventions for the duration of the study.
8. Have normal physical examination and laboratory test results at Screening/baseline. If abnormal, the finding(s) must be deemed not clinically significant by the Treating Clinician.

9. Ability to speak and understand English sufficiently to allow for the completion of all study assessments.
10. Ability to obtain written assent from the participant as well as written informed consent from their parent(s)/legal guardian.

Exclusion Criteria

1. Patients born prior to 35 weeks gestational age.
2. Patients with a primary psychiatric diagnosis other than ASD.
3. Patients with a medical history of neurological disease, including, but not limited to, epilepsy/seizure disorder (except simple febrile seizures), movement disorder, tuberous sclerosis, fragile X, and any other known genetic syndromes, or known abnormal brain MRI/structural lesion.
4. Pregnant female patients, sexually active female patients on hormonal birth control and sexually active females who do not use at least two types of non-hormonal birth control.
5. Patients with evidence or history of malignancy or any significant hematological, endocrine, cardiovascular (including any rhythm disorder), respiratory, renal, hepatic, or gastrointestinal disease.
6. Patients with one or more of the following: hemophilia (bleeding problems, recent nose and brain injuries), abnormal blood pressure (hypotension or hypertension), drug abuse, immunity disorder or severe depression.
7. Patients who are currently taking OXT or have taken IN-OXT in the past with no response.
8. Patients who have an Aberrant Behavior Checklist (ABC) Irritability subscale score > 19 at screening/baseline
9. Patients with sensitivity to OXT or any components of its formulation.
10. Patients unable to tolerate venipuncture procedures for blood sampling.
11. Patients in foster care for whom the state is defined as a legal guardian.
12. If they have an arrhythmia present on ECG, that upon consultation with a cardiologist, is deemed to be clinically significant.
13. Patients with any of the following clinical lab results
    a. ALT/AST levels of ≥ 5 times the upper limit of normal, or if clinical jaundice occurs
    b. Sodium levels of > 152 mmol/L or < 128 mmol/L
    c. Potassium levels of > 6 mmol/L in a non-hemolyzed sample
    d. Glucose levels of > 11 mmol/L or < 2.8 mmol/L
    e. Hemoglobin levels of < 100 g/L
    f. BUN levels of > 100 mmol/L
    g. Creatinine levels of > 100 µmol/L
    h. Osmolality levels of > 330 mmol/kg

3.4. Concomitant meds

Concomitant medications and non-pharmacological interventions will be allowed in the study, provided that participants are stable for 3 months prior to screening/baseline on non-pharmacological interventions and 30 days prior to screening/baseline on pharmacological interventions. For serotonergic agents, a minimal of 6 months is required to achieve stable, efficacious dosing in clinical samples. Participants will be strongly encouraged at the time of consent to keep concomitant treatments stable for the duration of the study, until completion of their 3 month follow-up visit. Hormonal birth control is contraindicated in this study.

3.5. Randomization

This pilot study will recruit 90, youth with ASD ages 8-11 years old into a randomized, parallel group design of 1) ION-ASD and 2) control group – facilitated play. A feasibility trial will be conducted with the first 10 participants to refine the protocol including finalizing measures to support Aim 2 (brain-based behavior

toolbox). If substantial protocol revisions are made, modifications to the protocol, consent, and IND will be made before proceeding with recruitment.

Participants will be randomized 1:1 to ION-ASD or control. After eligibility is confirmed and consent is obtained, eligible participants will be assigned a study randomization number according to computer-generated randomization list.

3.6. Blinding/Unblinding

Given the nature of behavioral intervention trials, concealing treatment allocation to participants and treating clinicians is not possible. Independent evaluators (IEs) will conduct blinded CGI ratings at baseline, midpoint, endpoint, and follow-up visits. IEs will be instructed to not ask about treatment allocation and will also be asked to predict treatment allocation for participants at week 12. IEs will not be unblinded at the end of study. If needed, these ratings will be completed via a HIPAA compliant platform (i.e. Zoom).

3.7. Participant Withdrawal Criteria

Participants will be withdrawn from the study treatment if they meet one or more of the following criteria:

1. CGI-I ≥ 6 on two consecutive visits
2. If they become pregnant during the study
3. If they have blood pressure above 99th percentile for age, gender, and height on two consecutive visits (*range in accordance with the Fourth Report on the Diagnosis, Evaluation, and Treatment of High Blood Pressure in Children and Adolescents (U.S. Department of Health and Human Services)[53]
4. If they have blood pressure above the 99th percentile for age, gender, and height on two consecutive visits
5. If they experience an adverse event that is deemed by the Medical Independent Evaluator (IE) to be an unacceptable safety risk
   - If the Study clinician feels it is in the participant's best interest to discontinue the study treatment

4. Study Assessments

4.1. Screening Assessments/Visits

Participants will complete the following assessments and procedures during the screening visit. Screening can be divided into two visits if necessary (estimated time=8-10 hours)

- Provide written informed consent/assent (subject and/or parent/caregiver/LAR).Consenting can take place virtually via a HIPAA compliant platform (i.e. Zoom) if needed to reduce the amount of in person interaction between study team and participant.
  - Parent/guardian and participant will be required to be on the virtual call. Physical signature(s) will be obtained in person during first screening visit before any assessment administration can begin. A physical copy of the signed consent/assent will be given to participant at this time.

- *Demographics:* Information including age, race, ethnicity, family status and income will be collected.

- *Inclusion/Exclusion criteria checklist:* A checklist will be completed to ensure all inclusion and none of the exclusion criteria are met.

- *GUID acquisition form*. This form is designed to obtain all the information necessary in order to assign a GUID such as full name, date of birth, city of birth (as it is written on one's birth certificate).

- *Physical and mental health assessment:* participants suitable for the study undergo comprehensive medical evaluation by the Treating Clinician, including the following:
  - *Family Medical History:* This form will ask for information about genetic, mental health and medical conditions of relatives of the subject.

- *Medical History:* This form will ask about the subject's medical history such as surgeries, medical procedures etc.
  - Females of childbearing potential will be asked to confirm the date of their last menstrual cycle and confirm contraception use if sexually active.
- *Adverse event ratings* systematic elicitation and screening of adverse events will be completed using a structured adverse events rating form. This will also be used at screening and baseline to obtain a comprehensive psychiatric and medical history of the patient; and assess suicidality.
  - *Suicidality Assessment:* The physician will use clinical judgment to determine if the participant understands the concepts of death and making one's self die or hurt. If the participant is deemed able to understand these concepts they will be asked if they have had any thoughts about wanting to die, wanting to hurt themselves, wanting to kill themselves and if he/she has done anything to hurt himself/herself so he/she would die or have done anything to hurt himself/herself for any other reason. If the participant is deemed not to understand these concepts, his/her caregiver will be queried with the thought questions replied by saying or doing anything that makes the parent think the child wanted to die, to hurt himself/herself or to kill himself/herself and asking about suicidal behaviors. The caregiver will also be asked if this is a significant change in severity or frequency from the participant's baseline. The clinician will determine whether the behaviors clearly do not appear stereotypic, might be stereotypic and are clearly stereotypic (e.g. chronic repeated head banging or self-biting).
- *Concomitant Medication Log*: At screening, the physician will make a list of any medications the subject is currently taking (i.e.: on the day of screening or that are prohibited medications and have been discontinued 30 days prior to ensure exclusion criteria). The physician will record any changes to the subjects' psychiatric and non-psychiatric medications from screening throughout the course of the study. PRNs will not be recorded unless:
  - they are taken for a period of 2 weeks or more AND they are taken more than 57% of the time (i.e.: 9 out of 15 days).
    OR
  - the clinician feels there is a compelling reason to document them in which case this can be documented in a note to file. Example: a PRN that is used to address an AE may be noted directly on the AE form for clinician reference in the future, but will not be data entered.
- *Physical examination:* A physical examination including height & weight on standard scales, tanner staging, vital signs (heart rate, blood pressure, and temperature), abdominal palpation to assess for liver enlargement, assessing for decreased tone and postural problems, increased drooling and poor orofacial tone, problems holding head up as well, difficulty with coordination of motor actions.

- *Electrocardiogram*. Trained staff will collect EKG on each subject and this will be read & confirmed by a pediatric cardiologist at screening/baseline for all participants and week 12 (optional for participants in social play condition, i.e. non-medication arm).

- *Safety Labs* (to the extent possible, blood samples will be obtained at approximately the same time of day and under non-fasting conditions). Blood monitoring will occur at screening/baseline and week 12 for safety purposes, and the time between safety labs and the start of the study medication must be not more than 30 days. Total blood collected is 8.5 ML in SST tube at each time point –screening and week 12. screening for all participants and week 12 (optional for participants in social play condition, i.e. non-medication arm).
  - Chemistry Panel: The chemistry panel will include glucose (random, non-fasting), CO2, CL, K, Na, Creatinine, BUN, Mg
  - Routine Hematology
  - Liver Profile: AST, ALT
  - Urinalysis

- Pregnancy test: All menstruating female participants will have a urine pregnancy test completed at screening and week 12 but the pregnancy test can be done at any time throughout the course of the study at the physician's discretion. In case of a positive pregnancy test, the participant will be dropped from the study and referred appropriately.

Routine hematology and blood chemistry and urinalysis will be done before final baseline visit. Due to the nature of ASD, it may be difficult to complete routine hematology and blood chemistry, liver profile and urinalysis at screening. Therefore, these tests can be done during the screening or baseline visit. Participants will not be randomized prior to the treating clinician reviewing and signing off on all results.

- *Diagnostic evaluation.*

  - *Autism Diagnostic Interview, Revised (ADI-R):* The ADI-R is a semi-structured, investigator-based interview for caregivers of children and adults for whom autism or pervasive developmental disorders is a possible diagnosis.. The detailed interview focuses on early development in social and communication and self-help skills of the child, and takes approximately 2 hours to administer. The ADI-R will be allowed to be administered virtually via a HIPAA complaint platform (i.e. Zoom) if needed to reduce in person interaction between clinician and participant caregiver.

  - *Autism Diagnostic Observation Schedule-2 (ADOS-2):* The ADOS is a semi-structured assessment used to assess and diagnose individuals suspected of having autism of varying ages, developmental levels, and language skills (from no speech to verbally fluent). The ADOS2 includes five modules, each requiring just 35 to 40 minutes to administer. The individual being evaluated is given just one module, depending on his or her expressive language level and chronological age. The rater will observe social and communication behaviors during various activities in the appropriate module. This assessment will be video recorded to ensure reliability.

  - Childhood Autism Rating Scale, Second Edition (CARS2) (Optional, alternative to ADOS2). The CARS2 is a well-validated, clinician completed rating form developed to assist in identifying individuals with autism spectrum disorders and distinguish them from individuals with other diagnoses. It includes two versions, the CARS2-Standard Version (CARS2-ST) and the CARS2-High-Functioning Version (CARS-HF). The version rated by the clinician is determined by the child's cognitive level and age. The CARS2 requires the clinician to rate 15 areas of behavior in terms of frequency, intensity, peculiarity, and duration, and yields a quantitative score reflecting a continuum of behavior problems related to autism that can be used in making comparative judgments regarding the level of autism-related behaviors present in a given individual or group. The CARS2 will be administered if in-person testing with the ADOS2 is compromised because of masking restrictions related to COVID-19 precautions.

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  - *DSM-V Checklist for Autism:* The proposed DSM-V criteria for autism will be documented in the form of a checklist for a clinician/physician to complete.

- *Clinical Global Impressions - Severity Scale – Global and Social (CGI-S)*[54]: The CGI-S employs a seven point scale (1 = normal, not at all ill to 7 = among the most extremely ill patients) to determine the subject's initial level of severity of impairment. The rating convention for assigning CGI scores is to use

all available data including direct observation, scales, and subject report to inform clinical judgment. The CGI-Severity will be used for eligibility criteria only.

- *Cognitive testing. The Weschler Abbreviate Scale of Intelligence (WASI-II),Wechsler Intelligence Scales for Children-Fifth Edition (WISC-V), or The Kaufman Brief Intelligence Test (KBIT)* will be administered to evaluate cognitive ability and eligibility. The selection of WASI-II,WISC-V, or KBIT will be determined by the clinician in conjunction with family input and informed by date of last cognitive testing (i.e. tests cannot be administered within 1 year) and family scheduling constraints, The WISC-V is a comprehensive test of intelligence in youth that yields five composite scores: Full, Verbal, Performance, Working Memory, & Processing Speed. An IQ index score of 80 or greater is required on VIQ, PIQ, or FSIQ scales on the WASI-II or WISC-V for inclusion.

- *Strange Stories Task*. These stories assess the ability to interpret nonliteral statements and interpret a speaker's communicative intent. Interpretation of intent in speech is a higher-level perspective taking skill and as such, performance on the Strange Stories Task will be used to evaluate and screen for deficits in complex social cognitive abilities in this study. The six mentalizing items will be used in screening. Individuals scoring 9/12 or less on the mentalizing items will be eligible for the study.

4.2. Efficacy Assessments

Efficacy assessments will be evaluated through questionnaires, direct assessments of social cognition, and questionnaires surveying quality of life, social functioning, anxiety, & repetitive behaviors.

Primary Outcome Measures. This study has two primary outcomes reflective of the dual treatment targets to underlie social learning in school-age children: social cognition and social behavior. Each outcome will be measured by a composite score developed through a factor analysis of measures in each domain in the Seaver-NET RCT[16].

1. Social Behavior Composite. (Baseline, Week 12, Week 16, Week 20)
    a. *Children's Communication* Checklist (CCC)[55]. The CCC[55] is a 70-item caregiver rating scale which includes language scales (e.g. speech, syntax) and pragmatic scales (e.g. scripted language, nonverbal communication, social relations).
    b. *Griffith Empathy Scale*.[56] The Griffith Empathy Measure[0] is a 23-item caregiver rating scale that assesses both affective and cognitive empathy in children and adolescents. The measure was adapted from Bryant's Index of Empathy[32] by Dadds and colleagues.

2. Social Cognition Composite (Baseline, Week 12, Week 16, Week 20)
    a. *Reading Mind in Eyes Test (RMET*). The RMET examines an individual's ability to determine what a person is thinking or feeling based on photographs of the eye region of male and female faces. A computerized, combined child and adult version of the RMET will be used in the trial reflecting clinical measurements employed in a larger, NIH-funded monotherapy trial of oxytocin vs. placebo[57].
    b. *Diagnostic Analysis of Nonverbal Accuracy-2 (DANVA2)*[58]. The DANVA2 examines emotion recognition using both visual and auditory stimuli. Participants were administered all subtests of the DANVA2.

Secondary Outcome Measures. While functional improvements are not expected to be clinically significant within the brief intervention, we will assess changes in social and quality of life metrics as secondary outcomes with the following measures.

1. Global functioning

Clinical Global Impressions – Improvement Scale - Global (CGI-I)[54]. The CGI-I employs a seven point (1 = very much improved to 7 = very much worse) to determine the patient's improvement in response to treatment. The CGI-I has been successfully used as an outcome measure in previous psychopharmacology trials including the RUPP trial of risperidone in children with ASD, and the STAART funded citalopram trial. The rating convention for assigning CGI scores is to use all available data including direct observation, scales, and patient report to inform clinical judgment. CGI-I, Global will be used to evaluate safety, with the requirement that individuals scoring CGI of 6 or greater for 2 or more weeks be discontinued from the study and provided with appropriate referrals. CGI ratings will be made by unblinded clinicians conducting safety evaluations and blinded raters.

Blinded CGI-I ratings will incorporate semi-structured questions to evaluate social conversational and social behavioral skills. Questions will be based on the Bates & Reilly conversational task (Reilly, Bates, & Marchman, 1998). Blinded CGI ratings will be recorded to code narrative discourse and quality in collaboration with the social communication lab of Molly Losh, PhD.Social Functioning

2. Social functioning
    a. *Social Skills Improvement System (SSIS).* The SSIS is an informant-report that evaluates social skills, problem behaviors, and academic competence in youth ages 3-18 years old. The SSIS is a multi-rater with reports provided by caregivers, teachers, and students. The form takes 10-25 minutes to complete. The scale is designed to identify social impairments for intervention development and tracking.

3. Quality of Life
    a. *Caregiver Strain*[59]*.* The CSQ assess family stress and was developed for caregivers of children with developmental disabilities.

<u>Exploratory Outcome Measures</u>

1. Social Function
    a. *Autism Impact Measure*[60]*.* The AIM is a 25-item caregiver report assessment of the frequency and impact of core ASD symptoms in children with ASD. This new measure is designed to assess recall of core symptoms within two-week intervals and will be used as an exploratory measure of changes in core ASD symptom presentation in this study.
    b. *Social Responsiveness Scale*[55]. The SRS has been developed to measure autism related symptoms and focuses more on social function than social cognition. The SRS measures social behaviors such as social awareness, information processing, and social motivation and yields a quantitative score that has been useful in endophenotyping studies of ASD. It is a well validated measure in this population, with an internal consistency 0.93 to 0.97, test-retest reliability of 0.77 to 0.85, and inter-rater reliability 0.74 to 0.91. Validity has been examined in terms of discriminant validity, concurrent validity, structural validation, and factor analytic studies.
    c.
2. Social behavior
    a. *Pervasive Developmental Disorders Behavior Inventory-Screening Version*[61]. The PDDBI-SV assesses both maladaptive and adaptive social behaviors presenting in children with ASD. The PDDBI-SV yields a measure of social impairment (SOCDEF raw & T scores). This is a co-primary outcome measure in a larger NIH-funded oxytocin monotherapy trial[57] and is included in this study to help provide consistency across studies in measuring critical social behavior outcomes.
    b. *Aberrant Behavior Checklist (ABC).* The ABC focuses on problem behaviors in 5 domains: irritability, attention, repetitive behaviors, unusual speech, and social withdrawal (SW). The ABC-SW is a co-primary outcome in the NIH-funded OXT monotherapy trial and will be used in this study to provide methods for comparison across trials.
    c. *Sensor-based measures of social-communication*. This assessment will utilize a computer-based package to automatically detect target social behaviors including eye-gaze, facial

expressions, use of emotion words, quality of speech, and gestural communication. The package includes three system components: wearable eye-tracking device (e.g. Tobii glasses), 3-D cameras, and audio recordings of voice recognition software.

3. *Social cognition*. Social cognition requires distinct but related mental mechanisms including perceiving people and actions, perceiving emotional states of others, evaluating beliefs and intentions, sharing attention, and reflecting others' beliefs and states (Perlman, Vander Wyk, & Pelphrey, 2012). Exploratory outcomes of specific social cognitive domains will be tested and and will inform development of a brain-based behavioral toolbox for use in future trials. All measures will be administered on a Tobii T-60 eye-tracker.
    a. *Biological motion*. Detecting human forms and predicting intent of actions are basic social information processing skills supporting social cognition, and have been linked to activation of the superior temporal sulcus. This study will utilize a point-light task in which forms are designed either in human or scrambled forms. Outcomes will evaluate differential attention to human vs. scrambled motion related to treatment.

    b. *Emotion Recognition*. The *Geneva Multimodal Emotion Portrayals (GEMEP)* is a 15 minute assessment of emotion recognition in faces, voices, and gestures will be evaluated using short-video clips extracted from the GEMEP database (Banziger, et al, 2007).

    c. *Mental State Attributions in others*. Mental state attributions of self are an advanced, critical social cognitive process. The [62,63] has been associated with neural correlates in the medial prefrontal cortex (mPFC), fusiform gyrus, and middle temporal gyrus. Performance on will be compared with mental state attributions in others as measured by the RMET and emotion & cognition ratings on narrative ability tasks (see below).
    d. *Affiliation*. OXT has consistently been shown to target affiliative tendencies across species.
        i. The *Trustworthiness of Face*[64] task is a measure of reaction to positive emotions associated with neural activation of the amygdala. Evaluating trust has been associated with theory of mind skills in a non-linear fashion in psychiatric and healthy populations[65].
    e. *Social motivation*. OXT has also been hypothesized to increase the reward value of social stimuli potentially through increasing the salience and incentive value of social stimuli. A handful of studies have demonstrated impact of single-dose OXT challenges on social reward processes in ASD[26]. An adapted version of the economic trust game[65] will be evaluated as a potential measure of social reward and affiliation in this trial.

4. Anxiety
    a. Multidimensional Anxiety Scale for Children-Second Edition[68]. The MASC-2 is a multi-rater assessment of anxiety-related symptoms in youth ages 8-19 years old used for identification, diagnosis, treatment planning, and monitoring.

5. Narrative communication
Narrative ability: a complex social-communication skill tapping into social-emotional, cognitive, and linguistic abilities. Narrative ability will be assessed using two tasks adopted from the social communication lab of Molly Losh, PhD. Tasks will be administered on the Tobii T60 eye tracker and will be analyzed for gaze patterns to social vs. non-social stimuli as well as several language variables.

    a. *Adapted Thematic Apperception Task*[71]. An adapted TAT will be used within a Tobii T60 eye-tracker to evaluate attention to emotional stimuli as well as narrative story telling abilities using ambiguous, emotionally evocative stimuli. Latent semantic analysis (LSA) is a quantitative assessment of narrative skills and will be used to evaluate potential to serve as targeted measure of change in complex, narrative story-telling skills.

6. Repetitive Behaviors
    a. *Repetitive Behavior Scale-Revised (RBS-R)*[72]. The RBS-R was developed to capture the breadth of repetitive behaviors that are specific to autism and is a parent report measure. In particular, the RBS-R consists of 43-items that tap six repetitive behavior subtypes: Stereotyped, Self-injurious, Compulsive, Ritualistic, Sameness, and Restricted Interests.

4.3. Safety Assessments

Adverse events will be systematically reviewed by study clinician and each study will have a back-up physician to assess safety. The study clinician will also do a general inquiry about health, other medications, and visits to medical care providers since the last visit. The following safety measures will be completed either in-person or may take place virtually via a HIPAA compliant platform (i.e. Zoom) if needed to reduce the amount of in person interaction between study team and participant.

- *Clinical Global Impressions – Improvement Scale – Global (CGI-I).* The CGI-I employs a seven point (1 = very much improved to 7 = very much worse) to determine the patient's improvement in response to treatment. The CGI-I has been successfully used as an outcome measure in previous psychopharmacology trials including the RUPP trial of risperidone in children with ASD, and the STAART funded citalopram trial. The rating convention for assigning CGI scores is to use all available data including direct observation, scales, and patient report to inform clinical judgment. CGI-I, Global will be used to evaluate safety, with the requirement that individuals scoring CGI of 6 or greater for 2 or more weeks be discontinued from the study and provided with appropriate referrals.
- *Adverse event rating form*
  This is a semi-structured interview that has been modified to assess OXT specific side effects for a recently completed pediatric trial of sustained oxytocin treatment in ASD [52]. It contains a general inquiry, drug-specific queries, as well as several questions about daily activities (e.g., sleep, appetite, energy level, bowel and bladder function). This instrument has been widely used in other autism related clinical trials. In addition to these measures, vital signs (heart rate, blood pressure) will be measured biweekly at the following visits: screening, baseline/week 1, week 3, week 5, week 7, week 9, week 11, week 12, week 16, and week 24 (or another biweekly schedule depending on start days/dates) Standardized procedures will be used for all physical measurements. We will attempt to do vital signs following a 4 minute period of rest, with the participant seated. Height and Weight will be taken at screening, baseline, Week 12, and Week 24 with all outer wear and shoes removed at every visit.

5. Study Treatment

5.1. Treatment Conditions

For both conditions, virtual group formats have been created and will involve adaptations of child activities to virtual sessions. Parent groups will be held prior to groups instead of concurrently to allow added staffing required to prompt and maintain engagement during virtual interventions. If groups are held in session during COVID-19 pandemic conditions, clinic policies for sanitation, PPE, and distancing will be followed for both parent and child groups.

Cognitive Behavioral Intervention for social cognitive deficits: ION-ASD

ION-ASD will be adapted from the manual established in the prior RCT of Seaver-NETT[16]. The NETT curriculum is manualized and anchored in CBI strategies, such as problem identification, affective education, performance feedback, and weekly homework activities to facilitate generalization. Each child group session follows a consistent written schedule; uses structured teaching to break down important elements of gestural

communication, nonverbal synchrony, emotional expression, and interpretation of intent; and introduces games and activities to practice each skill. A reinforcement system is used to reinforce participation and spontaneous skill use. The approach to the parent group is psychoeducational with a focus on reviewing skills, rationale for teaching target skills, homework, progress or obstacles, and planning for generalization. Parent groups will also be used to schedule individual meetings with study physicians to review safety and other concerns related to study drug administration. The first three sessions focus on nonverbal communication, and include activities that promote active listening and social referencing (i.e., following eye gaze, gestural communication). The second four-week module targets emotion recognition in the self and others, including a review of basic and complex emotions, and a focus on increasing children's lexicon of emotion words. Children are encouraged to use an awareness of their own emotions to empathize with others and find appropriate ways to respond. The last four-week module focuses on theory of mind, including and taking someone else's perspective and understanding the communicative intent behind nonliteral remarks, building upon skills taught in the emotion recognition module.

Oxytocin will be administered in an intranasal format using the synthesized peptide oxytocin in a solution formulated to promote absorption through the nasal mucosa. Intranasal oxytocin (OXT) will be administered at a dose of 24 IU (3 puffs per nostril), four times per week during the 12-week intervention. The first dose will be administered before the group session at week 1. One administration will occur 30 minutes prior to the weekly social skills group therapy session, and the other three administrations will occur 30 minutes prior to at-home homework/practice sessions. Parents and participants will undergo training modeled after SOARS-B procedures (e.g. parent medication administration handout, in-vivo practice with placebo). If groups are conducted virtually, medication will be mailed to families. Regardless if the group is virtual or in-person participant and guardian will be required to meet with study physician and study PI to review how to properly administer the medication. This review can occur virtually, if needed.

Control Condition: Facilitated Play

The facilitated play condition was manualized for use in the R21 and is a child-directed social play group. Therapists tailor available activities based on interests and abilities of group members. Standard educational practices for children with ASD including visual supports, schedules, and short-directed statements are also used. The concurrent parent group is supportive in nature. Groups will be led by doctoral level clinicians trained in eclectic therapeutic approaches (e.g. supportive psychodynamic). The treatment manual described methods to tailor child-directed play based on the interests and abilities of group members. Therapists established "stations" to support object play (e.g. legos, board games), motor/tactile (e.g., drawing), and dramatic play. The treatment manual also provided instruction on use of reflective statements to foster communication with the child and between peers. Each group session began with a review of a posted visual schedule, a check-in circle, activity-time, and wrap-up. The 30-minute concurrent parent group was supportive in nature and facilitated by the lead therapist.

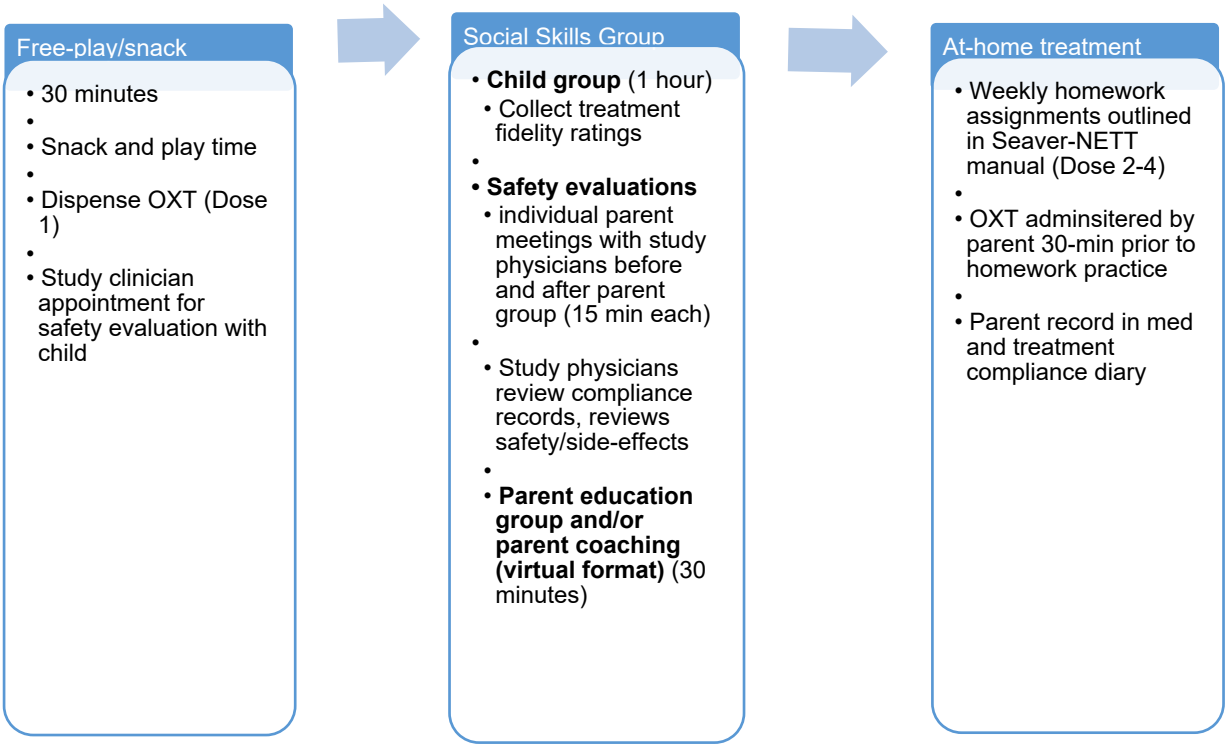

Figure 3. ION: Weekly study procedures

5.2. Study Procedures

Screening Visit(s)
Participants and their parent(s)/legal guardians will come in to sign informed consent and meet with a study clinician to establish eligibility. The following measures will be obtained: a diagnostic assessment, including the ADOS-2 (if required) and DSM-V interview, the ADI–R (if required), a cognitive assessment (WASI-II, KBIT, or WISC-V,if required), the ABC, the CGI-S (Global and Social), the AE rating form, height, weight, vital signs, temperature, a physical examination, review of medical and psychiatric health and concomitant therapies, and a pregnancy test (if applicable). The ADOS-2 (if required) will be video recorded. The screening visit is anticipated to take approximately 4-6 hours to complete. The visit can be split into two visits if this is better for the participant and may be combined with the baseline visit to facilitate scheduling and compliance with visit windows. If required, the following measures can be completed virtually: consenting, DSM-V interview, ADI-R, CARS2, the ABC, the CGI-S (Global and Social), the AE rating form, review of medical and psychiatric health and concomitant therapies.

ECG and safety lab assessments will be completed no more than 30 days before the first dose of medication is administered.

In addition, a physical exam will be conducted, and height, weight, temperature, and vital signs will be obtained. At the baseline visit, the participant will also be randomized to receive either ION-ASD or control (social play), and the research pharmacy will be notified by research staff to dispense a two week supply of study drug to families assigned to ION-ASD. Blood work will be collected prior to participants taking their first dose of study medication. The randomization form will be developed prior to screening/recruitment for each cycle and present randomization schema for 10-12 participants each time. Coordinators will open the Randomization form after the completion of baseline assessments.

Screening and baseline procedures may be combined to accommodate participant schedules.

Baseline Visit(s)
Participants entering the study will undergo the baseline assessments, which are anticipated to take approximately 6-10 hours to complete no more than 21 days from the last screening visit. The visit can be split into as many as three visits if this is better for the participant. The following measures will be completed: the CGI-S (Global and Social), AE rating form, the RMET, the CASI-5, the RBS-R, the SRS, the BASC-3, the PDDBI-SV, the Griffith Empathy Scale, the Children's Communication Checklist, the Caregiver Strain, the DANVA2, the TAT, and the Test, GEMEP emotion recognition test, and sensor-based social-communication monitoring tests..

Vineland Scales of Adaptive Functioning (3rd edition): The VABS-3 is a survey administered to a parent or caregiver is organized around four Behavior Domains: Communication, Daily Living Skills, Socialization, and Motor Skills. The VABS-3 will take the parent/caregiver/LAR approximately 20-60 (average 30) minutes to complete, depending on the subject's age and level of functioning.

Weekly Visits (1-11)
Interventions are delivered in 12-session modules occurring on a weekly basis. Groups will commence when all participants in a cycle have completed baseline evaluations and within 21 days of participant's last baseline visit. Participants will receive group treatment conditions as described in Section 5.1 (Treatment Conditions). Participants randomized to ION will also be prescribed 24 IU (3 puffs/nostril) of intranasal oxytocin 4 times/week to be administered by the parent 30-minutes prior to homework practice (3 x/week) and 30 minutes prior to each weekly group session.

<u>Safety ratings during group: Weeks 1, 3, 5, 7, 9, 11 (or biweekly schedule depending on group start day/date)</u>

Vital signs, temperature, and AE ratings will be completed in 6/12 sessions by a licensed physician on participants in all groups. In addition, the CGI-I (Global and Social) will be completed by the Study clinician. At

Week 6, the CY-BOCS, the CASI-5, the RBS-R, the PDDBI-SV, the Griffith Empathy Scale, the Children's Communication Checklist, the Caregiver Strain and the ABC will be completed. In addition, a two week supply of study drug will be dispensed at weeks 1, 3, 5, 7, 9, 11. Unplanned visits will be permitted if there are any concerns about AEs. Safety visits are expected to take 15-20 minutes.

Week 6 (Midpoint)
Caregiver reports on the ABC, PDDBI-SV, Griffith Empathy Scale, Children's Communication Checklist, CASI-5, Caregiver Strain, Vineland 3, SRS-2, RBS-R, and AIM will be collected at midpoint along with CGI-I ratings (blinded). Ratings may be conducted +/- 7 days from week 6 group depending on family schedules.

Week 12 (Endpoint)
The end of treatment visits occurs at Week 12 (to take place within ± 7 days from the target date). At these visits, blood work, vital signs, temperature, height, weight, and an ECG will be completed. In addition, participants will undergo a series of assessments, which are anticipated to take approximately 4-6 hours to complete. The visit can be split into as many as three visits if this is better for the participant. The following measures will be completed: the CGI-I (Global and Social), the AE rating form, the Bates & Reilly Conversational Task, the CY-BOCS, Let's Face It, the RMET, the CASI-5, the RBS-R, the SRS, the ABC, the BFRT, the BASC-3, the PDDBI-SV, the Griffith Empathy Scale, the CCC, the Caregiver Strain, the DANVA2, the TAT, , and the GEMEP emotion recognition test, and sensor-based social-communication monitoring tests.

Week 16 & Week 24 (Maintenance visits 1 & 2)
There will be an additional follow-up appointment 12 weeks (± 7 days) after the participant stops taking the study drug. This visit is primarily to examine safety of OXT discontinuation, and look for possible maintenance of any favorable effects of ION-, and longer term safety. The assessments completed during this visit are identical to the Week 12/end of treatment visit.

Termination Visit: A participant may voluntarily withdraw from the study at any time. The PI has the right to discontinue a subject from this study or withdraws a participant from the study at any time. Every effort will be made to obtain information on subjects who withdraw. Participants who discontinue study drug prematurely will be asked to return to the clinic and complete all end of study safety lab work (week 12 blood work). Participants will also be asked to complete all endpoint study procedures on their final visit before withdrawal to allow for true intent to treat analysis.

Missed Visits and Visit Windows

Subjects that miss a weekly group meeting will be asked to come in +/-2 days from the scheduled day to complete safety assessments. In the event the subject is unable to come in, we will ask the family to follow up with the study physician via phone to complete safety assessments.

Video Recording & Electronic Assessment

The ADOS-2 will be recorded during the study to ensure reliability. Additionally both the facilitated play and cognitive behavioral social skills groups will also be video recorded.

Additionally some tasks will be presented on a computer in electronic format including: DANVA2, Reading Mind in Eyes, TAT and GEMEP Emotion Recognition Test. Data will be captured, labeled with participant ID, and stored on the encrypted Rush network. The following assessments will be video recorded and eye tracking data will be recorded using the Tobii T-60 eye-tracker: CGI-S, CGI-I, Reading Mind in Eyes, DANVA2,TAT, and . Recordings will be used to analyze response time, attention to social regions of stimuli, and engagement with the examiner. The recording will be labeled with a code number, stored electronically on the encrypted Rush network in a password protected folder and destroyed when the study is completed. Sensor-based measures of social-communication will utilize a computer-based package and three system components: wearable eye-tracking device (e.g. Tobii glasses), 3-D cameras, and audio recordings of voice recognition software to automatically detect target social behaviors including eye-gaze, facial expressions, use of emotion

words, quality of speech, and gestural communication. These tools will be used to record target behaviors during CGI ratings by the Independent Evaluator at BL, Weeks 2, 6, 12 and, 16- & 24- week follow-up. Data recorded from these measures will be labeled and stored electronically on a secure server.

5.3. Treatment Fidelity and Compliance

Training
Licensed doctoral-level therapists and assistant therapists will be trained by the treatment developers (Drs. Latha Soorya & Drs. Danielle Halpern) using the manuals developed for Seaver-NETT. Training procedures include manual review, watching videos and weekly supervision meetings with the developers.

Safety evaluations will be conducted by a Medical Independent Evaluator (IE), i.e. licensed child and adolescent psychiatrists and/or doctoral-level nurse practitioners, with experience using the AE rating form. Training on study procedures for all clinicians will occur through a protocol review including safety evaluations and specific modifications to the AE rating form for OXT treatment. AE data will be collected across all groups. In addition, parents will be trained to administer OXT in the NETT-OT condition during the baseline visit using written instructions and in-vivo practice with placebo. Procedures are adopted from the on-going SOARS-B trial.

Neurocognitive Independent Evaluators (IE) will be trained on study assessments including the following: Reading Mind in Eyes Test (RMET), Diagnostic Analysis of Nonverbal Accuracy-2 (DANVA2), Test, Trustworthiness of Faces test, and the adapted Thematic Apperception Test.

Fidelity and Compliance
Behavioral intervention: Fidelity

Treatment fidelity ratings for the behavioral intervention component will occur weekly through use of treatment fidelity checklists completed by raters blind to study hypotheses. Checklists developed in the Sever-NETT RCT will be used in the current trial and are available for both behavioral intervention conditions, NETT and facilitated play. Raters are trained to 80% reliability prior to independent coding. Feedback is provided on a weekly basis to lead therapists in each condition.

Medication Compliance

Compliance in ION will be measured by providing a diary where the participant and/or parent(s)/legal guardian will note every dose after they take it, at what time they took it, and whether they have any comments. Homework and behavioral rehearsal of assigned weekly activities will also be recorded on the compliance log. In the case of non-compliance (i.e. 1 of 4 weekly doses missed) the Study clinician will review administration of medication and schedule of doses with the participant and will problem solve with the participant to improve compliance at the next visit. Compliance logs and homework can be completed via paper form or virtually on Google Classroom.

In addition, compliance will be assessed research staff who will weigh the bottles each time they are returned. At the end of the study, research staff will assess compliance by comparing the weight of the bottle before dispensing to the weight measured after the bottle was returned to the study team.

6. Laboratory Evaluations

6.1. Clinical Laboratory Evaluations

Clinical laboratory evaluations will be conducted at screening and Week 12.

- Total blood collected is 8.5 ML in SST tube at each time point –screening and week 12.
    - Chemistry Panel: The chemistry panel will include glucose (random, non-fasting), CO2, CL, K, Na, Creatinine, BUN, Mg

- Routine Hematology
- Liver Profile: AST, ALT
- Urinalysis

7. Ethical Considerations

7.1. Ethics Approval

This protocol and any amendments will be submitted to the Rush Institutional Review Board (IRB) for formal approval of the study conduct.

7.2. Informed Consent

Consent will be obtained by the PI or another delegated research staff member prior to participation in the study and conduct of any study-specific procedures, but after the study has been described and all information given to the participants and their caregivers, as per FDA and IRB policy. This will be done on the first day of screening. It will be the responsibility of the PI to ensure that potential participants understand the extent of their role in the research. Specifically, the written consent and child assent documents will be read through with all potential participants and his/her parent(s)/legal guardian(s), and focus will be placed on potential risks and benefits associated with study participation as well as any alternatives to participating in the research. Most notably, participants and parent(s)/legal guardians will be informed that a participant's opportunity to receive treatment will not depend upon research participation. In addition, the PIs will try to foster an open exchange of information, encouraging potential participants and their families to discuss study particulars and ask questions prior to research involvement; to take a copy of the consent form home to discuss with family and friends, if desired; and to continue asking any questions that might arise during participation. Details on written consent and child assent forms will be presented in simple language approved by the IRB. All key personnel are PHIPPA trained. For children and adolescents not capable of consenting, we will obtain written consent from the parent(s)/legal guardian, and assent from the child. Both consent and assent are required for participation in the study if the participant is unable to consent but able to provide assent. Informed consent review can be completed virtually, as needed, however informed consent signature can only be obtained in person.

7.3. Confidentiality

A site and participant number will be assigned for each participant. The database will only be identified by participant number and will not contain any personal identifiers. The results of all assessments will be kept strictly confidential unless an appropriate written release of information is provided by the participant and/or parent(s)/legal guardian.

The research team will do everything possible to keep others from learning about the participant's involvement in this research study. Each participant will be assigned a sequential identification number, and these numbers, rather than names, will be used to collect, store, and report participant information.

Data from this study may be submitted to the National Database for Autism Research (NDAR). NDAR is a computer system run by the National Institutes of Health that allows researchers studying autism to collect and share information with each other. During and after the study, the researchers will send information about your health and behavior, to NDAR. Before sending data to NDAR, however, information such as name, address, and phone number, will be removed and replaced with a code number. Other researchers nationwide can then file an application with the National Institutes of Health to obtain access to your study data for research purposes. Experts at the National Institutes of Health who protect health and science information will look at every request carefully to minimize risks to your privacy.

Data from this study will be shared with collaborators at Northwestern and Eotvos Lorand University in Hungary working on developing assessment tools designed to reduce time, burden and improve detailed analysis of treatment effects from this treatment.

8. Safety Reporting

8.1. Adverse Events (AEs).

ICH E6 defines an AE as any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product regardless of its causal relationship to the study treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding that is not directly obtained for purposes of the study), symptom, or disease temporally associated with the use of medicinal (investigational) product. The occurrence of an AE may come to the attention of study personnel during study visits and interviews of a study subject presenting for medical care, or upon review by a study monitor. All AEs including will be captured on the appropriate source documentation, the structured AE rating form. Information to be collected includes event description and clinician's assessment of severity.

8.2. Treatment Emergent Adverse Events (TEAEs).

Safety analysis will focus on TEAEs. Medical and behavioral conditions will be evaluated at screening and baseline, and will only be considered TEAEs if the severity increases significantly after a participant has completed a treatment session. All TEAEs will be documented during the course of the study regardless of relationship to treatment. All TEAEs will be followed to adequate resolution. If a TEAE is also a serious adverse event (SAE) and/or Unexpected Problem (UP), the SAE or UP forms will also be completed. If a TEAE is both a SAE and UP, only the SAE form and the AE FORM will be completed.

8.3. *Suicidality Assessment*.

Suicidality assessments will be completed for each participant during visits with Study clinicians. The clinician will determine if the participant understands the concept of death and making one's self die or hurt. If the participant is able to understand these concepts, they will be asked if they have any thoughts about wanting to die, wanting to hurt themselves, wanting to kill themselves, and if s/he has done anything to hurt himself/herself so s/he would die or have done anything to hurt himself/herself for any other reason. If the participant is deemed not to understand these concepts, his/her caregiver will be asked the above questions. The caregiver will also be asked to rate if this is a significant changes in severity or frequency from the participant's behavior at baseline.

8.4. Safety Reporting

The Study clinician will discuss events with the patient and caregiver; review the participant's history of any similar behaviors or suicidal ideation, and the caregiver's perception of any change in severity of frequency of the behavior. S/he will review alarm values of labs and ECGs and serious adverse events within 3 working days of their reporting and who will review all new treatment emergent adverse effects of moderate or greater severity three times a year prior to DSMB meetings. Cases may also be reviewed by the DSMB.

8.5. Adverse Event Ratings

Serious Adverse Events (SAE).

An SAE is defined as an AE that meets one of the following conditions:

- Death during the period of protocol-defined surveillance
- Life-threatening event (defined as a subject at immediate risk of death at the time of the event)
- An event requiring inpatient hospitalization or prolongation of existing hospitalization during the period of protocol defined surveillance
- Results in congenital anomaly or birth defect
- Results in a persistent or significant disability/incapacity
- Any other important medical event that may not result in death, be life threatening, or require hospitalization, may be considered a serious adverse experience when, based upon appropriate medical judgment, requires medical or surgical intervention to prevent one of the outcomes listed above. Examples of such medical events include allergic bronchospasm requiring intensive treatment in

an emergency room, blood dyscrasias or new onset of convulsions that do not result in inpatient hospitalization.

All SAEs will be:
•recorded on the appropriate SAE CRF
•followed through resolution by a study clinician
•reviewed and evaluated by a study clinician

Unanticipated Problems (UP). We will consider unanticipated problems involving risks to subjects or others to include, in general, any incident, experience, or outcome that meets all of the following criteria:

•unexpected (in terms of nature, severity, or frequency) given (a) the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document; and (b) the characteristics of the subject population being studied;
•related or possibly related to participation in the research (in the guidance document, possibly related means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and
•suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

Severity of Event. All AEs will be rated by the Study clinician using a protocol defined grading system. For events not included in the grading system, the following guidelines will be used to quantify intensity.

•*Mild*: events require minimal or no treatment and do not interfere with the subject's daily activities.
•*Moderate*: events result in a low level of inconvenience or concern with the therapeutic measures. Moderate events may cause some interference with functioning.
•*Severe*: events interrupt a subject's usual daily activity and may require systemic drug therapy or other treatment. Severe events are usually incapacitating.
•*Life-threatening*: Substantial risk dying or requires intervention to prevent death.
•*Death related to AE*: Subject died as a result of the event, self-explanatory.
Changes in the severity of an AE will be documented to allow an assessment of the duration of the event at each level of intensity to be performed. Adverse events characterized as intermittent require documentation of onset and duration of each episode.

Relationship to Study Treatment. Study clinicians will assess and document whether the AE is related to treatment condition. However, the relationship to study treatment will not be a factor in determining whether or not an AE is reported in this study. If there is any doubt as to whether a clinical observation is an AE, the event will be reported. All AEs must have their relationship to study product assessed using the terms: associated or not associated. In a clinical trial, the study product must always be suspect. To help assess, the following guidelines are used.

•*Certain*: The adverse event and administration of the study drug are related in time, and a direct association can be demonstrated.
•*Probably*: The adverse event and administration of the study drug are reasonably related in time, and the adverse event is more likely explained by the study drug than other causes.
•*Possible*: The adverse event and administration of the study drug are reasonably related in time, and the adverse event can be explained equally well by causes other than the study drug.

•*Unrelated*: The adverse event is clearly explained by another cause not related to the study drug.
•*Unknown or not applicable*

Reporting Procedures: Serious Adverse Events & Unanticipated Problems
Any AE considered serious by the PI or Sub-Investigator (Sub-I) or which meets the aforementioned criteria must be submitted on an SAE form to the Rush IRB. Additionally, any event which meets the aforementioned criteria for an unanticipated problem also needs to be reported to the Rush IRB. It should be reported to the following numbers. It is preferable to have a written report (fax or email) for documentation purposes.

IRB Contact Information:

Research and Clinical Trials Administration
707 S. Wood, Lower Level
Chicago, IL 60612

IRB Telephone: (312) 942-5498
IRB Fax: (312) 942-2874

The study clinician will complete and submit an SAE or a UP Form within the following timelines:

- All deaths and immediately life-threatening events, whether related or unrelated, will be recorded on the Serious Adverse Event Form and sent by fax to the medical monitor within 7 business days of site awareness.
- Serious adverse events other than death and immediately life-threatening events, regardless of relationship, will be reported by fax within 7 business days of site awareness. The study clinician will complete and submit SAE and UE forms within 7 business days.
- Unanticipated problems involving risk to subjects and subject complaints will be reported to the Rush IRB within 10 days of site awareness.
- Other supporting documentation of the event may be requested and should be provided as soon as possible. All SAEs will be followed until satisfactory resolution or until the PI or Sub-Investigator deems the event to be chronic or the patient to be stable.

Reporting to Other Regulatory Bodies

- Medical Monitor: The study team will report all SAEs, UPs, and other reportable events to the medical monitor within 14 days and receive acknowledgement that she has reviewed them.
- DSMB: The study team will report SAEs, Ups, and other reportable events that are considered possibly related to the study treatment to the DSMB within 14 days of being made aware of their occurrence.
- FDA: If the event meets FDA reporting criteria (below), the IND holder or investigator will follow specified reporting procedures. Reportable events will meet the following criteria:
    - Suspected Adverse Reaction: meaning there is reasonable probability that the drug caused the event. Meaning there is evidence to suggest a causal relationship between the drug and adverse event.
    - Serious (see section above for definition)
    - Unexpected (see section above for definition)

Timelines for reporting to the FDA

- Unanticipated fatal or life threatening adverse events (7 days)
- Unanticipated non-fatal/non-life threatening events (14 days)

Other reportable events

Other reportable will be reported during the annual review. Other reportable events with shorter reporting intervals include pregnancy of subject or the subject's sexual partner and serious intentional self-harm or serious suicidal ideation.

- In case of a pregnancy, investigators will follow the pregnancy until completing (either termination or birth). The baby will be followed until s/he is 1 month old to assess and record any medical complications. The initial report will be sent to the IRB within 7 days of the study team's awareness.
- In case of serious intentional self-harm or serious suicidal ideation, the physician/clinician should take necessary steps to ensure the safety of the participant including involuntary hospitalization (if needed). This should be reported (on the appropriate source document) to the IRB within 7 days of the study team's awareness.

Stopping procedures.
Subjects may be withdrawn from the study for any of the following reasons:

- At the participant or guardian's request
- If moderate or severe AEs occur that cannot be addressed by the PI, Study clinician or medical monitor
- If a subject is worsening clinically (i.e. have CGI-I = 6 (much worse) or 7 (much worse)) and there has been a return visit/call within 1-2 weeks to revisit/re-evaluate the CGI-I. If subject remains at a CGI-I = 6 or 7, the case can be discussed with the medical monitor to get approval for the participant to continue
- Study noncompliance
- Study physician/clinician discretion

8.6. Safety Oversight

Data Safety Monitoring Committee (DSMC)
A local DSMC was created to ensure that the safety of study participants is protected and that the scientific goals of the study are being met. To support those purposes, the DSMB will: 1) review any proposed amendments to the study protocol, 2) perform expedited monitoring of all SAEs, 3) perform on-going monitoring of drop-outs and non-serious adverse events; 4) determine whether study procedures should be changed or the study should be halted for reasons related to the safety of study subjects, and 5) perform periodic review of the completeness and validity of data to be used for analysis of safety & efficacy. The DSMB will also ensure subject privacy and research data confidentiality. The DSMB will consist of three members with experience in conducting clinical trials for childhood psychiatric and neurodevelopmental disorders, expertise in biostatistics, and a thorough knowledge of clinical trial ethics and human protection issues. The Rush-based DSMB is be led by Niranjan Karnik, MD a child and adolescent psychiatrist and will also include Elizabeth Berry-Kravis, MD, a pediatric neurologist with extensive clinical trial experience in neurodevelopmental disabilities and Lou Fogg, PhD, lead biostatistician in the Department of Nursing at Rush.

Cardiac Monitoring Plan
Based on the patient's age, gender, and height percentile, we have adapted blood pressure parameters where blood pressures above or below the set threshold will prompt the PI to consider doing an EKG. These parameters were derived from the National High Blood Pressure Education Program Working Group on High Blood Pressure in Children and Adolescents[73]. We have likewise adapted parameters from Pediatric cardiology for practitioners[74] and Normal ECG standards for infants and children[75] to establish heart rate guidelines where patients whose heart rate is above or below these thresholds will be considered for an EKG at every visit. Finally, we will incorporate clinical signs and symptoms of cardiac risk into our assessment with the vital sign measurement and specifically query for the presence of syncope, dizziness, palpitations, shortness of breath,

and bradycardia or tachycardia. The clinical parameters will be incorporated into the AE form. The specific parameters for heart rate, blood pressure are located in Appendix A. We believe, given the low cardiac risk of oxytocin, that careful monitoring of vital signs and clinical signs and symptoms at screening/baseline, biweekly during group, week 16, and week 24. Data Management & Statistical considerations

8.7. Site Monitoring and Data Management

The PI and Project Manager will monitor the study to ensure human subject protection, study procedures, study intervention administration, and data collection processes are maintained and meet regulatory guidelines. The Project Manager will monitor the database, with the PI and statistical consultant, in order to assess quality and control issues. The Project Manager will also review regulatory documents for completeness and accuracy (e.g. delegation log, 1572s, general and IRB correspondence, lab values). The Project Manager will also review case report forms (CRFs) to ensure data are complete and accurate, issue queries for data management team, and ensure queries are resolved. The database will be entered and then checked with CRFs by separate members of the data entry team. Data will also be collected on videos, eye-tracking software from wearable camera glasses (e.g. Google glasses), and 3-D cameras to detect motion. These data are kept on secure, local Rush servers and shared via encrypted, HIPAA compliant websites.

8.8. Statistical analysis

Specific Aim 1 & Exploratory Aim 1: Safety and tolerability will be analyzed by analyzing for group differences on total scores and domains of the AE form and satisfaction surveys respectively. Efficacy analysis will be follow analytic procedures used to derive social behavior and social cognition composites for the Seaver-NETT RCT[16]. First, a principal components analysis with varimax (orthogonal) rotation will be conducted on subscales of the primary social behavior measures - Griffith Empathy Scale and CCC-2; and raw total scores on primary social cognition outcomes – RMET and DANVA2. The PCA is used to develop composite scores in a field with no viable outcomes for higher-order core social impairments as well as to minimize floor and ceiling artifacts, effects of variability in response, and other sources of measurement error. Variables with high factor loadings for subscales will be entered into composite score development for the social cognition and social behavior composites. Missing, invalid, and/or incomplete data (i.e. more than 30% items missing when not pre-defined by scale) will be excluded from the factor analysis.

A linear mixed model analysis will be conducted on all participants with at least two of four available time points (Wk 0, Wk 12, 1-mon, 3-mo). Composite scores of co-primary outcomes social behavior and social cognition will be used as the response variable; treatment condition, time, and condition * time interactions will be covariates. Verbal IQ will also be entered into the model in exploratory moderator analyses. We will estimate effect sizes (Cohen's d) and confidence intervals (95%) acknowledging the likelihood that analyses may not permit definitive conclusions given the likely size of the confidence intervals.

8.9. Sample size considerations

Based on data from the NETT trial, published trials in sustained OT treatment[54], and single-dose challenge studies in autism[49] we expect our sample size (n=25/group with 5% attrition) to yield 80% power to detect a difference in means of 0.40, assuming a standard deviation of differences of 0.670, with a 0.050 two-sided significance level. Although potentially underpowered, we believe data from this trial will provide important information on safety, tolerability, and treatment targets from a novel, multi-dosing OT-combined treatment strategy. Specific Aim 2 (treatment targets, pre-treatment factors predictors). Repeated measures ANOVAs will be conducted to evaluate group differences on social behavior and social cognition composites, social recognition, mental state inference, affiliation, social reward and social competence (i.e. SSIS) in order to identify potential treatment targets for the combined treatment approach. Factors predicting treatment response will include global scores on all baseline measures. Responder status will be entered as a categorical variable as defined above (CGI-I≤2 + SSIS > 25%). PCA will be conducted on significant baseline predictors to develop composite variables and used to predict response using logistic regression.

Appendix A: Time & Events Schedule

	Scrn (1-2)	Scn/ BL (1-2)	WK1	WK2	WK3	WK4	WK5	WK6	WK7	WK8	WK9	WK10	WK11	WK12	WK16	Wk24
ADOS-2[1] or CARS2	X															
ADI-R	X															
WASI-II/WISC-V/KBIT	X															
DSM-V Checklist	X															
Inclusion/ Exclusion	X	X														
Demographics	X															
Med/Psych Intake	X															
Family Med Hx (NIH Form)	X															
Randomization		X														
GUID Acquisition/ Record Form	X															
Con Meds	X	X	X		X		X		X		X		X	X	X	X
Con Non Drug TX	X	X	X		X		X		X		X		X	X	X	X
Vital Signs	X	X	biweekly												X	X
Height	X	X												X		X
Weight	X	X												X		X
Physical Exam (NIH Form)	X	X														
Medical History (NIH Form)	X															
Tanner Staging	X															
Adverse Effects & Suicidality Ratings	X	X	X		X		X		X		X		X	X	X	X
Blinded CGI S/I[1]		S						X						X	X	X
Unblinded CGI S/I	S	S	S/I		S/I		S/I		S/I		S/I		S/I	S/I	S/I	S/I
Psychosocial Tx	X	X												X	X	X
Laboratory																
ECG		X												X		
Female Reproductive Status		X												X		
Urine Pregnancy		X												X		
Safety labs		X												X		

Social Skills Groups[1]			X	X	X	X	X	X	X	X	X	X	X	X		

[1]These assessments will be videotaped and/or administered electronically and recorded on a computer.

	Scrn (1-2)	Scn/ BL (1-2)	WK1	WK2	WK3	WK4	WK5	WK6	WK7	WK8	WK9	WK10	WK11	WK12	WK16	Wk24
Parent Questionnaires																
ABC	X							X						X	X	X
PDDBI-SV		X						X						X	X	X
Griffith Empathy Scale		X						X						X	X	X
Children's Communicatio n Checklist		X						X						X	X	X
CASI-5		X						X						X	X	X
Caregiver Strain		X						X						X	X	X
Vineland 3 (Survey Form)		X												X		X
SRS-2		X						X						X	X	X
RBS-R		X						X						X	X	X
Autism Impact Measure		X						X						X	X	X
Parent expectancy questionnaire		X												X	X	X
SSIS		X												X	X	X
Participant Assessments																
Strange Stories Test	X															
Reading Mind in Eyes Test[1]		X												X	X	X
DANVA2[1]		X												X	X	X
TAT [1]		X												X	X	X
Biological motion task[1]		X												X	X	X
AEDEA		X												X	X	X
Medication																
Dosing Instructions			X		X		X		X		X		X			
Medication Dispensing Log (staff)			X		X		X		X		X		X			
Medication Compliance			X		X		X		X		X		X	X		
Medication Diary Dispensed			X	X	X	X	X	X	X	X	X	X	X			

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