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HRP-503B – BIOMEDICAL RESEARCH PROTOCOL

(2016-1)

Protocol Title: Translating Neuroprediction into Precision Medicine via Brain Priming

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(*If applicable*) **Clinicaltrials.gov Registration #: NCT03370510**

INSTRUCTIONS

This template is intended to help investigators prepare a protocol that includes all of the necessary information needed by the IRB to determine whether a study meets approval criteria. **Read the following instructions before proceeding:**

1. Use this protocol template for a PI initiated study that includes direct interactions with research subjects. Additional templates for other types of research protocols are available in the system Library.

2. If a section or question does not apply to your research study, type “Not Applicable” underneath.

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APPROVED BY THE YALE UNIVERSITY IRB 8/5/2021

SECTION I: RESEARCH PLAN

1. **Statement of Purpose:** State the scientific aim(s) of the study, or the hypotheses to be tested.

Our overarching goal is to develop more precise treatments for children with ASD, guided by their distinct neural profiles. Informed by our published findings[1–11], we will conduct a double-blind, placebo controlled trial of intranasal oxytocin (OXT) as an enhancer of response to Pivotal Response Treatment (PRT) in young children with Autism Spectrum Disorder (ASD). This trial will test the key hypothesis that children with lower levels of activity in and functional connectivity among recently-discovered[10] PRT-response neuropredictive brain regions will benefit more from the administration of OXT vs. placebo as a social cognitive neural circuit enhancer to a 16-week trial of PRT, as evidenced by state-of-the art brain imaging[7,12] and eye tracking paradigms[13–22] as well as key behavioral outcomes[23-24]. **This study will advance personalized medicine in ASD and serve as a proof-of-concept of the use of pharmacological compounds to enhance evidence-based behavioral and cognitive treatments for ASD, while leveraging the inferential power of a randomized experiment to elucidate the brain mechanisms underlying social communication deficits in ASD.**

Aim 1. Evaluate the magnitude of response to PRT+OXT treatment versus PRT+placebo in a randomized controlled trial. **Hypothesis 1a.** Relative to the PRT+placebo group (*n*=40, ages 5-9 years), children with ASD randomized to the PRT+OXT (*n*=40 ages 5-9 years) condition will show greater improvement in social communicative skills from baseline to endpoint (16-weeks post-treatment onset) as quantified by our primary behavioral outcome measure, the Social Responsiveness Scale, 2nd Ed. (SRS-2[23]) Total Score, and the Brief Observation of Social Communication Change (BOSCC[24]), as the secondary behavioral outcome. **Hypothesis 1b.** PRT+OXT vs. PRT+placebo will exhibit greater PRT treatment-induced increases in coherent biological (BIO) > scrambled biological (SCRAM) activity/connectivity, measured by functional magnetic resonance imaging (fMRI), coinciding with the hypothesized behavioral improvements, in two sets of neural structures for social information processing: the Action Observation Network (AON; premotor cortex, inferior parietal lobule, occipitotemporal cortex & supplementary motor area) and the Theory-of-Mind network (ToM; temporoparietal junction, precuneus/posterior cingulate cortex, medial prefrontal cortex[25]).

Aim 2. Quantify the effects of OXT vs. placebo in children who are less biologically prepared to respond to PRT (i.e., those children with relatively BIO > SCRAM activity/connectivity in PRT-response neuropredictive brain regions[10]). **Hypothesis 2a.** The administration of intranasal oxytocin, by priming key neural circuits for social motivation and social perception, will serve to enhance the effectiveness of PRT in the children with lower levels (quantified across all children in the study) of pretreatment activation in the neuropredictive biomarkers regions. **Hypothesis 2b.** As outlined in **Figure 1**, we expect that in the absence of OXT, children who have relatively lower levels of pretreatment activation and connectivity in the neuropredictive circuits will show little or no behavioral improvement; however, when OXT (vs. placebo) is provided to these children prior to the PRT sessions, they will show more favorable behavioral responses to PRT, and the behavioral difference between OXT and placebo, conditional upon levels of pretreatment activation, will be significant.

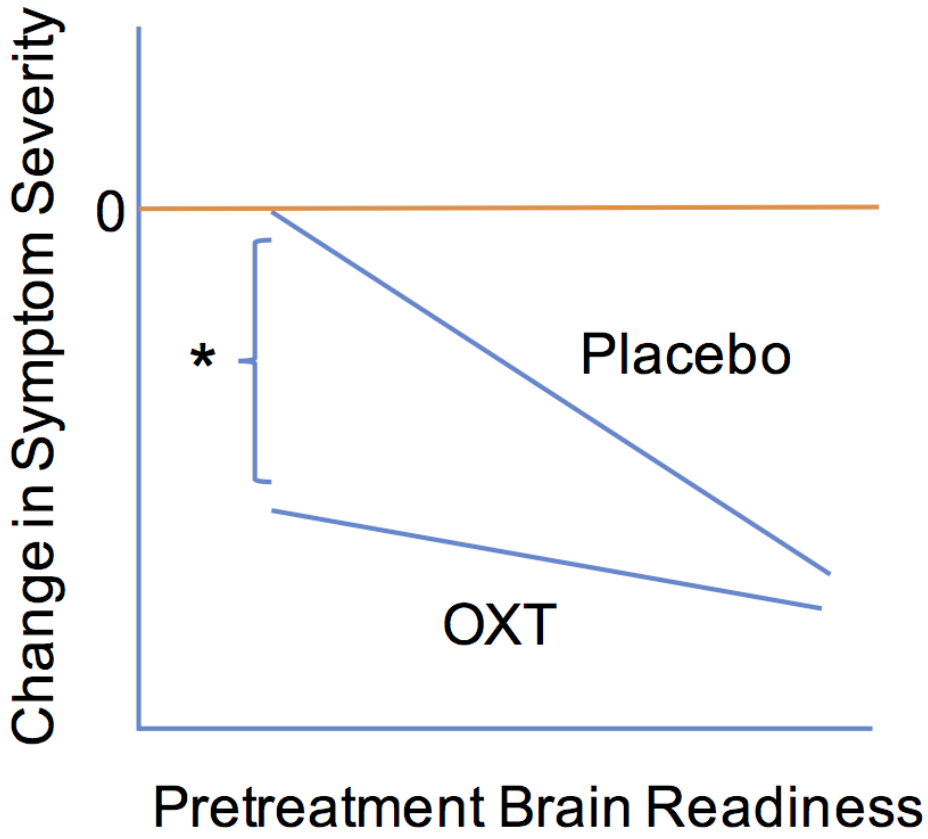

FIGURE 1. THE HYPOTHESIZED SIMPLE MAIN EFFECT OF A SIGNIFICANT OXT (VS. PLACEBO) EFFECT ON TREATMENT EFFECTIVENESS (MEASURED BY Δ IN SRS-2 TOTAL RAW SCORE) IN CHILDREN WITH ASD WITH LOWER LEVELS OF PRETREATMENT ACTIVATION IN THE BRAIN REGIONS OF PREDICTIVE BIOMARKERS.

Aim 3. Develop maximally "practicable" biomarkers by evaluating visual scanpaths as a surrogate for fMRI while testing the mediating influence of attentional preference and sensitivity on brain activation and treatment response. **Hypothesis 3a.** Recognizing that eye-tracking offers an advantage in scalability and ease of implementation, we will determine how changes in visual scanpaths as outcome variables are associated with concurrent changes in observable behaviors and how eye-tracking variables assessed at baseline and midpoint/endpoint are associated with ultimate treatment response as measured by the fMRI and clinical outcomes described in **Aims 1**-**2**.

2. **Probable Duration of Project:** State the expected duration of the project, including all follow-up and data analysis activities.

Three years: Month (M) M1-3: Study Start-up and recruitment. M4-30: Active Study. M30-36: Data Analysis and Grant/Paper Writing. Milestones include end of M3: first subject enrolled; M18: interim analysis; end of M30: last participant session; end of M33: data analysis complete; end of M36: paper submission.

Enrollment was closed due to COVID-19 during the spring of 2020. The expected end date is October 2021 to allow for data analysis, but we will not be enrolling any additional subjects.

3. **Background:** Describe the background information that led to the plan for this project. Provide references to support the expectation of obtaining useful scientific data.
   a) **Pivotal Response Treatment (PRT)** is an empirically-supported manualized treatment for individuals with autism, which was specifically designed to improve social communication by addressing core deficits in motivation by teaching communicative, social, and behavioral goals within a context that enhances the response-reinforcer relationship[26,27]. A large body of empirical research has demonstrated the effectiveness of the specific motivational procedures of PRT (i.e., providing clear prompts, incorporating child choice of materials, interspersing maintenance and acquisition tasks, providing contingent reinforcement for attempts, employing natural reinforcers) in improving social and communication skills in children with autism[27,28]. In addition, research on PRT has demonstrated improvements in a variety of skills, including improvements in communication, social interaction, positive affect, and reduction in disruptive behaviors[29-31], thus, improvements are likely to occur across developmental domains. Furthermore, PRT was identified by the National Research Council of the National Academy of Sciences[32] as one of 10 comprehensive model programs for children with autism. Therefore, this study will further add to the literature to support the effectiveness of PRT for school-aged children with ASD to improve language, play, and social functioning.
   b) **fMRI**. Additionally, given that we anticipate PRT to significantly improve a variety of areas of development, the nature of these changes will also be documented using fMRI and eye tracking. More specifically, as PRT is targeting critical social behaviors, we hope to examine the treatments effects on brain regions previously found to be involved in social perception. Social perception refers to the initial stages of evaluating the intentions of others by analysis of eye-gaze direction, facial expressions, body movements, and other kinds of biological motion[33]. This construct is part of a domain of skills variously referred to as theory-of-mind, mentalizing, social attention, mindreading, and social cognition, which has been defined by Brothers (1990) as the 'processing of information which culminates in the accurate perception of the dispositions and intentions of other individuals[34]. Cognitive neuroscientists have begun to identify key brain regions involved in aspects of social perception and cognition in typically developing individuals. The primary regions that have been consistently implicated are the amygdalae, the superior temporal sulcus (STS), and the fusiform gyrus (FFG), as well as other interconnected regions including the inferior frontal gyrus, inferior parietal lobule, medial prefrontal cortex, and orbitofrontal cortex[33,35,36].
   c) **Eye tracking**. Eye tracking is a flexible technique, applicable across the spectrum from toddlers to adults and from severe intellectual disability to high function, that could provide much-needed[37]

sensitive and reliable measures of change for individuals with ASD. A recent review notes that eye tracking has the potential to provide early indicators of efficacy in clinical trials by tapping into subtle changes in "social attention"[38]. However, to date, these capabilities and this potential exist only in theory. Several factors act as barriers to the practical applicability of eye tracking for intervention monitoring. First, no studies currently examine how eye tracking monitors the effects of longer-term, ongoing treatments. Pairing eye tracking with reliable, accepted, evidence-based treatments would be a first step in understanding the real-world performance and challenges of using eye tracking in a clinical context. Second, most eye-tracking work in autism research focuses on theoretical constructs (e.g., social engagement, joint attention, theory of mind). However, fine-tuning eye-tracking outcome measures to the properties of specific interventions may yield more proximal, sensitive measures of change, even while theoretically-motivated targets provide a framework for examining general changes in autism symptoms. Third, most eye tracking studies look at how individuals passively view information. However, the challenges individuals with autism face occur within the active moment-by-moment demands of real-world interactions. Gaze-contingent technologies pairing computer algorithms with eye tracking can react to looking patterns in real time, allowing us to explore more complex interactive scenarios while retaining the reproducibility and scalability of more standard eye-tracking paradigms. Fourth, the high cost of commercial eye trackers (often >$20,000 USD) and associated specialized expertise is a barrier to the widespread use of eye tracking for clinical purposes and for more routine monitoring, such as in homes. Recent advances in mobile device eye tracking may help surmount this barrier.

d) **Neurodevelopmental disorders and genetics**. It has been demonstrated that vulnerability to developmental neuropsychiatric disorders has a genetic component. Studies have shown that in autism, there is a 60-90% concordance rate for monozygotic twins and less than 5% concordance rate for dizygotic twins. However, recent findings of gene mutations in autism show that spontaneous deletions and duplications of genetic material are ten times more prevalent in sporadic cases of autism spectrum disorders than in healthy control subjects, but only twice as prevalent in autism cases from families with more than one affected member. These results implicate the anomalies as primary, rather than just contributory causes of the disorder in most cases when they are present. Some relatives of people with autism spectrum disorders may exhibit subtle behavioral and cognitive problems. Family members often share biological chemical, behavioral, and cognitive phenotypes with their more obviously affected relatives. For example, language and cognitive abnormalities are more common in relatives of children with autism than in the general population. In the case of neuropsychiatric developmental disorders, it is extremely unlikely that any gross genetic abnormality will represent a common pathway to disease. In a manner analogous to successes in other fields of medicine, the elucidation of a single causal genetic abnormality in a small proportion of patients, will point to the types of genes and physiologic pathways that are involved in more common forms of these disorders. The co-morbidity of impairments in regulation of emotional and social behaviors and the cognitive deficits seen in neurodevelopmental disorders point to the multifactorial nature of these syndromes and the need for a comprehensive approach to identifying and localizing genes that produce susceptibility to these disorders.

e) **Use of Oxytocin to enhance social skills in ASD**. The neuropeptide oxytocin plays a critical

role in social functioning. When given acutely, intranasal OXT leads to enhanced processing of social stimuli in typically developing (TD) adults, as evidenced by increased eye contact, in-group trust, and emotion recognition from facial expressions[53]. At the level of neural systems, intranasal OXT heightens activity in a set of neuroanatomical structures involved in processing socially meaningful stimuli in TD adults[54,55]. Behavioral studies demonstrate that in children and adults with ASD, a single administration of intranasal OXT leads to increased willingness to interact socially[56], better comprehension of affective speech[57], reduced repetitive behaviors[58], increased understanding of others' mental states[59], and improved social cognition[59]. There are several large-scale clinical trials currently underway (www.clinicaltrials.gov) to examine the effects of chronically administered OXT in ASD, but the results have been mixed[60-62]. For example, two recent studies of the effects of repeated daily administration for a period of weeks have resulted in only modest improvements in social behavior[61,62]. Studies of neural systems in ASD demonstrate that OXT vs. placebo: 1) increases activation in the right amygdala during social information processing; 2) increases, to a level approaching that observed in TD individuals, coherent biological motion vs. scrambled biological motion activity in brain regions known to be involved in perceiving and thinking about social-emotional information[11]; 3) enhances effective connectivity between nodes of the brain's reward and socioemotional processing systems[63] preferentially for coherent biological motion.

4. **Research Plan:** Summarize the study design and research procedures using non-technical language that can be readily understood by someone outside the discipline. **Be sure to distinguish between standard of care vs. research procedures when applicable, and include any flowcharts of visits specifying their individual times and lengths**. Describe the setting in which the research will take place.

Eighty children will be randomly assigned to receive PRT with either oxytocin (forty children) or a placebo (forty children).

Intervention. Behavioral treatment will consist of 8 intervention hours per week for sixteen weeks. These eight hours are broken down into three two-hour sessions perweek with the child and one two-hour session with the parent for parent training. The children will be randomly assigned to receive OXT or placebo nasal sprays for all sessions 45 minutes prior to the PRT sessions (three times weekly). We will apply a PRT protocol created by the developers of the approach[64], which focuses on enhancing social motivation, social communication, and language skills. The general approach for teaching that will be employed in this study is described by Koegel, O'Dell, and Koegel (1987) and incorporates the following motivational principles into the treatment: (1) child choice of stimulus materials used to evoke communication, (2) interspersal of maintenance and acquisition tasks, (3) reinforcement of goal-directed communicative attempts, (4) the use of natural and contingent reinforcers following communicative attempts, (5) attention to multiple cues. The specific procedures are outlined in the manual How to Teach Pivotal Behaviors to Children with Autism: A Training Manual[65]. Intervention will involve six hours of services delivered directly from a trained clinician to

the child. In addition, two hours of parent training will be provided per week. The parent-child intervention sessions will follow a "practice with feedback" format[66]. Specifically, the therapist will model techniques and provide opportunities for the parents to work with their children while offering them feedback related to their use of the procedures. Parents will be encouraged to incorporate learning trials into naturally occurring opportunities to facilitate their children's use of functional communication in their daily routines (e.g. mealtime and bedtime) and environments (e.g. at home, at the park). The specific intervention goals will be individualized based on child communication and social interaction skills and parent skill acquisition. Target behaviors will typically include expressive and receptive language development, play skills, and social interaction skills. Parent training services will be delivered at the YCSC. Direct clinician services will occur at the YCSC and may occur at the child's home. During the treatment procedures, the individuals may be videotaped for training purposes and to improve the delivery of the intervention. Only children whose parents consent via the Videography Consent Form will be videotaped. Parents do not need to consent to videotaping to be included in the study.

Randomization. Eligible children will be randomized to PRT+OXT or PRT+placebo using Wei's urn randomization. To assure concealment of allocation, the study pharmacist who is not involved in subject recruitment, evaluation, or treatment will carry out randomization of children. We will stratify on sex, given the recent findings by our group on sex differences in neural basis of social dysfunction in ASD. We will not stratify on other variables because in a study with *n*=80 more than two strata can lead to imbalance between treatment groups.

i. **Assessment Procedures**: Prior to intervention assignment, individuals will receive a developmental evaluation, including the below described assessments as well as an fMRI scan. These assessments, in-lab eye tracking, and fMRI scans will be administered again post-intervention. At the follow up session, assessments, fMRI, and EEG will again be administered 4 months after the conclusion of the intervention to measure longitudinal effects of the intervention, if any. The follow up session will take the same amount of time as the pre-treatment and post-treatment assessment days. Eye tracking will be administered before treatment, after treatment, and at follow up.

a. Diagnostic Assessments. In order to best characterize each subject and judge the severity of neurodevelopmental disorder, all study participants will receive a standard diagnostic workup composed of gold-standard clinical measures, including the Autism Diagnostic Interview-Revised (ADI-R) and Autism Diagnostic Observation Scale (ADOS), as carried out by trained clinicians at the YCSC. At times, these assessments will be video-recorded for training purposes. The parent does not need to consent to videotaping to be included in the study. Only children whose parents who consent via the Videography Permission Form will be videotaped.

b. Cognitive measures. All subjects will participate in a measure of developmental/cognitive ability, such as the Differential Ability Scales-Second Edition (DAS-II) or Mullen Scales of Early Learning and a language measure, such as the Clinical Evaluation of Language Fundamentals-Preschool, Second Edition (CELF-P-2).

c. Participant characterization / behavioral assessments. All participants and parents of children will be asked to fill out personality metrics and behavioral assessments, such as the Social Responsiveness Scale (SRS) and the Vineland Adaptive Behavior Scales- Second Edition

(Vineland-II). Parents will be asked to complete measures about their child and themselves (see list of measures below). In case of report of elevated symptoms of depression and/or anxiety, Dr. Denis Sukhodolsky, clinical psychologist, will be consulted and will take action based on his judgement. All participants will be asked to participate in a 10-minute free play and a 5 minute conversation with a research staff member or parent, which may be videotaped for training and feedback.

CHILD ASSESSMENTS: (administered by clinician or trained research staff)	**Timepoint Administered**
Autism Diagnostic Observation Schedule (ADOS)	Baseline and Endpoint
Autism Diagnostic Interview –Revised (ADI-R)	Baseline
Clinical Evaluation of Language Fundamentals, Second Edition (CELF-P-2)	Baseline
Vineland Adaptive Behavior Scales, Third Edition (Vineland-III)	Baseline and Endpoint
Differential Ability Scale – Second Edition (DAS-II)	Baseline
Clinical Global Impressions-Improvement (CGI-I) and CGI-Severity (CGI-S)	Baseline and Endpoint (CGI-S), Endpoint (CGI-I)
CELF Pragmatics	Baseline
PARENT ABOUT CHILD: (parent report)	
Social Responsiveness Scale-parent report (SRS)	Baseline and Endpoint
Aberrant Behavior Checklist (ABC)	Baseline and Endpoint
Child Behavior Checklist (CBCL)	Baseline and Endpoint
National Database for Autism Research (NDAR)	Baseline
Repetitive Behavior Scale-Revised (RBS-R)	Baseline and Endpoint
Child & Adolescent Symptom Inventory (CASI)	Baseline and Endpoint
Behavior Rating Inventory of Executive Function (BRIEF)	Baseline and Endpoint
Theory of Mind Interview (TOMI)	Baseline and Endpoint
Griffith empathy measure parent report	Baseline and Endpoint
Brief Observation of Social Communication Change (BOSCC)	Baseline and Endpoint

TEACHER ABOUT CHILD: (teacher report)	
Social Responsiveness Scale-teacher report (SRS)	Baseline and Endpoint
PARENT ABOUT SELF: (parent report)	
Beck Depression Inventory—II (BDI-II)	Baseline and Endpoint
Beck Anxiety Inventory (BAI)	Baseline and Endpoint
Parenting Stress Index-4 (PSI-4)	Baseline and Endpoint
Parent's Report of Parent Behavior Inventory (PRPBI)	Baseline and Endpoint

d. Magnetic Resource Imaging

ii. Functional Magnetic resonance imaging (fMRI). All subjects will participate in3 MRI scans one before treatment, one after treatment, and one at follow up visit. Images will be acquired on either 3.0 Tesla Siemens scanner located at the Yale Magnetic Resonance Research Center (MRRC). Each MRI visit will last from 60-120 minutes, including breaks, signing consent forms, and practicing the scan. The fMRI scan will last from 30-60 minutes, depending on the participant's comfort level. Child participants will not be expected to scan for longer than 60 minutes. At any point, participants can end the session immediately either by requesting it verbally or if the experimenter sees any sign of distress. Trained research assistants will perform the research procedures, and the MR technicians will run the fMRI machine. Experiments are computer controlled, as are all stimulus presentations and response measurements. An LCD projector provides visual stimuli. Auditory stimuli include digitized speech or non-speech sounds and will be presented to the subject through headphones. We will collect 40 axial (parallel to ac-pc) slices with 3.5 mm^3 voxel size using a single shot echo planar sequence (TR= 2320, TE = 30). We currently plan to use the pulse sequences described above. However, pulse sequence development is a key component of neuroimaging research, and thus the precise sequence utilized may change as improvements become available. We emphasize that all sequences will meet FDA limits for non-significant risk for specific absorption rate (SAR) and time-varying magnetic fields (db/dt). We will collect 2D in plane axial anatomical images with the same voxel dimensions and orientation for fMRI co-registration (T1 Flash, TR = 30, TE= 4, NEX = 2, Flip angle = 60, BW = 300) as well as volumetric analyses, including white matter density, ventricular volume, and head circumference calculations. Data for diffusion tensor imaging (DTI) will be collected with an 8-channel head coil and parallel imaging to gain signal at air-tissue interfaces where susceptibility artifacts are prominent with DTI via the standard single channel coil. DTI quality will be optimized on each subject using auto-shimming methods. Each subject will have water reference standards placed adjacent to the head to allow the diffusion gradient strengths (b-value) to be verified. Two acquisitions will be performed without diffusion sensitization (b = 0), followed by diffusion weighted (b = 1000 s/m^{m2}) images with gradients applied in 24 non-collinear directions (by increasing the number of gradient directions, data sampling becomes more uniform and less biased to any specific direction). We will make two averages for each direction per run using the following

parameters: 56 axial slices, 2.5 m^{m^3} isotropic voxels, skip 0. TR = 7100; TE = 88; GRAPPA = 3/24; partial Fourier = off, M = 128 ×128, BW = 1396 Hz/pixel with an inter-echo distance of 0.87 ms. We will acquire three 6' 14" runs, for a total of 6 averages and a total scan time of 18' 42".

iii. Laboratory Eye-Tracking Battery. Eye tracking will take place 3 times, at baseline, endpoint, and follow up visits. The laboratory eye-tracking battery will be comprised of **(a)** ***Standard Tasks***, i.e., tasks previously used and validated in our laboratory, reflecting the cumulative synthesis of a decade of eye-tracking work in autism research primarily motivated by theory: **(b)** ***Intervention Tasks***, i.e., eye-tracking tasks that are designed to tap into skills and capabilities that underlie improvements as the specific result of our intervention. These tasks do not necessarily act as surrogates for behavioral measurement, but rather reflect components that may be prerequisites to improvement. For instance, eye tracking cannot measure the appropriateness of a spoken response to an inquiry delivered verbally but can examine whether children will spontaneously direct their attention to actresses emulating the asking of a direct question to them; **(c)** ***Gaze-Contingent Tasks***, i.e., tasks that react in real time to the gaze patterns of participants, allowing for the construction of an interactive social simulation mediated by looking patterns and **(d) Behavioral Tasks**. All tasks will be administered on a commercial eye tracking system (such as a Tobii T60XL or an SR EyeLink 1000 Plus) in a room dedicated to eye-tracking studies. Eye tracking data will be collected before treatment, after treatment, and at your follow up visit. using the main in-laboratory eye-tracking battery.

1. Examples of **Standard Tasks** include:
    a. Biological Motion Preference: Though preferences for biological motion (biomotion) are evident from birth[67], individuals with ASD show recognition difficulties[68-71] and atypical preferences for biomotion vs. control motions[68,72,73]. We will use a set of trials which, after a central fixation (1.5s), children will be presented with two animated point light displays (CMU Graphics Lab, 2011): a biomotion *target* and a scrambled perceptual control (3-5s). Outcome variables will include % of time looking at the *target* (**Target%**) and proportion of trials where the first saccade was towards the target (**biopref_ratio**). This task taps into primitive social preferences.

    b. Activity Monitoring: Monitoring of the activities of others is compromised in children with ASD[74,75], with recent studies showing that atypical looking patterns during activity monitoring probes are present even in adults with ASD. In this task, children will be presented with multiple activity monitoring trials as developed in Shic et al., 2014[74]. Outcome variables will include % of time looking at the scene (**Scene%**), Faces (**Face%**), and activity (**Activity%**). This task taps into social motivation and understanding.

    c. Dynamic Naturalistic Scenes: Studies have shown the looking pattern differences between individuals with ASD and controls are most pronounced during viewing of complex, dynamic, naturalistic social scenes[76-78]. Recent studies have shown that, in children with ASD, these patterns change with intellectual and social impairments[79], suggesting that patterns of social attention could reveal subtypes of ASD[80].

Participants will be presented with socially complex clips from movies depicting children (e.g., The Sandlot and Welcome to the Dollhouse[79]). Outcome variables will include % of time spent looking at characters' eyes (**Eyes%**) and mouths (**Mouth%**). This task taps into narrative comprehension, social interest, and language processing.

2. Examples of **Intervention Tasks** include:

   a. Dyadic Bid Sensitivity*:* Recent studies have shown that children with ASD look less at faces of people trying to engage their attention through eye contact and child-directed speech (i.e., bids for dyadic engagement[81-83]) as compared to controls. In this task, we extend our previous work examining this phenomenon in toddlers with ASD[80-82] to a new, more complex and challenging environment for older children. In this task, multiple actors in naturalistic environments (e.g., standing in office hallways) will engage in conversation/interaction with one another. Periodically, one of the actors will look directly to the camera, "speaking" to the viewer, while the other actors continue their interactions. This task examines sensitivity to overtures for social engagement by others, a requisite for *speech to respond to inquiries* and *reciprocal conversation*. Outcome variables include % time looking at the dyadic bid actor's face (**DyadicFace%**).

   b. Conversational Flow: Designed as a subset of the Dyadic Bid Sensitivity task, this task examines how children with ASD follow the dynamic and flow of natural conversation between multiple actors. Outcome variables include % time looking at the current speaker's face (**SpeakerFace%**) and the % of time the participant predicts the next speaker (by looking at the person about to speak before he or she speaks, **SpeakerPredict%**). This task is relevant to *monitor nonverbal information, speech to inquire/comment, and reciprocal conversation.*

   c. Social Referencing: Social referencing, the process of seeking clarifying context from the faces of others regarding uncertain situations, develops throughout the first year of life[84] but can be deficient in older children with ASD[85]. To examine this social-information-seeking process, we will film multiple episodes in which actors are engaged in stressful activities (e.g., stacking a tall thin block tower, inflating a balloon till near-burst). These episodes will depict the activities' escalation (e.g., balloon continues to expand), critical event (e.g., balloon pops and the pieces begin flapping wildly), and resolution (e.g., actors sigh with relief). The central activity will be dynamic even after the critical event in order to provide an alternative target for attention. This task examines automaticity of information-seeking from others, a requisite for *speech to inquire* that is related to sharing behaviors in *speech to comment,* and *monitor nonverbal information.* Outcome variables will include % time looking at actors' faces (**Face%**).

   d. Theory of mind: Elegantly demonstrated by Senju and colleagues[86], the ability to automatically process others' beliefs as they translate into intentional actions is deficient even in adults with ASD. In this task, we create a fluid, naturalistic theory of

mind task in which a protagonist continuously engages in search/play activities with specific objects while a second, antagonist actor, unbeknown to the protagonist, constantly and randomly interferes with the protagonist's goals. For example, the protagonist, wanting to make breakfast, could pour the cereal and then get interrupted by the doorbell. The protagonist leaves, and the antagonist enters, taking the milk from the refrigerator, and putting it in the cabinet. The protagonist comes back, and goes to the fridge for milk. This task gauges spontaneous interest in others' intentions as well as comprehension of the mental frame and perspective of others and is relevant for *perspective taking* and associated skills. Outcome measures include % time looking at the active actor (**Actor%**), whether protagonist or antagonist, and the target area associated with the protagonists' false belief (**FalseBelief%**).

3. Examples of **Gaze Contingent Tasks** include:

    a. Normative Gaze Alignment Probe*:* We will deploy a gaze-contingent paradigm that has been created as part of our ongoing exploratory NIMH research grant, R21 MH102572. In this task, participants view naturalistic scenes including situations where an actress speaks directly to the camera with backgrounds varying in complexity (e.g., a backdrop of marathon runners or trains moving past). As the participant viewing the scene deviates from the scanning patterns of a normative sample, the program automatically reorients the participant by blurring and darkening the places that should not be attended to. This paradigm is similar to naturalistic interactions in which a child's attention is redirected. A clinician may deliberately capture a child's attention and redirect the child back to task or even remove a distraction from the environment. Outcome measures include **#Redirects**, the number of times adaptive darkening of the scene occurs, **%Redirect**, the amount of time spent in redirection, and **Deviation**, a mathematically defined measure describing deviance from prototypic scanning patterns.

    b. Social Cue Contingency: Designed as part of Dr. Shic's Postdoctoral Fellow's Autism Speaks fellowship, this task depicts an actress in the middle of the video screen flanked by two "targeted areas." The actress will gaze-shift to the target area that is of interest. If the child shifts his or her gaze into the correct target area, the display will seamlessly transition to a reinforcing cartoon, followed by the actress verbally praising the participant. Outcome variables will include the time from the start of the gaze cue to looking at the appropriate target. (**Time-to-target**). Gaze shifts will vary by their subtlety to accommodate a wide range of performance, with pre-programmed elevation of prompts in the event that the child does not follow a gaze cue.

4. Examples of **Behavioral Tasks** include:

    a. Social learning task (Preference Task, PT). In the social learning task, participants are asked to predict the preferences of two different persons for a number of items (food types, beauty products and leisure activities). One person's profile will be a more typical / predictable profile and the other will be atypical. Before each run participants

are introduced to each person. they subsequently rate preferences on a 10-point Likert scale (1='not at all' and 10='very much'; rating phase (5s)). After rating an item, participants receive trial-by-trial feedback about the person's actual rating.

b. Nonsocial learning task. The non-social condition (the Alien Language Task, or AT) will be designed to closely resemble the PT task. It will comprise the same items and rating scale as the PT. Here participants are introduced to two different aliens and their favorite 'alien language' word (i.e., novel pseudo-adjectives such as 'blickety'). Participants are asked to rate how much this word describes the task items (one word will be easier to infer from the items and the second one will be more difficult). Similar to the PT, participants receive trial-by-trial feedback indicating the alien's opinion about how well the word represents the particular item. Note that every pseudoword represents the "alien translation" of a real word (e.g., "blickety" for "shiny") and participants must deduce this translation from the feedback about items, which they receive during the task.

5. Genetic Testing N/A ☒

A. Describe

i. the types of future research to be conducted using the materials, specifying if immortalization of cell lines, whole exome or genome sequencing, genome wide association studies, or animal studies are planned *Write here*

ii. the plan for the collection of material or the conditions under which material will be received *Write here*

iii. the types of information about the donor/individual contributors that will be entered into a database *Write here*

iv. the methods to uphold confidentiality *Write here*

B. What are the conditions or procedures for sharing of materials and/or distributing for future research projects? *Write here*

C. Is widespread sharing of materials planned? *Write here*

D. When and under what conditions will materials be stripped of all identifiers? *Write here*

E. Can donor-subjects withdraw their materials at any time, and/or withdraw the identifiers that connect them to their materials? *Write here*

i. How will requests to withdraw materials be handled (e.g., material no longer identified: that is, anonymized) or material destroyed)? *Write here*

F. Describe the provisions for protection of participant privacy *Write here*

G. Describe the methods for the security of storage and sharing of materials *Write here*

6. **Subject Population:** Provide a detailed description of the types of human subjects who will be recruited into this study.

   The target sample for this project consists of 80 participants, ages five to nine years, with a diagnosis of ASD, and their primary caregiver.

7. **Subject classification:** Check off all classifications of subjects that will be specifically recruited for enrollment in the research project. Will subjects who may require additional safeguards or other considerations be enrolled in the study? If so, identify the population of subjects requiring special safeguards and provide a justification for their involvement.

☒ Children	☒ Healthy	☐Fetal material, placenta, or dead fetus
☐Non-English Speaking persons	☐ Prisoners	☐Economically disadvantaged
☒Decisionally Impaired	☐ Employees	☐Pregnant women and/or fetuses
☐Yale Students	☐ Females of childbearing potential	

This study evaluates the efficacy of a treatment specific for children with ASD. This is an age when development is unfolding, and we can have the most impact on later outcome.

NOTE: Is this research proposal designed to enroll children who are wards of the state as potential subjects?
Yes ☐ No ☒

8. **Inclusion/Exclusion Criteria:** What are the criteria used to determine subject inclusion or exclusion?

   Inclusion: Individuals ages five to nine years with ASD will be included to ensure that participating children will benefit from the intervention which specifically targets social communication development. Participants must also complete an eye tracking session and fMRI with success determined by the Principal Investigator.

   Exclusion: Individuals will be excluded from participation based on the presence of a physical or neurological disorder (e.g., cerebral palsy) which is likely to impact development and learning, as intervention procedures for these individuals may need to be modified beyond the standard approach to address more complex

developmental needs. We will also exclude children on psychotropic medications, psychotropic medication may affect social dysfunction and our neural targets. Parents/caregivers who do not speak fluent English or have previous training in PRT will be excluded. Also, individuals who must be excluded from fMRI or eye tracking will be excluded. These families will be offered the treatment clinically (i.e., fee-based), and they will be given referrals to outside agencies offering the treatment as well.

9. How will **eligibility** be determined, and by whom?

Eligibility will be determined by staff for this project based on the children's performance on standardized developmental assessments.

Group	Inclusion Criteria: Participants will:	Exclusion Criteria: Participants may not have:
General:	1. Fit the age requirement: age 5-9 2. Have been diagnosed previously with an ASD and meet criteria for ASD when characterized by research team 3. Be in good medical health 4. Be cooperative with testing 5. Speak English in the family 6. Successfully complete an fMRI scan 7. Full-scale IQ>70	1. Any metal or electromagnetic implants, including: a. Cardiac pacemaker b. Defibrillator c. Artificial heart valve d. Aneurysm clip e. Cochlear implants f. Shrapnel g. Neurostimulators h. History of metal fragments in eyes or skin 2. Significant hearing loss or other severe sensory impairment 3. A fragile health status. 4. Current use of prescription psychotropic medications that may affect cognitive processes under study. 5. A history of significant head trauma or

		serious brain or psychiatric illness

10. **Risks:** Describe the reasonably foreseeable risks, including risks to subject privacy, discomforts, or inconveniences associated with subjects participating in the research.

Treatment:

PRT + Placebo Group: There is the possibility that parents may experience some inconvenience in the frequency of the visits during this study. There is the possibility that some children may become upset/fussy and may begin to cry during the assessments and/or intervention. However, this risk is estimated to be minimal as the motivational intervention procedures of PRT are designed to promote positive affect and interactions and the researchers are experienced in helping children at such times. It is possible that some parents may not feel comfortable learning and using the intervention procedures. The information provided to us will be kept strictly confidential. Although every measure will be taken to maintain confidentiality, there may be unknown breaches of confidentiality.

PRT + Oxytocin Group: All of the risks previously stated are possible. Additionally, although Oxytocin is quite safe when used in a proper dosage, it has the following possible side effects:

Oxytocin intranasal inhalation: The hormone Oxytocin is produced naturally in the body and has a few natural effects: Oxytocin is used in medical practice as a drug with a few functions: to induce labor in pregnant women, to increase muscle tone in the uterus in case of postpartum bleeding. When used in a proper dosage the administration of Oxytocin is quite safe. Previous studies have shown that intranasal Oxytocin administration does not dry up nasal pathways or cause retainment of fluids.

The hormone, Oxytocin, is produced naturally in the body and the brain. In the brain, Oxytocin has some effects on behavior, it has been associated with sociability, love, parental behavior, reduced anxiety, increased trust, memory and learning abilities.

When Oxytocin is used in a proper dosage, like we are doing in this study, it is quite safe. Side effects are rare, and no serious side effects have been recorded for intranasal application.

Oxytocin has two possible side effects:

1.Disruption of urinary function
2. Increased heart rate and blood pressure

There is always the possibility of unknown or unexpected side effects that may result from the administration of the Oxytocin.

fMRI studies: There are no known risks to the use of fMRI and related protocols *per se*; however, there are several areas of concern. The first is the potential risk of the main magnetic field attracting ferromagnetic objects toward the magnet, which includes internal electronic devices. The second is the discomfort some participants encounter by the confinement within the bore of the MRI system, as well as the loud noise made by the gradients during imaging. These risks occur for all clinical MRI exams and are not increased by the proposed research.

This MR study is for research purposes only and is not in any way a healthcare examination. The scans performed in this study are not designed to find abnormalities. The principal investigator, the lab, the MR technologist, and the Magnetic Resonance Research Center are not qualified to interpret the MR scans and are not responsible for providing a healthcare evaluation of the images. If a worrisome finding is seen on a scan, a radiologist or another physician will be asked to review the relevant images. Based on his or her recommendation (if any), the principal investigator or consulting physician will contact the participant, inform them of the finding regarding your child, and recommend that they seek medical advice as a precautionary measure. The decision for additional examination or treatment would lie only with the family and their physician. The investigators, the consulting physician, the Magnetic Resonance Research Center, and Yale University are not responsible for any examination or treatment that children receive based on these findings. The images collected in this study are not a healthcare MR exam and for that reason, they will not be made available for healthcare purposes.

Eye-tracking: There are no known risks to the use of infrared oculography. The eye tracking systems are completely non-invasive. There is the possibility that some children may become upset in a new environment during the testing. However, the researchers are experienced in helping children at such times.

Breach of confidentiality: In the unlikely chance that someone outside our group gains access to the subject's information, there is a risk of breach of confidentiality.

11. **Minimizing Risks:** Describe the manner in which the above-mentioned risks will be minimized.

Treatment: Each visit will be organized and involve breaks for the family and child to rest between procedures. Experienced clinicians will always be available to help in efforts to reduce anxiety. Parents will always be present either in the same room where the child assessments and intervention occur or in a nearby observation or assessment room. Research staff working on the project all have experience working with children and will therefore do everything possible to accommodate the children who may come to our laboratory as part of this study. Parental concerns regarding the intervention procedures will be readily addressed within the intervention sessions. Clinicians working with parents have extensive clinical experience working with parents and young children. Those parents who do not wish to continue the procedures will be provided with appropriate alternative referrals.

Oxytocin Drug Administration: After administration of all pharmacological agents, the well-being of participants will be checked regularly. A research team member will be accompanying the participants and available at all times to call for medical assistance if required. Procedures will be immediately stopped if subjects request to stop or exhibit any sign of significant distress. Participants will be free to leave the laboratory at the end of the study day provided they are feeling fit and well as determined by their subjective reports and careful observation by the research staff. If participants experience or report any adverse effects or if the research staff feels that at the end of testing the participant has any adverse effects, participants will be asked to remain until effects wear off (likely only for a short period) and medical assistance will be called. Participants will be free to withdraw from the study whenever they wish. In addition, if any participant presents with an adverse reaction to the psychopharmacological agent as reported by them or assessed by a member of the research team, we will discontinue the study and call for medical assistance. Finally, the participant may be withdrawn from the study

if his/her withdrawal is in the best interests of either the participant or of the research (e.g., due to a medical condition making the procedures unsafe or any condition making interpretation of the results difficult). No subjects with any known medical or psychological risk factors will be enrolled. To minimize risks, participants will be closely monitored by clinical staff and appropriate medical care will be provided in the event of significant side effects. Participants with any history of cardiovascular, renal or other significant acute or chronic medical disease will be excluded from the study. The phone and on-site screening will minimize the risk to the subject with regard to the OXT administration. We will provide snacks and water after participation.

Adverse events are monitored in the study on a weekly basis by interview with parent that is conducted by study investigators. No serious or unexpected adverse events have been reported during the first year of the study. These was also no reports of serious adverse effects in studies of intranasal oxytocin in autism (101-108). However, given that increased heart rate and blood pressure were noted as possible side effects, we will also measure blood pressure and heart rate at study baseline and then monthly during the sixteen-week trial. Dr. David Grodberg, a child an adolescent psychiatrist who prescribes oxytocin in this study, will measure blood pressure and heart rate on a monthly basis.

Guidance on Blood Pressure and Cardiac Monitoring: Based on the participant's age, gender and height percentile, we have obtained blood pressure parameters where blood pressures above or below the set threshold will prompt the clinician to recommend an ECG. We have adapted these values from "The fourth report on the diagnosis, evaluation and treatment of high blood pressure in children and adolescents. National Institutes of Health. May 2005." "Growth Charts: Stature-for-Age Percentiles for Use in Blood Pressure Assessments" (Attachment A) and "95th Percentile Blood Pressure Levels For Sex by Age and Height" (Attachment B) will be used to determine the 95th percentile for both the systolic (SBP) and diastolic blood pressures (DBP) by sex, age and height percentile.

Instructions for Use of charts:

1. Use Growth chart to determine the height percentile. Subjects who fall below the 5th or above the 95th percentile for their age, sex, and height should be evaluated using the parameters for the 5th or 95th percentile.

2. Measure and record the blood pressure of the child or adolescent.

3. Use the table below to identify the 95th percentile for both systolic and diastolic blood pressure, by sex, according to age and height.

If the predose SBP and/or DBP is abnormal on any dosing day, blood pressure measurement should be repeated after the subject rests for at least 5 minutes in a supine or semi-supine position to confirm the measurement. If SBP and/or DBP remain ≥ the 95th percentile for sex, age and height or < the 5th percentile for age, sex and height, dosing should be postponed and rescheduled on the following day or within the given visit window. If blood pressure elevation persists at the next visit, the subject should be scheduled for a consultation by cardiologist, other specialist, or primary care physician prior to further dosing. If an elevation is observed, continue monitoring until blood pressure returns to normal.

Electrocardiogram (ECG): A 12-lead ECG will be performed if the participant is found to have cardiac risk. We define cardiac risk as abnormal heart rate and/or blood pressure according to The Fourth Report on the Diagnosis, Evaluation and Treatment of High Blood Pressure in Children and Adolescents and the Normal ranges of heart rate and respiratory rate in children from birth to 18 years: a systematic review of observational studies (Fleming, S. 2013, ref # 109) as well as the presence of specific cardiac symptoms such as: syncope, dizziness, palpitations, and shortness of breath. These clinical parameters are also incorporated into the Adverse Events monitoring checklist. Given the low cardiac risk associated with Oxytocin, we believe that with careful monitoring of vital signs and clinical symptoms at every visit we will be able to ensure the safety of our participants.

During the collection of ECGs, subjects should be in a quiet setting without distractions (eg, television, cell phones). Subjects should rest in a supine or semi-supine position for at least 5 minutes before ECG collection and should refrain from talking or moving arms or legs.

Physical Examination, Body Weight and Height: Physical examinations and measurement of body weight and height will be performed at baseline by a study physician and results will be documented using a structured form.

fMRI: Prior to arrival, familiarization will begin with the use of social stories and videos that will be available on our website. To minimize potential problems of anxiety during behavioral testing, the exam will not begin until the participant is comfortable with the office, laboratory room, and experimenter. Breaks will be provided for snacks and computer or board games as needed. The length of the test sessions will be adjusted to each individual. The experimenters demonstrate competence in test administration and scoring, but also for a pleasant, calm, and reassuring demeanor. The experimenters are able to communicate in a succinct, clear manner, recognize the needs of young children, and respond appropriately using behavior management techniques. Participants will be provided with frequent positive feedback and will be reinforced for all efforts.

Staff and a parent or parents will accompany participants to the MR scans. We will have the participant practice the procedures in the mock scanner beforehand in order to reduce anxiety associated with the MR procedures. The mock scanner includes a recording of the noise associated with imaging; practice versions of the study procedures also can be practiced in the mock scanner. Participants may be shown a social story (storyboard and/or video media) associated with the imaging procedures prior to testing. During testing, the MR technologist and experimenter will provide information to the participant through an intercom about the progress of the examination. The participant can communicate during the procedure with the technologist and the experimenter. During the structural MRI (sMRI) and DTI sequences, the participant may listen to and watch a DVD of his/her favorite movie or a choice from our laboratory's library of children's movies. Sedation will not be used and the participant may abort the examination at any time.

There are no known biological risks to exposure to magnetic fields from MRI exams using the techniques described. All MR studies will follow guidelines set by the FDA with regard to specific absorption ratio, limits on gradient slew rate (dB/dt), and noise. The MR studies involve a 3.0 MR scanners that conform to FDA safety guidelines. All scanner adaptations are FDA approved. Such scanners are available for patient studies at other major medical centers and have been used for a number of years without problems. Safety procedures are rigorously enforced. Staff members receive intensive training with periodic retraining to ensure implementation of the most rigorous safety procedures. This is a standard requirement of the MR imaging facility. The presence of a metal foreign object implanted in the participant will be determined by the screening procedure, orienting session, interview with each potential participant and (if necessary) parent(s), and completion of a detailed screening questionnaire. Participants and staff are instructed to remove all metal objects, including clothing with metal clasps, before entering the magnet room.

Risks associated with the MRI procedures are minimized by: (1) checking participants for possession of ferromagnetic objects prior to entry into the scanner room, both physically and with the use of a metal detector.

The magnetic properties of unknown material are tested outside the magnet room with a strong permanent magnet. MR staff members and all persons entering the room are instructed to enter the magnet room slowly and pause at the entrance to determine if any items on their person may be pulling toward the magnet; (2) verification that there are no contraindications to MRI (cardiac pacemaker, aneurysm clip, cochlear implants, pregnancy, IUD, shrapnel, history of metal fragments in eyes, neurostimulators, weight of 250 pounds or more, or claustrophobia) and that all participants are medically healthy; (3) use of adequate padding around the head for comfort and noise reduction; (4) requiring the participants to insert earplugs and/or headphones during scanning to reduce noise levels below FDA limits (the sound levels within the scanners are compliant with FDA guidelines); (5) direct observation of participants throughout the procedure by a familiar staff person in the room; and (6) having trained radiological imaging technologists available at all times.

Some participants may feel uncomfortable or confined once positioned within the bore of the MRI system. This potential reaction will be reduced by having subjects participate in the orientation/mock-scanner session, by discussing the procedure prior to entry into the magnet room, by providing the participant with a panic squeeze ball which they can use to immediately signal the experimenter and the MR technician, and by frequently communicating with the participant over the intercom during the scan session. During set-up and anatomical imaging, participants will watch a movie on the projector and screen. However, if participants feel uncomfortable, the imaging procedure will be terminated and the participant will be removed from the magnet.

When appropriate, parents will receive audiovisual materials that provide prior exposure/desensitization to visual and auditory stimuli that are encountered during a scan. We will ask parents to expose their child to these materials several times before the scan. Participants will be placed in the MRI scanner (with foam bolsters position around the body and head and a vacuum pack used to discourage movement). The child will be fitted with earplugs and/or headphones to diminish the sounds of the scanner. An experienced experimenter will remain in the scanner control room so that they can always hear or talk to the child should they become frightened or uncomfortable. In such instances, the attending experimenter will be readily available to quickly comfort the child or remove the child from the scanner. If the child requests, the experimenter can also remain in the scanner room during the actual scan.

12. **Data and Safety Monitoring Plan:** Include an appropriate Data and Safety Monitoring Plan (DSMP) based on the investigator's risk assessment stated below. (Note: the HIC will make the final determination of the risk to subjects.)

    a. What is the investigator's assessment of the overall risk level for subjects participating in this study?

    PRT + OXY ARM: 45 CFR 46.405, 21 CFR 50.52: Research involving greater than minimal risk but presenting the prospect of direct benefit to the individual child subjects involved in the research

    PRT + PLACEBO ARM: 45 CFR 46.404, 21 CFR 50.51: Research not involving greater than minimal risk to the children

b. If children are involved, what is the investigator's assessment of the overall risk level for the children participating in this study?

PRT + OXY ARM: 45 CFR 46.405, 21 CFR 50.52: Research involving greater than minimal risk but presenting the prospect of direct benefit to the individual child subjects involved in the research

PRT + PLACEBO ARM: 45 CFR 46.404, 21 CFR 50.51: Research not involving greater than minimal risk to the children

c. Include an appropriate Data and Safety Monitoring Plan. Examples of DSMPs are

available here http://your.yale.edu/policies-procedures/forms/420-fr-01-data-and-safety-monitoring-plans-templates for

i. Minimal risk

ii. Greater than minimal

The principal investigator is responsible for monitoring the data, assuring protocol compliance, and conducting the safety reviews monthly. During the review process the principal investigator will evaluate whether the study should continue unchanged, require modification/amendment, continue or close to enrollment.
Data and Safety Monitoring Boards will be authorized to monitor the conduct of the study and will have access to data.
The principal investigator, the Human Investigation Committee (HIC) have the authority to stop or suspend the study or require modifications.

2. The risks associated with the current study are deemed greater than minimal for the following reasons:

1. We do not view the risks associated with the use of oxytocin in children as minimal risks.
2. We do not view the risks associated with the combined use of intranasal oxytocin and pivotal response training as minimal risks.
3. Given the now established safety and validity of the current use of intranasal oxytocin in our prior work, we do not view the proposed studies as high risk.
4. Given our experience with the combined co-administration of intranasal oxytocin and pivotal response training we do not view the proposed studies as high risk.

Although we have assessed the proposed study as one of greater than minimal risk, the potential exists for anticipated and/or unanticipated adverse events, serious or otherwise, to occur since it is not possible to predict with certainty the absolute risk in any given individual or in advance of first-hand experience with the proposed study methods. Therefore, we provide a plan for monitoring the data and safety of the proposed study as follows:

3. Attribution of Adverse Events:

Adverse events will be monitored for each subject participating in the study and attributed to the study procedures / design by the principal investigator (*Denis Sukhodolsky*) according to the following categories:

a.) Definite: Adverse event is clearly related to investigational procedures(s)/agent(s).

b.) Probable: Adverse event is likely related to investigational procedures(s)/agent(s).

c.) Possible: Adverse event may be related to investigational procedures(s)/agent(s).

d.) Unlikely: Adverse event is likely not to be related to the investigational procedures(s)/agent(s).

e.) Unrelated: Adverse event is clearly not related to investigational procedures(s)/agent(s).

4. Plan for Grading Adverse Events:

The following scale will be used in grading the severity of adverse events noted during the study:

1. Mild adverse event
2. Moderate adverse event
3. Severe

5. Plan for Determining Seriousness of Adverse Events:

Serious Adverse Events:

In addition to grading the adverse event, the PI will determine whether the adverse event meets the criteria for a Serious Adverse Event (SAE). An adverse event is considered serious if it results in any of the following outcomes:

1. Death;
2. A life-threatening experience in-patient hospitalization or prolongation of existing hospitalization;
3. A persistent or significant disability or incapacity;
4. A congenital anomaly or birth defect; OR
5. Any other adverse event that, based upon appropriate medical judgment, may jeopardize the subject's health and may require medical or surgical intervention to prevent one of the other outcomes listed in this definition.

An adverse event may be graded as severe but still not meet the criteria for a Serious Adverse Event. Similarly, an adverse event may be graded as moderate but still meet the criteria for an SAE. It is important for the PI to consider the grade of the event as well as its "seriousness" when determining whether reporting to the IRB is necessary.

6. Plan for reporting UPIRSOs (including Adverse Events) to the IRB

The principal investigator will report the following types of events to the IRB:

Any incident, experience or outcome that meets ALL 3 of the following criteria:

1. Is unexpected (in terms of nature, specificity, severity, or frequency) given (a) the research procedures described in the protocol-related documents, such as the IRB-approved protocol and informed consent document and (b) the characteristics of the subject population being studied; AND
2. Is related or possibly related to participation in the research (*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
3. Suggests that the research places subjects or others at greater risk of harm (including physical, psychological, economic, legal, or social harm) than was previously known or recognized.

Unanticipated Problems Involving Risks to Subjects or Others (UPIRSOs) may be medical or non-medical in nature, and include – but are not limited to – *serious, unexpected, and related adverse events* and *unanticipated adverse device effects*. ***Please note*** that adverse events are reportable to the IRB as UPIRSOs **only** if they meet all 3 criteria listed above.

These UPIRSOs/SAEs will be reported to the IRB in accordance with IRB Policy 710, using the appropriate forms found on the website. All related events involving risk but not meeting the *prompt* reporting requirements described in IRB Policy 710 should be reported to the IRB in summary form at the time of continuing review. If appropriate, such summary may be a simple brief statement that events have occurred at the expected frequency and level of severity as previously documented**.** In lieu of a summary of external events, a current DSMB report can be submitted for research studies that are subject to oversight by a DSMB (or other monitoring entity that is monitoring the study on behalf of an industry sponsor).

7. Plan for reporting adverse events to co-investigators on the study, as appropriate the protocol's research monitor(s), e.g., industrial sponsor, Yale Cancer Center Data and Safety Monitoring Committee (DSMC), Protocol Review Committee (PRC), DSMBs, study sponsors, funding and regulatory agencies, and regulatory and decision-making bodies.

For the current study, the following individuals, funding, and/or regulatory agencies will be notified (choose those that apply):

X All Co-Investigators listed on the protocol.

☐ Yale Cancer Center Data and Safety Monitoring Committee (DSMC)

☐ National Institutes of Health

2000021581

☐ Food and Drug Administration (Physician-Sponsored IND #_____)

☐ Medical Research Foundation (Grant_____)

☐ Study Sponsor

☐ Other Data Safety Monitoring Board (DSMB) or Committee (DSMC)

The principal investigator (*Denis Sukhodolsky*) will conduct a review of all adverse events upon completion of every study subject. The principal investigator will evaluate the frequency and severity of the adverse events and determine if modifications to the protocol or consent form are required.

Please note: For any study that may be considered high risk, the IRB will be more focused on the safety requirements for the study and a DSMB will likely be required.

13. **Statistical Considerations:** Describe the statistical analyses that support the study design.

For Aim 1, our power analysis of clinical outcome is based on the number of subjects needed to detect the difference in outcome between PRT and WLC on the sex-norm-a adjusted SRS-2 Total Score. Our preliminary data from 28 subjects showed that the effect size associated with the treatment group difference (two-sided) before treatment as assessed using the SRS-2 Total Score is d=.80. Accordingly, to have at least 85% power to detect the effect, we need 60 subjects total. Our power analyses are also based on actual effect sizes from our previous research (e.g., r=.54+ for neural prediction and r=.80 for neural mechanism, suggesting n=40 per group is sufficient at β=.80). On the basis of our prior published findings[11], we predict sufficient variability in the PRT-response neuropredictive brain regions as well as sufficient variability in behavioral responses to PRT to allow us to adequately test our two key (and most complex) hypotheses under Aim 2. For eye tracking analyses (Aim 3), the proposed sample sizes are sufficient based on identified effects from prior work evaluating multi-level social information processing via eye-tracking in adults with ASD (Shic et al., in prep; data from Roche, Ltd. study) (e.g., d=.92 for orienting to biological motion under the effects of a novel vasopressin V1a antagonist, suggesting n=16 per group is sufficient at β=.80). Furthermore, based on *ongoing* work, we found significant diagnostic group differences for novel eye-tracking tasks in n=21 ASD and n=25 TD participants, d=.68 to d=.97, suggesting n=28 per group. Therefore, taken together, a sample size of 80 is appropriate for this study.

Data Analyses for Hypotheses Testing.

Our overall approach to data analysis relies on the framework provided by the class of mixed (random and fixed) effects models referred to as hierarchical linear models (HLMs). HLMs were developed to deal

APPROVED BY THE YALE UNIVERSITY IRB 8/5/2021

with clustering of observations within groups of participants (for instance, of children within schools) and within-participant clustering of observations (for instance, in longitudinal studies, or studies with repeated stimulus presentations). HLMs provide the means to deal uniformly with methods typically used in ROI-based fMRI analysis, voxel-based whole-brain fMRI analysis, and longitudinal data analysis of behavioral measures. In each of these cases, the differences in approach to analysis concern the ways in which non-independence of observations is dealt with. Any neuroimaging experiment involves repeated individual voxel measurements nested within multiple scan process and experimental condition-induced levels (the conceptual ordering of the levels of clustering is actually analysis dependent). The result is that each individual measured value from a single measurement location is correlated with other values from that location and neighboring ones. Therefore, it is not an independent observation in the statistical sense. Furthermore, large numbers of statistical tests comparing individual voxel signals are performed in these analyses; hence, the use of random-effects models in standard voxel-based fMRI analysis packages to correct for these problems. The effects of repeated measurement are typically treated as nuisance effects, but they can also be modeled as effects of interest, for instance, to examine patterns of fMRI signal habituation over multiple stimulus presentations. ROI analyses typically deal with the issue of the non-independence of repeated voxel measures by averaging over voxels, time slices, and trials in pre-specified brain regions. This “collapsing” over levels of clustering reduces the HLM to a simple fixed-effects model; typically, an ANOVA (the simplest form of which is a *t*-test) in fMRI analyses, but the same logic applies to linear regression with continuous predictors.

Statistical models are, in all of these analyses, fit at the level of brain region, with each participant having a single value for each experimental condition. However, at this level, and across all of these types of predictors, we can also fit multivariate models (involving multiple outcome variables) to model multiple fMRI measures simultaneously. An example of this would-be path-analytic approaches to examine functional connectivity of brain regions. In the proposed project, we will often take the ROI approach to our hypotheses testing, but we have the added complication of having multiple scans from our participants, before and after our treatments of interest. In other words, we will have within-participants clustering of scan data. This is easily dealt with in HLM by treating participant identity as a random effect while continuing to average across all lower nested levels, resulting in analysis at the level of repeated scans. The simplest form of analysis here will treat the within-participant correlations between scan occasions as a nuisance factor, providing what is essentially an ANOVA (or multiple regression) with correction for the repeated measures. However, within-participant slopes (i.e., change over time) can also be modeled to determine whether different children are changing in different ways (i.e., growth curve analysis), as a function of treatment condition. For example, we will examine change as a function of treatment condition. Such analyses can be performed in many packages, including SAS PROC MIXED (or with the SAS GLIMMIX macro if the outcomes are non-normal), Stata, and HLM. Latent growth curve analysis is a further extension that seeks to detect classes of individuals with different patterns of change over time empirically (such analyses can be performed using PROC TRAJ in SAS or GLAMM in Stata). An advantage of these methods is that, unlike repeated measures ANOVA, they can handle the presence of individuals with different numbers of repeated measures. In other words, they use whatever data are available from each individual.

The first aim of the study is to compare efficacy of PRT+OXT vs. PRT+placebo in a randomized controlled trial. A strict intent-to-treat approach, in which all randomized subjects are included in the analysis, will be adopted to test ***Hypothesis 1a***. The primary approach to analysis of the SRS-2 (the

primary dimensional outcome measure administered by the IE at baseline, midpoint and endpoint) will be the mixed effects linear model also called a random regression model. The model assumes that missing data are missing at random and includes all available data. This approach is more robust than other alternatives such as analysis of completers only or using the last observation carried forward. Each subject's response during the trial is modeled by regressing the SRS-2 score against time. The intercept and slope of the regression are estimated for each subject. Subjects who show a strongly positive response (indicated by lower scores on the SRS-2) over time will have a steeper regression slope than subjects who barely change over time. The average slope of the regression line (i.e., clinical response) over time will be compared across the treatment groups (PRT+OXT vs. PRT+placebo) and tested for statistical significance.

Hypothesis 1b parallels Hypothesis 1a but focuses on brain responses. It will be tested in a similar way, but we will utilize a multivariate model that includes measurements of activity and connectivity from the brain networks of interest. This hypothesis focuses on examining the neural effects of adding OXT vs. placebo to PRT treatment, by collecting fMRI during rs-fcMRI and tb-fMRI. Focusing first on the tb-fMRI data: ***Hypothesis 1b*** will be tested using hierarchical linear models (HLM) with treatment assignment (PRT+OXT vs. PRT+placebo), assessment visit (baseline and endpoint) as independent variables and the BIO vs. SCRAM BOLD signal in the AON, ToM, and SMN networks and levels of functional connectivity (PPI regressor) as dependent variables. The association of change in the activity/connectivity in the BOLD signal with the change in SRS-2 scores will be examined by calculating the bivariate correlations between the pre- to post-treatment change in the BOLD signal in each network as well as their level of functional connectivity with change in the SRS-2 total score (the primary continuous outcome measure).

Resting-state connectivity analyses will follow steps described by Hampson and colleagues[87,88]. We will conduct seed-based analyses with seeds in amygdala and nACC to test ***Hypothesis 1b***. For example, to examine amygdala-pSTS connectivity, the time-course of activity for all voxels in the amygdala will be averaged to obtain a reference (seed) time-course. This seed time-course will be correlated with the time-course of each other voxel in the brain, and the resulting *r*-values will be transformed to z-values. These maps will be transformed to a common reference space via a concatenation of nonlinear and rigid transformations as described previously[89]. A pSTS ROI will be defined in reference space as a sphere centered around the peak activation falling within pSTS in the BIO > SCRAM contrast from our independent, preliminary studies. The average value within this sphere will be computed for the pre- and post-treatment seed maps of amygdala connectivity from each subject. These values will represent amygdala-pSTS connectivity pre- and post-treatment for that subject and will be used as described to test ***Hypothesis 1b***. A seed map analysis of connectivity to the PCC will also be computed from the resting state data collected pre-intervention from each subject. After normalization to the common space, a standard template of the default mode network[90] will be overlaid on each subjects' PCC seed map and the values of all voxels in the network (excluding the PCC) will be averaged. These numbers will represent pre-treatment connectivity in the default mode network (DMN) for each subject. In addition to these hypothesis-driven analyses, we will analyze the data using an exploratory approach that assesses the network measure of degree in a voxel-wise manner. This technique allows an exploration of rs-fcMRI patterns unbiased by *a-priori* assumptions regarding ROIs.

The second aim is to evaluate the moderating effects of neuropredictive fMRI indices of social perception on response to OXT vs. placebo. ***Hypothesis 2a*** will be tested by adding the levels of

activity/connectivity in the previously identified neuropredictive regions from the BIO vs. SCRAM contrast at baseline as a covariate into our HLM analyses of the effects of PRT treatment on social communication symptoms (quantified via the SRS-2 Total Score). The model will include fixed effects for treatment and time, random effects for subjects and time, interaction of treatment with time, and three-way interaction of time, treatment and moderator variable. Significant three-way interactions will indicate the presence of moderating effects.

The third aim focuses on providing an easier/lower cost approach to biomarkers of treatment response by evaluating how changes in visual scanpaths as outcome variables are associated with concurrent changes in observable behaviors and how eye tracking variables assessed at baseline and midpoint/endpoint are associated with ultimate treatment response as measured by the fMRI and clinical outcomes. ***Hypothesis 3a*** will be evaluated by correlating pretreatment and treatment midpoint eye-tracking metrics of attentional preference for, and sensitivity to, more vs. less socially meaningful stimuli, with brain and behavioral responses to PRT. ***Hypothesis 3b*** involves modeling both PRT participants' change in visual scanpaths from baseline to treatment midpoint, *and* their change in behavioral and brain outcomes from baseline to treatment midpoint. Individual-specific estimates of changes in visual scanpaths will be calculated and used to predict the magnitude of improvement in SRS-2 scores and enhancements in target brain networks from baseline to treatment endpoint

As detailed above, the primary clinical outcome measure will be the SRS-2 Total Score. Symptom inventories and behavioral measures, such as the Vineland-II, BOSCC, and ABC, will also be administered to assess outcomes related to adaptive and maladaptive behavior, respectively. All *a priori* hypothesis testing will occur with the primary clinical outcome (SRS-2 Total Score). However, we will conduct additional exploratory analyses utilizing these other outcome variables. Our primary hypotheses, and data analysis plan will be pre-published, prior to the beginning of data collection, with our registration of this trial on clinicaltrials.gov.

SECTION II: RESEARCH INVOLVING DRUGS, BIOLOGICS, RADIOTRACERS, PLACEBO AND DEVICES

If this section (or one of its parts, A or B) is not applicable, check off N/A and delete the rest of the section.

A. RADIOTRACERS ☒N/A

If NO, an FDA issued IND is required for the investigational use unless RDRC assumes oversight.

1. Check one: ☐IND# *Write here* or ☐RDRC oversight (RDRC approval will be required prior to use)

B. DRUGS/BIOLOGICS ☐N/A

1. If an **exemption from IND filing requirements is** sought for a clinical investigation of a drug product that is lawfully marketed in the United States, review the following categories and complete the category that applies (*and delete the inapplicable categories)*:

Exempt Category 1: The clinical investigation of a drug product that is lawfully marketed in the United States can be exempt from IND regulations if all of the following are yes:	
1. The intention of the investigation is NOT to report to the FDA as a well-controlled study in support of a new indication for use or to be used to support any other significant change in the labeling for the drug.	XX
2. The drug that is undergoing investigation is lawfully marketed as a prescription drug product, and the intention of the investigation is NOT to support a significant change in the advertising for the product.	XX
3. The investigation does NOT involve a route of administration or dosage level or use in populations or other factor that significantly increases the risks (or decreases the acceptability of the risks) associated with the use of the drug product	XX
2. The investigation will be conducted in compliance with the requirements for institutional (HIC) review and with the requirements for informed consent of the FDA regulations (21	XX

CFR Part 50 and 21 CFR Part 56).	
3. The investigation will be conducted in compliance with the requirements regarding promotion and charging for investigational drugs.	XX

2. **Background Information:** Provide a description of previous human use, known risks, and data addressing dosage(s), interval(s), route(s) of administration, and any other factors that might influence risks. If this is the first time this drug is being administered to humans, include relevant data on animal models.

OXT is a nonapeptide hormone produced in the supraoptic nucleus and paraventricular nucleus of the hypothalamus by magnocellular neurosecretory cells and released into the blood from the neurohypophysis. OXT is a key regulating hormone of innate human emotions and behaviors including aggression, attachment, fear, and social cognition. In recent studies, OXT has been shown to act on the limbic system, most notably the amygdala.

The proposed 3-year study will use targeted manipulations with intranasal OXT administrations in conjunction with neurocognitive and neuroimaging paradigms to assess how OXT may impact social brain functioning in children and young adults with ASD and typically developing adults.

OXT has been considered to be heavily involved in the processing of information relating to social cognition, interaction, and approach behavior. We are particularly interested in how children and young adults interpret the social world and how their brain functions when they process social gaze, touch, vocalization and biological motion. People with ASD have known impairments in such social functioning and differential brain activation when they are faced with social tasks. In addition, promising recent studies have shown that OXT has a positive effect on emotion recognition in young men with ASD[45] and that the polymorphism in the OXT receptor gene is related to the prevalence of autism. In light of the above, we would like to assess if administration of OXT will alter brain function in social paradigms in children and young adults with ASD. Eventually, with a better understanding of the functional role of OXT, predictions of novel treatment strategies can be made.

We believe that an understanding of the functional role of OXT in children with ASD is of clear importance. Should modulation OXT levels produce specific impacts on brain functioning or behavioral social functioning in tasks linked to the social world, novel treatment strategies could be suggested. Thus, the purpose of this protocol is to determine whether administration of OXT in children with ASD will alter performance on those social tasks.

The greatest potential risk to the participants involves the possibility of alterations in blood pressure and heart rate; however, these effects are mild in the majority of studies in healthy volunteers. Several studies

have been conducted in which between 20 and 60 IU of OXT were administered (comparable to the 24 IU to be used in the present study), and no serious side effects have been reported[91-95].

Dosing Justification: One study in which participants daily received 4 doses of either 40 or 80 IU of OT for 7 days reported no significant side effects in participants, including no significant changes in blood pressure, pulse, or serum osmolality[92]. Additionally, Anagnostou and colleagues (2014) completed a study where children with ASD ages 10-17 received two doses per day of intranasal oxytocin (administered at home) over 12 weeks. Dosing was 0.4 IU/kg / dose. No severe or serious adverse events were reported. Furthermore, in this study, several measures of social cognition/function, repetitive behaviors and anxiety showed sensitivity to change with some measures suggesting maintenance of effect 3 months past discontinuation of intranasal oxytocin[96]. Anagnostou (2012) completed a similar study with adults, and the results were consistent[97].

Yatawara et al (2016) administered OT intranasally to children as young as 3 years old and no significant side effects were reported. Dosage for this sample was 12 IU in the morning and 12 IU at night[98]. A dose of 48 IU was used in children ages 6-12 (24 IU twice daily) with minimal side effects, and no significant difference in reported side effects between the Oxytocin and placebo groups[99]. In a review published in 2017 no adverse events were linked to the use of intranasal oxytocin in school-aged children[100.]

In ongoing work, Brentani is leading a study on intranasal oxytocin in 11-17-year-old children. The dose is 24IU twice per day for 8 weeks. Alaerts and colleagues are enrolling for a study in adults with ASD. The individuals receive 24IU of oxytocin per day for 4 weeks. Joshi is enrolling children aged 12 years through adulthood. Dosing is 48IU/day for 8 weeks. These studies are evaluating the effects of oxytocin compared to placebo on core symptoms of ASD.

Soorya and colleagues are enrolling children with ASD aged 8-11 in an RCT comparing oxytocin plus behavioral therapy to play therapy. Dosing is 24IU administered before weekly (12 weeks) behavioral therapy sessions.

Hardan and colleagues are recruiting children aged 6-12 years with ASD in an RCT of vasopressin on social communication functioning. Participants aged 6 to 9.5 years of age will receive the maximum dose of 24 IU (12 IU twice daily). Participants aged 9.6 to 12 years of age will receive the maximum dose of 32 IU (16 IU twice daily) for 4 weeks. No severe or significant adverse events are known for the above studies.

Several studies are currently ongoing involving children as young as 3 years of age. Studies at UNC Chapel Hill are investigating oxytocin in children ages 3-17 (mean age 10.3) using a dose of 24IU for children ages 3-10. Another ongoing study is testing children ages 6-17 using doses of 8 IU, 24 IU, or 40 IU depending on study phase. Duke University is currently testing doses of up to 80 IU per day, 24 IU per dose in children ages 3-17 with no significant side effects reported.

For our study, dosage was decided by an ASD- and drug-trial-experienced psychiatrist (Dr. Roger Jou, Assistant Clinical Professor at the Yale Child Study Center). Participants in our study will receive 50% of the typical adult dose, amounting to a full dose of 24IU, three times a week for sixteen weeks (12 IU or one puff per nostril). Oxytocin will be administered at the Yale Child Study Center before each treatment

session, three times a week for 16 weeks. The dosing is below or consistent with the large body of current work on oxytocin. All published findings on oxytocin have reported no severe adverse events. Ongoing studies are continuing with the above dosing with no known severe adverse events.

The children with ASD may experience significant benefit from receiving oxytocin in conjunction with evidence-based behavioral treatment (PRT). We hypothesize that the oxytocin will enhance response to PRT, minimizing the developmental impact of ASD on young children. This hypothesis is consistent with prior work[96-98] as well as ongoing work in the field (e.g., Soorya; Sikich; Dawson; Hardan).

3. **Source:** Identify the source of the drug or biologic to be used. *Write here*

a) Is the drug provided free of charge to subjects? ☒**YES** ☐**NO**

If yes, by whom? Through the grant funding agency: Simons Foundation

iii. **Storage, Preparation and Use:** Describe the method of storage, preparation, stability information, and for parenteral products, method of sterilization and method of testing sterility and pyrogenicity.

The YNHH IDS will prepare the drug according to the procedure outlined in HIC protocol #1011007689. The preparations as noted in the previous protocol #1011007689 have been reviewed by the YNHH IDS and the OXT will be prepared and dispensed accordingly.

Check applicable Investigational Drug Service utilized:

☒ **YNHH IDS** ☐ **CMHC Pharmacy**

☐ **West Haven VA**

☐ **PET Center** ☐ **None**

☐ **Other:**

Note: *If the YNHH IDS (or comparable service at CMHC or WHVA) will not be utilized, explain in detail how the PI will oversee these aspects of drug accountability, storage, and preparation*.

iv. Use of Placebo: ☐Not applicable to this research project

If use of a placebo is planned, provide a justification which addresses the following:

a) Describe the safety and efficacy of other available therapies. If there are no other available therapies, state this.
All children will receive intensive evidence-based behavioral treatment for the duration of study participation. They can also continue participation in school-based intervention while participating in the study. We require that additional treatment not be added from baseline to endpoint, and as much as possible, type and intensity remain stable during study participation. Given the nature of the population (children), we cannot require no change in additional treatment, as school schedules will fluctuate while children are in the study. We account for this in sample size and enrollment periods.

b) State the maximum total length of time a participant may receive placebo while on the study.
16 weeks

c) Address the greatest potential harm that may come to a participant as a result of receiving placebo.
Although there is a placebo administration in this protocol, no standard of care medications for ASD will be withheld from participants. The use of OXT and the placebo in this study are to evaluate the administration of OXT on brain function. The OXT is not being given for a therapeutic purpose.

d) Describe the procedures that are in place to safeguard participants receiving placebo.

Because the study is double blinded, study personnel will monitor all participants

v. Continuation of Drug Therapy After Study Closure ☐Not applicable to this project

Are subjects provided the opportunity to continue to receive the study drug(s) after the study has ended?

☐ **Yes** If yes, describe the conditions under which continued access to study drug(s) may apply as well as conditions for termination of such access. *Write here*

☒ **NO** If no, explain why this is acceptable. Oxytocin is not currently available by prescription for ASD. If need is indicated, we may provide psychiatric referrals for consultation with respect to treatment with other medications.

B. DEVICES ☒N/A

SECTION III: RECRUITMENT/CONSENT AND ASSENT PROCEDURES

1. Targeted Enrollment: Give the number of subjects:

a. Targeted for enrollment at Yale for this protocol: 80

b. If this is a multi-site study, give the total number of subjects targeted across all sites: Not applicable.

2. Indicate recruitment methods below. Attach copies of any recruitment materials that will be used.

☒ Flyers	☒ Internet/web postings	☐ Radio
☒ Posters	☒ Mass email solicitation	X Telephone
☒ Letter (mailout to doctors and school professionals)	☒ Departmental/Center website	☐ Television
☐ Medical record review*	☒ Departmental/Center research boards	☒ Newspaper
☒ Departmental/Center newsletters	☒ Web-based clinical trial registries	X Clinicaltrails.gov
☒ YCCI Recruitment database	☒ Social Media (Twitter/Facebook):	
☐ Other:		

* **Requests for medical records should be made through JDAT as described at** http://medicine.yale.edu/ycci/oncore/availableservices/datarequests/datarequests.aspx

3. Recruitment Procedures:

a. Describe how potential subjects will be identified.

- At Yale Child Study Center Specialty Clinics, clinician may contact their patient to inform them of this study. If their patient is interested, they will have the option of contacting the recruitment coordinators for this study, Krista Drapalik and Martha Jane Williams, or have a recruitment coordinator contact them.
- Prior participants at the Yale Child Study Center who have expressed an interest in remaining considered for research studies by indicating on their past consent forms will be contacted by phone, mail or email when a study is available for which they are eligible. We will only contact if their consent was provided (see protocol **#1206010363, #1106008625,** #1004006656 or #0711003222). Eligibility is determined by querying our research database.
- Local private and public schools may contact us seeking options for their students and their parents.

b. Describe how potential subjects are contacted. Potential subjects will receive a telephone call or email describing the study.

c. Who is recruiting potential subjects?

If the subjects are contacted by phone, our intake and recruitment coordinators, Krista Drapalik and Martha Jane Williams will make the initial contacts with the families.

4. Assessment of Current Health Provider Relationship for HIPAA Consideration:

Does the Investigator or any member of the research team have a direct existing clinical relationship with any potential subject?

☐Yes, all subjects

☐Yes, some of the subjects

☒No

If yes, describe the nature of this relationship.

5. Request for waiver of HIPAA authorization: (When requesting a waiver of HIPAA Authorization for either the entire study, or for recruitment purposes only. Note: if you are collecting PHI as part of a phone or email screen, you must request a HIPAA waiver for recruitment purposes.)

Choose one:

☐ For entire study

☒ For recruitment/screening purposes only

☐ For inclusion of non-English speaking subject if short form is being used and there is no translated HIPAA research authorization form available on the University's HIPAA website at hipaa.yale.edu.

i. Describe why it would be impracticable to obtain the subject's authorization for use/disclosure of this data:

ii. If requesting a waiver of **signed** authorization, describe why it would be impracticable to obtain the subject's signed authorization for use/disclosure of this data: requesting a waiver of signed authorization for telephone screening. This is for potential participants who contact us with an interest in the study

The investigator assures that the protected health information for which a Waiver of Authorization has been requested will not be reused or disclosed to any person or entity other than those listed in this application, except as required by law, for authorized oversight of this research study, or as specifically approved for use in another study by an IRB.

Researchers are reminded that unauthorized disclosures of PHI to individuals outside of the Yale HIPAA-Covered entity must be accounted for in the "accounting for disclosures log", by subject name, purpose, date, recipients, and a description of information provided. Logs are to be forwarded to the Deputy HIPAA Privacy Officer.

6. **Process of Consent/Assent:** Describe the setting and conditions under which consent/assent will be obtained, including parental permission or surrogate permission and the steps taken to ensure subjects' independent decision-making.

Verbal consent from families upon initial screening and recruitment will be obtained. Upon recruitment, the family will be mailed copies of the consent forms prior to their initial visit so that these forms may be reviewed ahead of time. Consent will be obtained in-person at the Child Study Center during the child's first study visit. The researcher will answer any questions regarding the study and remind the subject that participation in the study is voluntary and that it can be terminated by the parent at any time with no obligations to continue and no penalties whatsoever. Refusal to participate will in no way influence the individual's relationship with the Clinics at the Yale Child Study Center.

7. **Evaluation of Subject(s) Capacity to Provide Informed Consent/Assent:** Indicate how the personnel obtaining consent will assess the potential subject's ability and capacity to consent to the research being proposed.

   Given the age and functioning level of the children (5-9 years old with delayed language skills and ASD), the children's level of language comprehension and developmental/intellectual functioning preclude the ability to sign assent/consent. Therefore, the consent personnel will verbally explain in simple, child-friendly terms the procedures and what participation in the study entails. Written parental permission will be obtained prior to explaining the procedures to the child.

8. **Non-English-Speaking Subjects:** Explain provisions in place to ensure comprehension for research involving non-English speaking subjects. If enrollment of these subjects is anticipated, translated copies of all consent materials must be submitted for approval prior to use.

   The current study does not enroll non-English speaking participants.

As a limited alternative to the above requirement, will you use the short form* for consenting process if you unexpectedly encounter a non-English speaking individual interested in study participation and the translation of the long form is not possible prior to intended enrollment? YES ☐ NO ☐

Note* If more than 2 study participants are enrolled using a short form translated into the same language, then the full consent form should be translated into that language for use the next time a subject speaking that language is to be enrolled.

Several translated short form templates are available on the HRPP website (yale.edu/hrpp) and translated HIPAA Research Authorization Forms are available on the HIPAA website (hipaa.yale.edu). If the translation of the short form is not available on our website, then the translated short form needs to be submitted to the IRB office for approval via modification prior to enrolling the subject. ***Please review the guidance and presentation on use of the short form available on the HRPP website.***

If using a short form without a translated HIPAA Research Authorization Form, please request a HIPAA waiver in the section above.

9. Consent Waiver: In certain circumstances, the HIC may grant a waiver of signed consent, or a full waiver of consent, depending on the study. If you will request either a waiver of consent, or a waiver of signed consent for this study, complete the appropriate section below.

☐Not Requesting any consent waivers

☐Requesting a waiver of <u>signed</u> consent:

☒ **Recruitment/Screening only** *(if for recruitment, the questions in the box below will apply to recruitment activities only)*

☐ **Entire Study (**Note that an information sheet may be required.)

For a waiver of signed consent, address the following:

- Would the signed consent form be the only record linking the subject and the research? YES ☐ NO ☐
- Does a breach of confidentiality constitute the principal risk to subjects? YES ☐ NO ☐

OR

- Does the research pose greater than minimal risk? YES ☒ NO☐
- Does the research include any activities that would require signed consent in a non-research context? YES ☐ NO ☒

☐ Requesting a waiver of consent:

☐ **<u>Recruitment/Screening</u> only** *(if for recruitment, the questions in the box below will apply to recruitment activities only)*

☐ **<u>Entire Study</u>**

For a full waiver of consent, please address all of the following:

- Does the research pose greater than minimal risk to subjects?

  ☐ Yes *If you answered yes, stop. A waiver cannot be granted.*

  ☐ No

- Will the waiver adversely affect subjects' rights and welfare? YES ☐ NO☐
- Why would the research be impracticable to conduct without the waiver? *Write here*
- Where appropriate, how will pertinent information be returned to, or shared with subjects at a later date? *Write here*

SECTION IV: PROTECTION OF RESEARCH SUBJECTS

Confidentiality & Security of Data:

1. What protected health information (medical information along with the HIPAA identifiers) about subjects will be collected and used for the research?

   Name, date of birth, names of parents and siblings, home address, history of interventions, developmental/medical history (provided by parents), full face photographs and videos, and prior evaluations from other health care providers.

2. How will the research data be collected, recorded and stored?

   Data will be collected by the clinicians, research associates, and research assistants trained to collect the research measures. All personnel are HIC trained and HIPAA certified. Once data are collected, the hard copies of all the testing measures, materials, and observation notes will be stored in a locked file cabinet within a locked room. After each visit, we will de-identify all data and assign a subject number instead. A master list matching the subject name to the subject number will be kept separately in a restricted-access file cabinet; only the P.I. and select research personnel will have access to the list. In addition, the data from the hard copies will be selected and entered into the password-protected computer database (Prometheus Research database) by a limited number of staff members. All this will be stored on a secure server which is password-protected and will allow for audits and backups

by the PI, Project Coordinator, and IT partner working on the research team.

We are also asking participants consent to provide research data and related findings to the NIH/NIMH Data Repositories (formerly known as the National Database for Autism Research (NDAR)). NIH/NIMH Data Repositories are a biomedical informatics system and data repository, created by the National Institutes of Health to assist biomedical researchers working to develop a better understanding of autism and/or to develop more effective methods to diagnose, treat and prevent autism spectrum disorders. Data entered into NIH/NIMH Data Repositories will be kept confidential. With NIH/NIMH Data Repositories being designed for access by researchers only, data provided to NIH/NIMH Data Repositories will be de-identified.

3. How will the digital data be stored? ☐CD ☐DVD ☐Flash Drive ☒Portable Hard Drive ☒Secured Server ☒Laptop Computer ☒Desktop Computer ☐Other

4. What methods and procedures will be used to safeguard the confidentiality and security of the identifiable study data and the storage media indicated above during and after the subject's participation in the study?

All electronic data will be stored on encrypted devises or password protected Yale network servers.

All information obtained will be kept confidential. Information obtained will not be discussed with anyone outside of the research team. This information will only be accessible to the investigators collaborating in this study and their research study staff. All video recordings will be stored in a secure location labeled by subject number. Presentations or publications based on these data will not identify subjects by name. All hard copies of records pertaining to the participant's involvement in this research will be de-identified and stored in a locked file cabinet.

Limits to confidentiality include cases of suspected child abuse or neglect or suspected elder abuse or neglect. In case of this, study members are required to report reasonable suspicion of child abuse or neglect to the Connecticut Department of Children and Families.

All portable devices must contain encryption software, per University Policy 5100. If there is a technical reason a device cannot be encrypted please submit an exception request to the Information Security, Policy and Compliance Office by clicking on url http://its.yale.edu/egrc or email it.compliance@yale.edu

What will be done with the data when the research is completed? Are there plans to destroy the identifiable data? If yes, describe how, by whom and when identifiers will be destroyed. If no, describe how the data and/or identifiers will be secured.

Permission will be secured from parents in the Parental Permission form to keep all research records for at least 10 years after the child's participation, or until the data is to be destroyed by the PI.

5. If appropriate, has a Certificate of Confidentiality been obtained? *Not applicable.*

SECTION V: POTENTIAL BENEFITS

Potential Benefits: Identify any benefits that may be reasonably expected to result from the research, either to the subject(s) or to society at large. (Payment of subjects is not considered a benefit in this context of the risk benefit assessment.)

Research findings from this study will add to the knowledge of treatment for children with ASD. Based on all the assessments conducted during the study, families will receive a developmental evaluation at no charge, and will be placed in touch with appropriate community service providers so that they can access additional intervention services. Additionally, there may be direct benefits to families who are assigned to receive the intervention procedures, as children will receive direct intervention and parents will learn techniques which may also enhance their child's development. It is possible that children who receive Oxytocin may see additional benefit from receiving the hormone prior to treatment. In addition, this work has considerable potential to benefit society, as it may lead to the dissemination of further information regarding treatment for ASD. Results of the research will be made available to the public through journal publications and /or conferences.

SECTION VI: RESEARCH ALTERNATIVES AND ECONOMIC CONSIDERATIONS

1. **Alternatives:** What other alternatives are available to the study subjects outside of the research?

   Participants are not required to enroll in the study in order to receive a comprehensive developmental assessment for the presence of autism spectrum disorder. Alternative to participation involves treatment in community agencies. Another alternative is to not participate.

2. **Payments for Participation (Economic Considerations):** Describe any payments that will be made to subjects, the amount and schedule of payments, and the conditions for receiving this compensation.

Participants will be reimbursed for parking fees during study visits as well as $50 for each MRI scan attempted. Participants will also receive $40 for each of the eye-tracking visits.

3. **Costs for Participation (Economic Considerations):** Clearly describe the subject's costs associated with participation in the research, and the interventions or procedures of the study that will be provided at no cost to subjects.

There is no cost for participation in the research. All assessments and interventions conducted by clinicians or other research staff will be at no cost to the family. Parking during the course of study visits will be validated or reimbursed as indicated above in bullet #2.

The family may however encounter costs of participating in this study in terms of gas, childcare, and missing work time equivalent in monetary spending. These costs will not be covered by the study.

4. **In Case of Injury:** This section is required for any research involving more than minimal risk, and for minimal risk research that presents the potential for physical harm (e.g., research involving blood draws).

    a. Will medical treatment be available if research-related injury occurs? There are no anticipated injuries related to participation in this protocol. However, in case of injury as a result of participation, treatment will be provided.

    b. Where and from whom may treatment be obtained? *Treatment will be at the YNHH or to the location of parents' or caregivers' choice.*
    c. Are there any limits to the treatment being provided? *Limits on treatment are only those placed on the subjects by parents' insurance.*
    d. Who will pay for this treatment? Parents' insurance carrier or they themselves will be expected to pay the costs of this treatment. No additional financial compensation for injury or lost wages is available.
    e. How will the medical treatment be accessed by subjects? *Parents or caregivers will be responsible for transporting subjects to receive treatment.*

IMPORTANT REMINDERS

Will this study have a billable service? **Yes** ☐ **No**☒

A billable service is defined as any service rendered to a study subject that, if he/she was not on a study, would normally generate a bill from either Yale-New Haven Hospital or Yale Medical Group to the patient or the patient's insurer. The service may or may not be performed by the research staff on your study, but may be provided by professionals within either Yale-New Haven Hospital or Yale Medical Group (examples include x-rays, MRIs, CT scans, specimens sent to central labs, or specimens sent to pathology). Notes: 1. There is no distinction made whether the service is paid for by the subject or their insurance (Standard of Care) or by the study's funding mechanism (Research Sponsored). 2. This generally includes new services or orders placed in EPIC for research subjects.

If answered, "yes", this study will need to be set up in OnCore, Yale's clinical research management system, for Epic to appropriately route research related charges. Please contact oncore.support@yale.edu

Are there any procedures involved in this protocol that will be performed at YNHH or one of its affiliated entities? **Yes ☐ No ☒**

If Yes, please answer questions a through c and note instructions below.

a. Does your YNHH privilege delineation currently include the **specific procedure** that you will perform? **Yes ☐ No ☐**

b. Will you be using any new equipment or equipment that you have not used in the past for this procedure? **Yes ☐ No ☐**

c. Will a novel approach using existing equipment be applied? **Yes ☐ No ☐**

If you answered "no" to question 4a, or "yes" to question 4b or c, please contact the YNHH Department of Physician Services (688-2615) for prior approval before commencing with your research protocol.

IMPORTANT REMINDER ABOUT RESEARCH AT YNHH

Please note that if this protocol includes Yale-New Haven Hospital patients, including patients at the HRU, the Principal Investigator and any co-investigators who are physicians or mid-level practitioners (includes PAs, APRNs, psychologists and speech pathologists) who may have direct patient contact with patients on YNHH premises must have medical staff appointment and appropriate clinical privileges at YNHH. If you are uncertain whether the study personnel meet the criteria, please telephone the Physician Services Department at 203-688-2615. **By submitting this protocol as a PI, you attest that you and any co-investigator who may have patient contact has a medical staff appointment and appropriate clinical privileges at YNHH.**

References

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