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CLINICAL RESEARCH PROTOCOL
A PHARMACO-IMAGING APPROACH TO PREDICTING SOCIAL FUNCTIONING AND CLINICAL RESPONSES TO OXYTOCIN ADMINISTRATION IN SCHIZOPHRENIA

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LIST OF ABBREVIATIONS

AE	adverse event
CAINS	Clinical Assessment Interview for Negative Symptoms
CFR	Code of Federal Regulations
CRF	case report form
DMC	Data Monitoring Committee
DSMB	Data Safety Monitoring Board
DSM-V	Diagnostic and Statistical Manual of Mental Disorders, 5th Edition
FACES	Facial Expression Coding System
FDA	Food and Drug Administration
fMRI	Functional Magnetic Resonance Imaging
GCP	Good Clinical Practice
HIPAA	Health Insurance Portability and Accountability Act of 1996
ICF	informed consent form
ICH	International Conference on Harmonisation
IEC	Independent Ethics Committee
IRB	Institutional Review Board
IU	International Unit
OT	Oxytocin
PI	Principal Investigator
rTPJ	Right Temporal Parietal Junction
SAE	Serious Adverse Experience
SFS	Social Functioning Scale
ToM	Theory of Mind

PROTOCOL SYNOPSIS

TITLE	A pharmaco-imaging approach to predicting social functioning and clinical responses to oxytocin administration in schizophrenia
SPONSOR	Joshua Woolley, M.D., Ph.D.
FUNDING ORGANIZATION	VA Clinical Science Research and Development Program
NUMBER OF SITES	2
RATIONALE	The purpose of this project is to investigate the neural mechanisms of oxytocin's (OT) prosocial effects in patients with schizophrenia. Schizophrenia is a prevalent disorder that severely impacts social function in affected individuals – it deprives patients of precious relationships, undermines lifelong occupational function, and causes great stress for families and caregivers. Current treatments for schizophrenia are inadequate and have substantial side effects. Thus, novel pharmacologic interventions that target key neurophysiological deficits in the disease are desperately needed. Patients with schizophrenia have significant social cognitive deficits including impaired theory of mind (ToM; i.e., impaired understanding of other peoples' mental states) as indexed by poor recognition of facial emotion [1],[2]. These deficits interfere with social relationships, impact community functioning, and are more common and more strongly associated with quality of life and functional outcomes than "positive" symptoms such as hallucinations and delusions, and "cold cognition" such as executive functioning and working memory. Abnormal neural responses underlie these social deficits. Current antipsychotic medications are ineffective at treating social deficits, improving social functioning, or normalizing neural responses in patients with schizophrenia. However, oxytocin, a neuropeptide involved in attachment, parenting, and sociality in mammals, has promise as a treatment for these deficits. Intranasally administered oxytocin is safe for humans and has prosocial and beneficial neurophysiological effects in healthy and patient populations. The investigators propose to use functional magnetic resonance imaging (fMRI) to elucidate the neural mechanisms of oxytocin's prosocial effects on critical social cognitive processes. These experiments will: 1) Provide high-impact data on how oxytocin affects neural mechanisms that underlie social cognition in healthy individuals and in patients with schizophrenia; and 2) Lead to a deeper understanding of how to intervene to improve functioning in these neural systems.
STUDY DESIGN	This is a double-blind, placebo-controlled, counterbalanced order with a cross-over design one week apart, followed by a randomized,

	double-blind, three week (18-24 days) study design. We will also be implementing a response adaptive randomization (RAR) design after the first 60 participants complete the study. This will change the allocation ratio to favor the drug treatment arm with greater improvement in social functioning, while keeping adequate statistical power in all arms.
PRIMARY OBJECTIVE	The objective is to clarify how and in whom exogenous oxytocin improves social functioning in schizophrenia as measured by acute neural response patterns during social cognitive tasks and social functioning as measured by the Social Functioning Scale (SFS).
SECONDARY OBJECTIVES	Social skills will be assessed during the chronic period over the phone or video chat. Participants will answer questions about their social interactions and experiences. Theory of Mind (ToM) will be assessed using the Hinting Task a well validated audiovisual tool that incorporates social interferences. Negative symptoms will be assessed with Clinical Assessment Interview for Negative Symptoms (CAINS), comprising two subscales reflecting the negative symptom factors. Data will be analyzed with manual and computerized approaches.
NUMBER OF SUBJECTS	100 total participants
SUBJECT SELECTION CRITERIA	Inclusion Criteria: 18-70 years of age; meet DSM-V criteria for schizophrenia schizophreniform, schizoaffective disorder or brief psychotic disorder; no medication changes or psychiatric hospitalizations in the past month; SFS raw score of no more than 75. Exclusion Criteria: Active substance use disorder in the past month except mild to moderate cannabis use disorder, schizoaffective, schizophreniform disorder, or brief psychotic disorder; DSM-5 diagnosis other than schizophrenia; Significant neurological/medical disorders; a pacemaker; extensive dental work; claustrophobia; deafness; inability

	to read; current participation in a psychosocial intervention; taking high dose testosterone, estrogen/progesterone. Currently pregnant, breastfeeding, or unwilling to practice birth control during participation.
TEST PRODUCT, DOSE, AND ROUTE OF ADMINISTRATION	fMRI phase: Single dose 40 IU of intranasal oxytocin Longitudinal phase: Twice-daily for approximately 3 weeks (18- 24 days) of 40 IU of intranasal oxytocin
CONTROL PRODUCT, DOSE AND ROUTE OF ADMINISTRATION	fMRI phase: Single dose of intranasal placebo Longitudinal phase: Twice-daily for approximately 3 weeks (18-24 days) of intranasal placebo
DURATION OF SUBJECT PARTICIPATION AND DURATION OF STUDY	*Subjects will be on study for up to 42 days* **Screening:** The investigators will contact subjects to conduct a phone screening interview to verify eligibility. Screening will take at most twenty minutes. In order to reduce number of in-person visits, Visit 1 has been adapted to be completely remote. Participants will verbally consent at the start of Visit 1, before completing any study tasks or assessments. Clinical diagnosis will be obtained prior to participation and will take up to 4 hours. Written informed consent will be obtained during Visit 2, the first in-person visit, before study drug administration. **Treatment:** Subjects will participate in the study for up to 7 weeks. The first two visits will be separated by at least 1 week. The third and fourth visit will be separated by at least 3 weeks (18-24 days). Each visit will be between one to four hours. The total direction of the study is expected to be 5 years. 54 months for subject recruitment.
CONCOMMITANT MEDICATIONS	Allowed: atypical antipsychotic medication of a consistent, therapeutic dose Prohibited: high dose testosterone or estrogen/progesterone
EFFICACY EVALUATIONS	The epidemiologist will be evaluating social functioning at multiple points throughout the study to determine the drug treatment arm with greater improvement which will then be implemented in the RAR design. Results will remain blinded to the study staff.
PRIMARY ENDPOINT	• Neural activity from fMRI

	• Social Functioning Scale (SFS)
SECONDARY ENDPOINTS	• Social Skills • Negative Symptoms • Theory of Mind
OTHER EVALUATIONS	
SAFETY EVALUATIONS	No serious adverse effects have been demonstrated in studies examining intranasal administration of OT in numerous patient and healthy populations. Therefore, we don't predict any serious adverse events to occur during this study. Documented side effects include runny, stuffy, or irritated nose, and more rarely, nausea and vomiting. Participants may experience distress due to the nature of personal questions about mental health, substance use, and life experiences. They may also experience anxiety and/or fatigue due to length of the study days.
PLANNED INTERIM ANALYSES	Interim analysis will be conducted after the first 60 participants have completed the study and at multiple points over the course of the study. Resultswill remain blinded to all other study staff.
STATISTICS **Primary Analysis Plan**	Linear regression analyses with SFS change and rTPJ activation as the primary outcomes and drug modeled as a 2-level dummy variable comparing the study drug vs. placebo. Similar analyses will be conducted for the secondary clinical outcomes including social ability, negative symptoms (CAINS and Theory of Mind (HintingTask).
Rationale for Number of Subjects	All power and effect size estimates were calculated using STATA/SE v. 15.1 using 80% power and two-sided alpha=0.05. To be conservative, the investigators used the one-way command with two or three groups. The proposed sample size of 100 participants enables us to detect effect sizes of 0.23 for the primary hypotheses, which is clinically meaningful.

1 BACKGROUND

Oxytocin (OT) is a neuropeptide produced in the hypothalamus that promotes affiliative behavior in animals[1], and is sometimes called "the bonding hormone." The OT system has multiple interactions with dopaminergic reward pathways and this interplay likely mediates social behavior, permitting a range of social behaviors including trust, aggression, pair bonding and parenting[2].

2 STUDY RATIONALE

Oxytocin plays an important role in social behavior in mammals, including humans, increasing prosocial behavior and improving social cognition in healthy and autistic individuals[3]. Evidence from animal and human models suggests that oxytocin may also play a specific role in the social deficits of schizophrenia[4,5,6,7]. For example, peripheral oxytocin levels increase after a trust-related interaction in healthy subjects but not in patients with schizophrenia and this lack of response predicts negative symptom[6,7] severity. Intranasal oxytocin administration has powerful prosocial effects and is extremely well tolerated. After intranasal oxytocin administration, healthy subjects gaze more at the information-rich eye region of faces[8] and have better ToM[9]. These data indicate that exogenous oxytocin selectively improves various aspects of social cognition and raises the possibility that exogenous oxytocin may be an effective adjunct treatment for improving social deficits in patients with schizophrenia. Indeed, several small recent preclinical studies and our own preliminary data demonstrate that oxytocin has positive effects on social cognition in schizophrenia, improving ToM [10,11,12]. Furthermore, a recent, small clinical trial found that three weeks of intranasal oxytocin decreased negative symptoms of schizophrenia[13]. While early work is promising, sample sizes are small and thus far there has been no integration of the observed behavioral effects of oxytocin with assessment of neural activity in schizophrenia.

2.1 Risk / Benefit Assessment

Potential side effects of intranasal OT include a runny, stuffy, or irritated nose. Studies using intranasal administration of OT of this dosage have reported no side effects [13,14]. Pintocin® and Syntocinon® are synthetic and injectable forms of naturally occurring OT regularly used to induce uterine contractions in labor. The compounds may cause nausea and vomiting. No serious adverse effects have been demonstrated in studies examining intranasal administration of OT in numerous patient and healthy populations.

To minimize the risk of side effects and discomforts, study participants will be closely monitored for any adverse reactions to the intranasal administration of OT. The proper usage of an intranasal device will be demonstrated and explained to participants who may not be familiar with this type of drug administration. A medically licensed physician will be available by pager throughout the study.

The risks associated with intranasal OT use are minimal. However, all study participants will be monitored for any physical side effects and will be provided with additional rest, a drink of water, or the option to discontinue the study should these effects occur.

3 STUDY OBJECTIVES

3.1 Primary Objective

The primary objective is to clarify how and in whom oxytocin improves social functioning in schizophrenia. The study will assess the effects of exogenous oxytocin on social functioning, as measured by the Social Functioning Scale (SFS). Neural activation patterns, as measured will be measured by fMRI rTPJ responses during well-characterized social cognitive tasks. This will help determine if acute fMRI rTPJ responses to single oxytocin administration predicts clinical responses to chronic oxytocin treatment in schizophrenia participants.

3.2 Secondary Objectives

The secondary objective is to compare the acute effects of a single administration oxytocin vs. placebo, to the clinical and behavioral effects of chronic OT treatment vs. placebo in schizophrenia participants. The study will assess the effects of exogenous oxytocin on laboratory-based social interactions, Theory of Mind (ToM), and negative symptoms in participants with schizophrenia.

4 STUDY DESIGN

4.1 Study Overview

100 subjects are planned. During the fMRI phase, intranasal oxytocin and placebo will be administered to each subject in a randomized, double-blind, counterbalanced order with a cross-over design, at least one week apart. Testing will commence 45 minutes after drug administration and continue for no longer than 3 hours.

During the longitudinal phase, participants will then be randomized to receive approximately three weeks (18-24 day range) of twice daily administration of either OT or placebo. Testing will commence on baseline and after threeweeks (18 to 24 days) of drug administration.

Screening data will be reviewed to determine subject eligibility. Subjects who meet all inclusion criteria and none of the exclusion criteria will be entered into the study.

The following treatment regimens will be used:

- Experimental treatment: Intranasal oxytocin
- Placebo: Saline solution

Total duration of subject participation will be 15 hours over 2 months.

5 CRITERIA FOR EVALUATION

5.1 Primary Efficacy Endpoint

The primary efficacy endpoint will be social functioning measured by the Social Functioning Scale (SFS). SFS is one of the most commonly used social functioning measures in schizophrenia research, yields good information about abilities and activities, and is reliable, valid, and sensible and responsive to change. The endpoint will be assessed from baseline to end of the task. We will also assess activation of the rTPJ during social cognitive tasks measured with fMRI.

5.2 Secondary Efficacy Endpoints

- Social skills will be assessed during the chronic period over the phone or video chat. Participants will answer questions about their social interactions and experiences.
- Theory of Mind (ToM) will be assessed using The Hinting Task a well validated audiovisual tool that incorporates social inferences. The endpoint will be assessed from baseline to end of the task.
- Negative symptoms will be assessed with Clinical Assessment Interview for Negative Symptoms (CAINS), comprising two subscales reflecting the negative symptom factors. The endpoint will be assessed from baseline to end of the task.

5.3 Safety Evaluations

- No serious adverse effects have been demonstrated in studies examining intranasal administration of OT in numerous patient and healthy populations. Therefore, we don't predict any serious adverse events to occur during this study. Documented side effects include runny, stuffy, or irritated nose, and more rarely, nausea and vomiting. Participants may experience distress due to the nature of personal questions about mental health, substance use, and life experiences. They may also experience anxiety and/or fatigue due to the length of the study days.

5.4 Other Evaluations (include only if applicable)

- Positive symptoms will be assessed with the 34-item, interview-based, Scale for the Assessment of Positive Symptoms, which focuses on hallucinations, delusions, disorganized behavior and thought disorder
- Quality of life will be assessed with the Quality of Life Scale (QLS), a 7-item assessment that measures domains such as interpersonal relations, occupation role functioning and intrapsychic foundations

- The Experience in Close Relationships (ECR) questionnaire is a 9-item questionnaire that will be used to assess attachment style in respect to a variety of close relationships.

6 SUBJECT SELECTION

6.1 Study Population

Subjects with a diagnosis of schizophrenia, schizoaffective, schizophreniform disorder, or brief psychotic disorder, who meet the inclusion and exclusion criteria will be eligible for participation in this study.

6.2 Inclusion Criteria

1. Veteran
2. Male or female 18≥70 years of age at Visit 1.
3. Must comprehend English
4. Meet DSM-V criteria for schizophrenia, schizophreniform, schizoaffective disorder, or brief psychotic disorder.
5. Clinically stable, no medication changes or psychiatric hospitalizations in past month
6. Social Functioning Scale raw score ≤75
7. Written informed consent obtained from subject or subject's legal representative and ability for subject to comply with the requirements of the study.

6.3 Exclusion Criteria

1. Active substance use disorder in the past month – except Mild to Moderate Cannabis use disorder.
2. Significant neurological/medical disorder
3. Pacemaker, extensive dental work, or any metal implants
4. Claustrophobia, deafness, or inability to read
5. Current participation in a psychosocial intervention
6. Taking high dose of testosterone, estrogen/progesterone

7. Pregnant, breastfeeding, or unwilling to practice birth control during participation in the study.
8. Presence of a condition or abnormality that in the opinion of the Investigator would compromise the safety of the participant or the quality of the data.
9. Congestion or sinus problems that could interfere with the study as per the opinion of the investigator

7 CONCURRENT MEDICATIONS

All subjects should be maintained on the same medications throughout the entire study period, as medically feasible, with no introduction of new chronic therapies.

7.1 Allowed Medications and Treatments

Standard therapy for schizophrenia is allowed except for treatments noted in the exclusion criteria described above and as noted in the prohibited medications section below.

7.2 Prohibited Medications and Treatments

The following medications are prohibited during the study and administration will be considered a protocol violation.

- High dose testosterone or estrogen/progesterone

8 STUDY TREATMENTS

8.1 Method of Assigning Subjects to Treatment Groups

Randomization sequences will be generated using a response adaptive randomization study design. Enrollment would end when at least 55 participants have been assigned to the drug treatment arm and 40 participants have been assigned to the placebo arm.

8.2 Blinding

Due to the objectives of the study, the identity of test and control treatments will not be known to investigators, research staff, or participants. The following study procedures will be in place to ensure double-blind administration of study treatments.

- Access to the randomization code will be strictly controlled.
- Packaging and labeling of test and control treatments will be identical to maintain the blind. The oxytocin or placebo will be repackaged into smaller bottles containing 1 mL of either the oxytocin or placebo. Designated study staff, who will not be present during the study sessions and will not be involved with running any of the study sessions, will be responsible for repackaging the drugs and keeping track of each bottle's contents for unblinding.

The study blind will be broken on completion of the clinical study and after the study database has been locked.

- Investigators will be made aware of their subject treatment assignments at the completion of the study.

During the study, the blind may be broken **only** in emergencies when knowledge of the participant's treatment group is necessary for further participant management. When possible, the Investigator should discuss the emergency with the Medical Monitor prior to unblinding. During the study, the blind may be broken to the designated unblinded study staff **only** in emergencies when knowledge of the participant's treatment group is necessary for further participant management.

8.3 Formulation of Test and Control Products

8.3.1 Formulation of Test Product

Syntocinon is a formulation of oxytocin, developed originally by Novartis Pharmaceuticals, for intranasal administration to stimulate of milk ejection and prevent of mastisis. Syntocinon is a clear colorless solution that requires no reconstitution. See Table for formulation and measured pH of Syntocinon and Placebo.

Oxytocin and placebo will be ordered from one of the following manufacturers/pharmacies depending on supply and ability to guarantee prompt and cooled shipment:

1. Wellspring Compounding Pharmacy, Berkeley, CA – licensed pharmacy which produces research drugs to many large hospitals and research centers in the San Francisco Bay Area. Use of USP pharmaceutical grade chemicals in a State of California licensed sterile compounding facility.

If the shipment arrives in larger bottles (not packaged for individual-use), Abbotts Pharmacy, or designated study staff will repackage the oxytocin & placebo solution into smaller bottles

containing 40 IU/1ml of oxytocin or placebo. Abbotts Pharmacy or designated study staff, who will not be present during the study sessions and will not be involved with running anyof the study sessions, will be responsible for repackaging the drugs and keeping track of each bottle's contents for unblinding. All labels indicating that the drug is oxytocin or placebo will be removed.

Table 1: Wellspring Pharmacy Formulation and Measured pH of Oxytocin and Placebo

	Oxytocin	**Placebo**
Active Ingredient, mg/mL	Oxytocin USP 40 IU/ml	Water
Other ingredient, mg/mL	0.05% methylparaben 0.025% propylparaben Distilled water	0.05% methylparaben 0.025% propylparaben
pH	4 – 4.5	

8.3.2 Formulation of Control Product

A placebo solution will be provided by one of the three manufacturers/pharmacies described above in amber glass bottles with a nasal spray pump applicators and caps ready for administration. The formation for the placebo is listed in Tables 1 and 2. If the shipment arrives in larger bottles (not packaged for individual-use), Wellspring Pharmacy or designated study staff will repackage the nasal sprays into individual syringes (to be administered with nasal spray atomizers) and remove any labels that the vendors have placed

8.3.3 Packaging and Labeling

Packaging: Each nasal spray is supplied in glass bottles with nasal spray pump applicator and cap. If the shipment arrives in larger bottles (not packaged for individual-use), Abbotts Pharmacy or designated study staff will repackage the nasal sprays into 40 IU/1 ml doses in glass bottles with nasal spray pump applicators and caps.

Labeling: Wellspring Compounding Pharmacy or designated staff will remove any labels that the vendors have placed.

8.4 Supply of Study Drug at the Site

One of the three pharmacies/manufacturers described above will ship the oxytocin and placebo bottles to the investigational site, San Francisco VA Medical Center. The initial study oxytocin and placebo shipment will be shipped after site activation (i.e., all required regulatory documentation has been received by the Principal Investigator and a contract has been executed). Subsequent study drug shipments will be made after initial supply of oxytocin and placebo has run low. Care will be taken to ensure that the study drug supplier is never changed during the course of an active study. However, unpredictability in obtaining the study drug in a timely

fashion from the original manufacturer has led us to institutealternative manufacturing options.

8.4.1 Dosage/Dosage Regimen

Oxytocin and placebo (saline) solution will be administered intranasally followed by a resting period in a neutral environment. The oxytocin dose is comparable to that used previously in human studies of behavior (Davies et. al. 2019). In the first phase of the study,subjects will receive one administration of either oxytocin or placebo on two separate sessions at least one week apart. No adjustments for weight, age, and meals will be made. In the second phase, subjects will self-administer oxytocin or placebo twice daily for three weeks (18-24 days). Again, no adjustments for weight, age, and meals will be made.

8.4.2 Dispensing

The investigator has the authority to dispense the drug. Trained study staff will be instructing participants to administer the nasal spray from a safe distance to limit close contact.

8.4.3 Administration Instructions

It is preferable to sit when using the nasal spray.

During the acute phase, study staff will administer the nasal spray to participants with these instructions:

Remove the flip-off cap. Holding the bottle upright, insert the nozzle into one nostril and actuate while the participant gently inhales through nose and exhales through mouth. Switch nostrils and repeat. Keep switching nostrils, actuating, inhaling, and exhaling until all the oxytocin or placebo solution has been used up.

During the chronic phase, subjects will administer the nasal spray themselves with these instructions:

Remove the flip-off cap. Holding the bottle upright, insert the nozzle into one nostril and actuate while gently inhaling through nose and exhaling through mouth. Switch nostrils and repeat. Keep switching nostrils, actuating, inhaling, and exhaling until all the oxytocin or placebo solution has been used up.

8.4.4 Storage

Study drug should be stored at the study site in a refrigerator (2-8oC). If the temperature of the study drug storage exceeds or falls below this range, this should be reported to the Principal Investigator and captured as a deviation. Subjects will be instructed to store the medication in provided packaging and in a refrigerator.

8.5 Study Drug Accountability

An accurate and current accounting of the dispensing and return of study drug for each subject will be maintained on an ongoing basis by a member of the study site staff. The number of study drug dispensed and returned by the subject will be recorded on the Investigational Drug Accountability Record. The study monitor will verify these documents throughout the course of the study.

8.6 Measures of Treatment Compliance

Adherence to and administration technique of administering the study drug will be directly observed for all groups via secure video calls between participants and study coordinators during Video Observed Therapy (VOT) procedure. Participants may use tablets provided by the VA or their own devices.

In order to safeguard the well-being of all subjects enrolled in the study, each subject will be monitored for the following:

a. Adverse Event: Any untoward medical occurrence in a subject that does not necessarily have a causal relationship with the assessment procedures. Will include any unfavorable and unintended clinical sign, symptom, or illness temporally associated with the use of the assessment, whether or not considered related to it.
b. Serious Adverse Event: Any adverse experience that results in any of the following outcomes: death, a life-threatening experience, acute psychiatric decompensation, inpatient hospitalization, or a persistent or significant disability/incapacity.
c. Unexpected Adverse Event: Any adverse experience the specificity or severity of which is not consistent with the risks information described in this study protocol or consent/assent document

9 STUDY PROCEDURES AND GUIDELINES

A Schedule of Events representing the required testing procedures to be performed for the duration of the study is diagrammed in Appendix 1.

Prior to conducting any study-related activities, written informed consent and the Health Insurance Portability and Accountability Act (HIPAA) authorization must be signed and dated by the subject.

9.1 Clinical Assessments

9.1.1 Concomitant Medications

All concomitant medication and concurrent therapies will be documented at Baseline/Screening and at Study Days when applicable. Dose, route, unit frequency of administration, and indication for administration and dates of medication will be captured.

9.1.2 Demographics

Demographic information (date of birth, gender, race) will be recorded during the visit.

9.1.3 Medical History

Relevant medical history, including history of current disease, other pertinent respiratory history, and information regarding underlying diseases will be recorded during the visit.

9.1.4 Vital Signs

Blood pressure and pulse will be recorded during the visit.

9.1.5 fMRI Testing

Subjects will perform theory of mind tasks in the San Francisco Veterans Affairs Medical Center's on-site MRI research facility. Participants are asked to lie in the scanner with only their head placed in the fMRI and visual stimuli are presented on a screen. The task administrator is able to communicate through an intercom system. Task specific instructions are displayed on a computer screen and responses are collected through a button box.

9.1.6 Social Functioning Test

Subjects will perform social functioning tasks in Dr. Joshua Woolley's Bonding and Attunement in Neuropsychiatric Disorders laboratory. Participants will be asked to complete several measures of social functioning, social ability, negative symptoms, and Theory of Mind.

9.1.7 Adverse Events

Information regarding occurrence of adverse events will be captured throughout the study. Duration (start and stop dates and times), severity/grade, outcome, treatment and relation to study drug will be recorded on the case report form (CRF).

9.2 Clinical Laboratory Measurements

9.2.1 Pregnancy Test

For those who can become pregnant, a urine pregnancy test will be obtained during each in-person visit.

10 EVALUATIONS BY VISIT

10.1 Visit 1 (Day 1, Remote Visit)

1. Review the study with the subject (subject's legal representative) and obtain verbal informed consent and HIPAA authorization, if appropriate.
    1. During the covid-19 pandemic, Visit 1 tasks and procedures have been adapted to be remote. We will be collecting verbal consent by video call at the beginning of this visit. Written informed consent will be obtained during Visit 2, the first in-person visit, before study drug administration.
2. Assign the subject a unique screening number.
3. Record demographics data.
4. Record medical history, including a history of psychosis, diagnosis date, and prior antipsychotic treatments.
5. Record concomitant medications.

6. Structured Clinical Interview for DSM-5 (Diagnostic and Statistical Manual of Mental Disorders, 5th Edition)
7. Social functioning assessments.
8. Schedule subject for Visit 2 in one week from their first visit if visit hasn't been scheduled yet.

10.2 **Visit 2 (Week 1)**

1. Review the study with the subject (subject's legal representative) and obtain written informed consent and HIPAA authorization, if appropriate.
2. Urine Pregnancy Test
3. Oxytocin/placebo administration.
4. fMRI testing.

10.3 Visit 3 (Week 2)

1. Urine Pregnancy test
2. Oxytocin/placebo administration.
3. fMRI testing.

10.4 Visit 4 (Week 3)

1. Urine Pregnancy Test
2. Record changes to concomitant medications.
3. Record participant weight
4. ToM, negative and positive symptoms, and social ability assessments
5. Oxytocin/placebo administration
6. Oxytocin self-administration training.

10.5 Chronic Period (Remote procedures between Visit 5 and Visit 6)

1. VOT

2. Social Functioning assessment

10.6 Visit 5 (Week 6)

1. Urine Pregnancy test
2. Record changes to concomitant medications.
3. Record participant weight
4. Perform and record vital signs.
5. Oxytocin/placebo administration.
6. ToM, negative and positive symptoms, and social ability assessments.

10.7 Early Withdrawal Visit

1. Record any Adverse Experiences and/or Review subject diary for adverse experiences and exclusionary medication use.
2. If given, collect and return unused oxytocin to pharmacy.

11 DISCONTINUATION AND REPLACEMENT OF SUBJECTS

11.1 Early Discontinuation of Study Drug

A subject may be discontinued from study treatment at any time if the subject, the investigator, or the Sponsor feels that it is not in the subject's best interest to continue. The following is a list of possible reasons for study treatment discontinuation: *(The list should match the Study Completion/Discontinuation CRF page.)*

- Subject withdrawal of consent (or assent)
- Subject is not compliant with study procedures
- Adverse event that in the opinion of the investigator would be in the best interest of the subject to discontinue study treatment
- Protocol violation requiring discontinuation of study treatment

- Lost to follow-up
- Sponsor request for early termination of study
- Positive pregnancy test (females)

If a subject is withdrawn from treatment due to an adverse event, the subject will be followed and treated by the Investigator until the abnormal parameter or symptom has resolved or stabilized.

All subjects who discontinue study treatment should come in for an early discontinuation visit as soon as possible and then should be encouraged to complete all remaining scheduled visits and procedures.

All subjects are free to withdraw from participation at any time, for any reason, specified or unspecified, and without prejudice.

Reasonable attempts will be made by the investigator to provide a reason for subject withdrawals. The reason for the subject's withdrawal from the study will be specified in the subject's source documents Refer to Section 10 for early termination procedures.

12.3 Withdrawal of Subjects from the Study

A subject may be withdrawn from the study at any time if the subject, the investigator, or the Dr. Woolley feels that it is not in the subject's best interest to continue.

All subjects are free to withdraw from participation at any time, for any reason, specified or unspecified, and without prejudice.

Reasonable attempts will be made by the investigator to provide a reason for subject withdrawals. The reason for the subject's withdrawal from the study will be specified in the subject's source documents. As noted above, subjects who discontinue study treatment early (i.e., they withdraw prior to Visit X) should have an early discontinuation visit. Refer to Section 10 for early termination procedures. Subjects who withdraw after Visit X but prior to Visit X should be encouraged to come in for a final visit (and the procedures to be followed would include those for their next scheduled visit).

12.4 Replacement of Subjects

Subjects who withdraw from the study treatment will be replaced.

Subjects who withdraw from the study will be replaced.

12 STATISTICAL METHODS AND CONSIDERATIONS

Prior to the analysis of the final study data, a detailed Statistical Analysis Plan (SAP) will be written describing all analyses that will be performed. The SAP will contain any modifications to the analysis plan described below.

12.1 Data Sets Analyzed

All eligible patients who are randomized into the study and receive at least one dose of the study drug (the Safety Population) will be included in the safety analysis.

12.2 Demographic and Baseline Characteristics

12.3 Analysis of Primary Endpoint

The investigators will perform linear regression analyses with SFS change as the primary outcome and drug modeled as a 2-level dummy variable (drug vs. placebo). Identical analyses will be run using accuracy scores for the FBT and PDT instead of rTPJ activity to determine if acute OTimproves ToM ability versus placebo.

12.4 Analysis of Secondary Endpoints

Similar analyses as stated in 15.3 will be conducted for the secondary clinical outcomes including social ability, negative symptoms (CAINS), and ToM(Hinting Task).

12.5 Interim Analysis

Interim analysis will be conducted after the first 60 participants have completed the study and at multiple points after over the course of the study. Results will remain blinded to all other study staff.

12.6 Sample Size and Randomization

All power and effect size estimates were calculated using STATA/SE v. 15.1 using 80% power and two-sided alpha=0.05. To be conservative, the investigators used the one-way command with two or three groups. The proposed sample sizes enable us to detect effect sizes ranging from 0.23 to 0.27 for the primary hypotheses, which are clinically meaningful and consistent with the preliminary data.

13 DATA COLLECTION, RETENTION AND MONITORING

13.1 Data Collection Instruments

The Investigator will prepare and maintain adequate and accurate source documents designed to record all observations and other pertinent data for each subject treated with the study drug.

Study personnel will record data corresponding to a subject's visit on a physical study document. Subjects will not be identified by name in the study database or on any study documents to be

collected by Dr. Woolley (or designee), but will be identified by a subject number.

The Investigator is responsible for all information collected on subjects enrolled in this study. All data collected during the course of this study must be reviewed and verified for completeness and accuracy by the Investigator.

13.2 Data Management Procedures

The data will be entered into a validated database. The Data Management group will be responsible for data processing, in accordance with procedural documentation. Database lock will occur once quality assurance procedures have been completed.

All procedures for the handling and analysis of data will be conducted using good computing practices meeting FDA guidelines for the handling and analysis of data for clinical trials.

13.3 Data Quality Control and Reporting

After data have been entered into the study database, a system of computerized data validation checks will be implemented and applied to the database on a regular basis. Query reports (Data Clarification Requests) pertaining to data omissions and discrepancies will be forwarded to the Investigators and study monitors for resolution. The study database will be updated in accordance with the resolved queries. All changes to the study database will be documented.

13.4 Archival of Data

The database is safeguarded against unauthorized access by established security procedures; appropriate backup copies of the database and related software files will be maintained. Databases are backed up by the database administrator in conjunction with any updates or changes to the database.

At critical junctures of the protocol (e.g., production of interim reports and final reports), data for analysis is locked and cleaned per established procedures.

13.5 Availability and Retention of Investigational Records

The Investigator must make study data accessible to the monitor, other authorized representatives of the Sponsor (or designee), IRB, and Regulatory Agency (e.g., FDA) inspectors upon request. A file for each subject must be maintained that includes the signed Informed Consent, HIPAA Authorization and copies of all source documentation related to that subject. The Investigator must ensure the reliability and availability of source documents from which the information on the CRF was derived.

All study documents (participant files, signed informed consent forms, copies of CRFs, Study File Notebook, etc.) must be kept secured for a period of two years following marketing of the investigational product or for two years after centers have been notified that the IND has been discontinued. There may be other circumstances for which the Sponsor is required to maintain study records and, therefore, the Sponsor should be contacted prior to removing study records for any reason.

13.6 Monitoring

Monitoring visits will be conducted by VA Personnel according to the U.S. CFR Title 21 Parts 50, 56, and 312 and ICH Guidelines for GCP (E6). By signing this protocol, the Investigator grants permission to the Sponsor (or designee), and appropriate regulatory authorities to conduct on-site monitoring and/or auditing of all appropriate study documentation.

13.7 Subject Confidentiality

In order to maintain subject confidentiality, only a subject number will identify all study subjects on CRFs and other documentation submitted to the Sponsor. Additional subject confidentiality issues (if applicable) are covered in the Clinical Study Agreement.

14 ADMINISTRATIVE, ETHICAL, REGULATORY CONSIDERATIONS

The study will be conducted according to the Declaration of Helsinki, Protection of Human Volunteers (21 CFR 50), Institutional Review Boards (21 CFR 56), and Obligations of Clinical Investigators (21 CFR 312). To maintain confidentiality, all laboratory specimens, evaluation forms, reports and other records will be identified by a coded number and initials only. All study

records will be kept in a locked file cabinet and code sheets linking a participant's name to a participant identification number will be stored separately in another locked file cabinet. Clinical information will not be released without written permission of the subject, except as necessary for monitoring by the FDA. The Investigator must also comply with all applicable privacy regulations (e.g., Health Insurance Portability and Accountability Act of 1996, EU Data Protection Directive 95/46/EC).

14.1 Protocol Amendments

Any amendment to the protocol will be written by Dr. Woolley and his research group. Protocol amendments cannot be implemented without prior written IRB/IEC approval except as necessary to eliminate immediate safety hazards to participants. A protocol amendment intended to eliminate an apparent immediate hazard to participants may be implemented immediately, provided the IRBs are notified within five working days.

14.2 Institutional Review Boards and Independent Ethics Committees

The protocol and consent form will be reviewed and approved by the IRB of each participating center prior to study initiation. Serious adverse experiences regardless of causality will be reported to the IRB in accordance with the standard operating procedures and policies of the IRB, and the Investigator will keep the IRB informed as to the progress of the study. The Investigator will obtain assurance of IRB compliance with regulations.

Any documents that the IRB may need to fulfill its responsibilities (such as protocol, protocol amendments, Investigator's Brochure, consent forms, information concerning participant recruitment, payment or compensation procedures, or other pertinent information) will be submitted to the IRB. The IRBs written unconditional approval of the study protocol and the informed consent form will be in the possession of the Investigator before the study is initiated.

Protocol and/or informed consent modifications or changes may not be initiated without prior written IRB approval except when necessary to eliminate immediate hazards to the participants or when the change(s) involves only logistical or administrative aspects of the study. Such modifications will be submitted to the IRB and written verification that the modification was submitted and subsequently approved should be obtained.

The IRB must be informed of revisions to other documents originally submitted for review; serious and/or unexpected adverse experiences occurring during the study in accordance with the standard operating procedures and policies of the IRB; new information that may affect adversely the safety of the participants of the conduct of the study; an annual update and/or request for re-approval; and when the study has been completed.

14.3 Informed Consent Form

Informed consent will be obtained in accordance with the Declaration of Helsinki, ICH GCP, US Code of Federal Regulations for Protection of Human Subjects (21 CFR 50.25[a,b], CFR 50.27,

and CFR Part 56, Subpart A), the Health Insurance Portability and Accountability Act (HIPAA, if applicable), and local regulations.

A properly executed, written, informed consent will be obtained from each subject prior to entering the subject into the trial. Information should be given in both oral and written form and subjects must be given ample opportunity to inquire about details of the study.
If a subject is unable to sign the informed consent form (ICF) and the HIPAA authorization, a legal representative may sign for the subject. A copy of the signed consent form (and assent) will be given to the subject and the original will be maintained with the subject's records.

14.4 Publications

The preparation and submittal for publication of manuscripts containing the study results shall be in accordance with a process determined by mutual written agreement among the study Sponsor and participating institutions. The publication or presentation of any study results shall comply with all applicable privacy laws, including, but not limited to, the Health Insurance Portability and Accountability Act of 1996.

14.5 Investigator Responsibilities

By signing the Agreement of Investigator form, the Investigator agrees to:

1. Conduct the study in accordance with the protocol and only make changes after notifying the Sponsor (or designee), except when to protect the safety, rights or welfare of subjects.
2. Personally conduct or supervise the study (or investigation).
3. Ensure that the requirements relating to obtaining informed consent and IRB review and approval meet federal guidelines, as stated in § 21 CFR, parts 50 and 56.
4. Report to the Sponsor or designee any AEs that occur in the course of the study, in accordance with §21 CFR 312.64.
5. Ensure that all associates, colleagues and employees assisting in the conduct of the study are informed about their obligations in meeting the above commitments.
6. Maintain adequate and accurate records in accordance with §21 CFR 312.62 and to make those records available for inspection with the Sponsor (or designee).
7. Ensure that an IRB that complies with the requirements of §21 CFR part 56 will be responsible for initial and continuing review and approval of the clinical study.
8. Promptly report to the IRB and the Sponsor (or designee) all changes in the research activity and all unanticipated problems involving risks to subjects or others (to include amendments and IND safety reports).
9. Seek IRB approval before any changes are made in the research study, except when necessary to eliminate hazards to the patients/subjects.
10. Comply with all other requirements regarding the obligations of clinical investigators and all other pertinent requirements listed in § 21 CFR part 312.

APPENDIX 1. EXAMPLE OF SCHEDULE OF STUDY VISITS

	VISIT 1 (Day/Week/Month #)[a]	VISIT 2 (Day/Week/Month #)[a]	VISIT 3 (Day/Week/Month #)[a]	VISIT 4 (Day/Week/Month #)[a]	VISIT 5 (Day/Week/Month #)
Verbal Consent	X				
Informed Consent	X*	X			
Medical History	X				
Concomitant Medication Review	X	X	X	X	X
Height	X				
Weight				X	X
Vital Signs	X			X	
Urine Tests (pregnancy)		X	X	X	X
Randomization	X				
Administration of Study Drug		X	X	X	X
Dispensing of Study Drug				X	
Counting of Returned Study Drug					X
fMRI Testing		X	X		
Social Functioning Tests	X			X	X
Adverse Experiences					

*written informed consent will be collected during Visit 1 when possible.

[a] ±2 days