DECIPHERING THE ROLE OF OXYTOCIN IN MOTIVATION: AN FMRI STUDY. PART II.

Study Agents: Syntocinon® (intranasal oxytocin)

IND#: 115812

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PROTOCOL SUMMARY

Background and Rationale

We propose to ascertain intranasal oxytocin's effect on neural processes associated with the processing of reward stimuli. The neuropeptide oxytocin is best recognized for its effects on labor and lactation and has been well established to influence a wide-range of social behaviors including pair bonding and social interaction. Animal models demonstrate oxytocin's ability to influence social activity in part via its effects on neural circuits responsible for the processing of motivationally relevant stimuli. Whether oxytocin has similar effects in humans has not been tested. This research will utilize a state of the art neuroimaging technique, pharmacological functional magnetic resonance imaging (fMRI), in order to characterize the effects of intranasal oxytocin on brain activity elicited by motivationally relevant stimuli (social and monetary rewards).

Ongoing clinical trials are examining the use of intranasal oxytocin for the treatment of multiple psychiatric disorders including substance dependence, depression, and schizophrenia; disorders which reward system dysfunction appears to play a significant role. As such, it is important that we obtain a better understanding of the neurobiological effects this drug may have on reward circuitry functioning. To this end, in this study, we will examine healthy control participants and participants diagnosed with Alcohol Use Disorder (AUD).

Objectives and Endpoints

Our primary objectives: examine the effects intranasal oxytocin administration has on brain activity elicited by social and monetary incentives using incentive delay tasks, which are designed to engage circuits that process natural and artificial rewards, in a group of healthy participants and participants diagnosed with AUD. This will allow us to establish whether intranasal oxytocin can influence brain activity associated with the processing of rewards and given the social nature of the hormone, whether oxytocin can influence such reward-related activity in a general sense or only when the reward has direct social relevance, and whether oxytocin produces similar alterations to reward-related activity in individuals diagnosed with AUD. Our study end will correspond to the final scan follow-up of our final participant.

Assessments

Participants will be assessed at several different time points: first, at intake to collect preliminary information such as demographics, general history, drug use, social activity and trait measurements of mood, personality and well-being; then participants will be scanned using fMRI twice, once self-administering placebo and once self-administering intranasal oxytocin; participants will be followed-up with a phone call 24 hours following each scan to record and respond to any adverse events; finally, participants will be contacted for follow-up surveys 30, 60, and 90 days following their second fMRI visit.

Statistical Methods

We will utilize standardized techniques to analyze the neuroimaging data obtained during the fMRI scans. Data will be analyzed using a widely used neuroimaging software package (e.g. Statistical Parametric Mapping, SPM).

1. INTRODUCTION

1.1. Indication

The proposed study will investigate the effects of intranasal oxytocin administration on neural activity associated with social and non-social motivation. Oxytocin is a well-known social and reproductive hormone demonstrated to have a variety of prosocial effects in humans including enhancing trust and generosity, improving positive communication, increasing eye gaze, and reducing anxiety [1-5]. Oxytocin is hypothesized to facilitate social behaviors via its modulation of reward networks [6]. With this study, we will characterize oxytocin's effects on the neural processing of reward in a healthy control population and in individuals diagnosed with Alcohol Use Disorder (AUD). We will utilize a noninvasive brain imaging technique, functional magnetic resonance imaging (fMRI), to assess brain activity while participants perform tests designed to engage neural circuits associated with the processing of social and non-social rewards.

1.2. Background and Rationale

The brain's ability to appropriately identify the significance of everyday stimuli, assign a suitable incentive value and determine the appropriate action is critical for healthy functioning. When this system is disrupted, as it is with substance dependence, natural incentives, like social interactions with friends and family, may be devalued. Understanding the mechanisms that shape individual reactions to incentive stimuli is imperative to realizing the nature of the pathophysiological alterations that occur in many psychiatric disorders.

Recently, it has been suggested that the neuropeptide oxytocin, best known for its role in facilitating social and reproductive behaviors, may influence networks relevant to psychiatric illness. Animal work has indicated that oxytocin administration can induce dopamine release within the mesolimbic dopamine system [7,8] which has been hypothesized to be one mechanism by which oxytocin could impact incentive salience attribution to social stimuli (e.g. infants, conspecifics, mates) and to ultimately influence an organisms' drive towards such objects (e.g. [8-12]). Oxytocin's modulation of the neural circuits that control motivational processing does not appear to be limited to the social realm. Oxytocin can also alter central dopaminergic responses associated with non-social behaviors, including addictive behaviors (e.g. self-administration, tolerance, and dependence) (see review [13-18]). Although the animal literature suggests anatomical and functional interactions between oxytocinergic and motivational systems, there have been few human studies to date investigating the role of oxytocin in motivational processing.

1.2.1. Study Rationale

Studies on the neural effects of oxytocin administration in humans have been largely limited to brain studies examining emotional responsiveness, social cognition and trust [19-22]. This proposal seeks to establish, for the first time, the role oxytocin plays in the neural circuitries associated with reward and motivation in a human population. Though oxytocin has been primarily considered in the social realm, as indicated previously, some evidence suggests oxytocin can interfere with non-social processes (for review [13-18]). As such, we propose to characterize the effects of oxytocin on the neural processing of social and non-social rewards.

Several clinical trials are currently underway seeking to utilize oxytocin as a therapeutic agent to treat psychiatric illness (including autism, schizophrenia and substance use disorder (e.g.

[23,24])). As oxytocin is considered for this purpose, it is essential that we more completely characterize oxytocin's effects on systems relevant to psychiatric illness. Interestingly, recent research suggests oxytocinergic functioning may be altered in persons diagnosed with an alcohol use disorder (AUD). However, very little is known about how oxytocin administration may affect some of the key processes widely acknowledged to be disrupted in addiction -- particularly reward processes. As such, we propose to continue our work examining the influence intranasal oxytocin has on reward-related activity in control subjects and explore whether intranasal oxytocin produces similar alterations to reward-related activity in recently abstinent persons with AUD.

In order to achieve these research goals, we propose to employ a state of the art neuroimaging technique, pharmacological functional magnetic resonance imaging (pharmaco-fMRI). In this method, participants are given a drug prior to fMRI scanning which allows the investigation of drug effects on neural activity. This is an ideal method for studying neural modulation by drug and is safely done in healthy human populations.

1.2.2. Previous Human Experience

Oxytocin was first synthesized in the laboratory in the 1950s and since that time has been primarily utilized for stimulation of milk ejection and uterine contraction [25]. Synthetic oxytocin is widely prescribed in the United States in injectable form and, prior to 1997, in intranasal form (the manufacturer of the intranasal form of synthetic oxytocin [trademark name Syntocinon®, NDA 012285], Novartis, let FDA approval of the product lapse, presumably for financial reasons: http://www.fda.gov/ohrms/dockets/dockets/06p0068/06p-0068-cp00001-01-vol1.pdf [26]). In 1968 the FDA, National Academy of Sciences and National Research Council concluded oxytocin administered intranasally was effective for the indication of initial milk letdown. Intranasal oxytocin is still marketed and prescribed throughout Europe for the stimulation of milk ejection (see review [27]).

Syntocinon® and other forms of synthetic intranasal oxytocin are commonly used agents in human research studies. As recently reviewed by MacDonald and colleagues, between 1990 and 2010, 38 randomized control trials were conducted which investigated the effects of intranasal oxytocin in humans [27]. A vast majority of the studies reviewed (79%) utilized Syntocinon® produced by Novartis and were conducted in healthy adults. In addition, in reviewing ongoing clinical trials (via clinicaltrials.gov), we note there are currently over 25 active clinical trials utilizing intranasal oxytocin. In our review, we noted IND exemptions obtained from the FDA to utilize intranasal oxytocin in humans (i.e. #77,774, Dr. Pedersen, University of North Carolina; #78,246, Dr. Kelly, University of Maryland).

1.2.3. Safety

A review of the literature reports that short-term use of intranasal oxytocin administered in doses ≤ 40 IU, produces minimal side effects and adverse outcomes in healthy adults [27]. Intranasal oxytocin has also been safely used in multiple trials including persons diagnosed with psychiatric conditions including alcohol use disorder (AUD) and other addictive disorders [e.g. 43-44], schizophrenia [45-47], and depression [48]. In reviewing ongoing clinical trials (via clinical trials.gov), we note 8 separate trials who have or are currently utilizing intranasal oxytocin in AUD populations (i.e. NCT02275611, NCT01212185, NCT02251912, NCT02058251, NCT02407340, NCT02711189, NCT02469259,

NCT02742532). However, Syntocinon® is not advised for use in women who are pregnant or planning to become pregnant (particularly as oxytocin is indicated for the induction and augmentation of labor). Also, it should not be given to patients with impaired hepatic or renal function and should not be administered to individuals with allergies to oxytocin or to the preservatives contained in the nasal spray. To reduce these risks, female subjects who are pregnant, nursing, or unable or unwilling to use effective methods of birth control during the study will be excluded from these studies. In addition, in reviewing PubMed, there was a single case-study indicating a significant adverse effect of long-term usage of intranasal oxytocin: in this case study, a 55-year old patient with obsessive-compulsive disorder self-administered oxytocin nasal spray multiple times a day, each day for 4 weeks and developed psychotic symptoms and decreased plasma sodium and osmolality [28]. In our protocol, participants will receive a single dose in a controlled setting. Finally, we will exclude participants with any acute or uncorrected medical illness, including participants who exhibit nasal obstruction or report using intranasally administered medications that cannot be stopped 48 hours prior to each scanning session.

1.2.4. Risks

There are some risks associated with the use of oxytocin. Frequencies are as follows: Very common (≥1/10), common (≥1/100 to <1/10), uncommon (≥1/1,000 to <1/100), rare (≥1/10,000 to <1/1,000), very rare (<1/10,00).

- Common: headache, tachycardia, bradycardia, nausea, vomiting
- Uncommon: arrhythmia
- Rare: rash, anaphylactoid reactions associated with dyspnoea, hypotension and shock
- Very rare: hypertension, one case of psychotic reaction and one case of seizure have been reported.
- Prolonged use of excessive doses with infusion of large volumes of fluid may cause water intoxication with hyponatraemia

Please refer to the attached document titled "Syntocinon® Novartis Pharmaceuticals" [29] provided by PharmaWorld and the document "Oxytocin (Systemic)" [30] obtained from http://www.drugs.com/mmx/syntocinon.html?printable=1, for more information on the safety and toxicology of oxytocin. Oxytocin is commonly given to induce or augment labor or to stimulate milk ejection, as such, Syntocinon will not be given to women who are pregnant, suspected pregnant, or breastfeeding.

In order to appropriately evaluate and respond to any potential adverse events, participants will self-administer the doses at the University of Utah while being monitored by study staff within the Imaging and Neuroscience Center. Female participants will be screened for pregnancy (urine test) prior to drug administration. Participants will be monitored by study staff at the University of Utah for approximately 2 hours following drug administration, given contact information in case of an adverse event, and contacted 24 hours following scanning to track any potential drug related effects.

1.2.5. Dose Rationale

A vast majority of the studies utilizing intranasal oxytocin have noted minimal side effects and

significant effects on mood, anxiety, and social behavior with a dose between 20-24 IU [27]. In keeping with this dosing, we propose to utilize a dose of 24 IU units delivered intranasally in a series of 6 puffs (3 puffs per nostril at 4 IU per puff) over 5 minutes.

2. STUDY OBJECTIVES AND ENDPOINTS

2.1. Objectives

Our main goal is to characterize the effects of intranasal oxytocin on brain activity associated with reward. We will utilize fMRI in order to assess changes in neural activity as a result of processing social and non-social reward stimuli. We will characterize this activity in healthy individuals as well as in individuals diagnosed with Alcohol Use Disorder. Our objectives are as follows:

2.1.1. Primary Objectives

- To determine the effect of intranasal oxytocin (Syntocinon®) on brain activity associated with social rewards using fMRI.
- To assess the effects of intranasal oxytocin (Syntocinon®) on the neural processing of non-social (i.e. monetary) rewards.
- To determine whether the neural responses to social cues and rewards are altered in recently abstinent persons with AUD.
- To determine whether the effect of intranasal oxytocin (Syntocinon®) on brain activity associated with social cues and rewards differs between control participants and recently abstinent persons with AUD.

2.2. Endpoints

2.2.1. Primary Endpoint

- The primary endpoint will correspond to the final scan follow-up of the last participant. All group level analyses will be conducted at that time.

3. STUDY DESIGN

This is a double-blind, placebo-controlled, randomized study of the effects of Syntocinon® on brain activity elicited during the processing of reward. Each participant will be studied twice using fMRI; once self-administering Syntocinon® and once self-administering placebo intranasally approximately 30 minutes prior to being scanned. Participants will be randomly assigned to self-administer either Syntocinon® or placebo first. Scans will be performed on separate days approximately 1-2 weeks apart.

Participants will be evaluated thoroughly at screening for eligibility and will only be entered into the study if they meet all inclusion criteria and none of the exclusion criteria.

88 participants are planned to complete the study (44 controls, 44 AUD). Total duration of the study is expected to be 60 months.

4. SUBJECT SELECTION

4.1. Subject Recruitment

Recruitment will take place through advertisement in the University and surrounding communities in newspapers, brochures, radio advertisements, social media advertisements (e.g., Facebook ads) and bulletin boards. We will also utilize the University of Utah's Enterprise Data Warehouse (EDW) and chart review to identify patients who are appear to meet our inclusion criteria and are scheduled for treatment. For these potential participants, we will either contact the patient's physician/treatment team and ask for the opportunity to share information about the study with the patient or send a letter and brochure to these individuals prior to contacting them. Recruitment for the study will also include ResearchMatch.org, a national health volunteer registry that was created by several academic institutions to support recruitment for research studies. In order to target participants diagnosed with AUD, recruitment materials will be distributed to affiliated centers which assess and treat persons with AUD including the University Neuropsychiatric Institute (UNI), Assessment Referral Services (ARS), and Recovery Ways.

After an initial brief screen (typically conducted over the phone), subjects will undergo written informed consent procedures for study participation. Once an individual is considered suitable for study participation, the Mini-International Neuropsychiatric Interview will be administered to ensure study inclusion/exclusion criteria are met. Weight and height will be recorded and the body mass index computed.

4.2. Inclusion Criteria

General Inclusion Criteria for All Participants:

- 18-45 years of age at the time of screening
- No current or past history of neurological illness
- No acute medical illness

Additional Inclusion Criteria for Control group:

- No current or past history of psychiatric illness, including substance use disorder (except nicotine)

Additional Inclusion Criteria for AUD group:

- Meet DSM-5 criteria for current moderate-to-severe AUD
- Abstinent from alcohol for 2-8 weeks prior to study enrollment
- Express a desire to achieve abstinence or to greatly reduce alcohol consumption

4.3. Exclusion Criteria

General Exclusion Criteria for All Participants:

- Known allergies to oxytocin or to preservatives in the nasal spray

- Participants unable to tolerate the scanning procedures or would be unfit for scanning purposes (e.g. metal implants, claustrophobic, unable to lie still for the duration of the scan)
- Female subjects who are pregnant, trying to become pregnant, or nursing
- Any current or past history of any serious medical or neurological illness
- Acute or uncorrected medical illnesses, including history of hepatic or renal dysfunction, hyponatremia, traumatic brain injury, atrophic rhinitis, recurrent nose bleeds, and cranial-surgical procedures (hypophysectomy).
- Abnormal MRI (except if due to technical factors)
- Participants reporting use of an intranasal medication that could not be stopped 48 hours prior to each scanning session
- Participants taking medications including any current treatment with antipsychotics, antidepressants, mood stabilizers, psychostimulants and psychostimulant appetite suppressants, hormone use (i.e. testosterone, DHEA), opioid drugs, isoniazid, glucocorticoids, centrally active antihypertensive drugs (e.g., clonidine, reserpine), or sedative hypnotic medications (e.g. benzodiazepines, barbiturates, within 1 week prior to study enrollment).
- Unable to comply with study procedures or protocols

Additional Exclusion Criteria for Control group:

- Any reported current (within the last 2 months) use of any category of illicit drugs

Additional Exclusion Criteria for AUD group:

- History of, or current, psychotic disorder, antisocial personality disorder, or bipolar disorder
- Concurrent posttraumatic stress disorder
- Dependence (moderate to severe) to any other substance within the last 2 months other than alcohol or nicotine

Written informed consent will be obtained. Participants who exhibit nasal obstruction or upper-respiratory tract infection at the time of scanning, test positive on drug and/or ethanol screens, or are affected by a condition or abnormality that in the opinion of the Investigator would compromise the safety of the patient or the quality of the data will be withdrawn from the study. Additionally, female subjects of childbearing potential must agree to use effective methods of birth control discussed during the consenting process throughout the duration of the study in order to participate. Acceptable methods of birth control include hormonal birth control (e.g. birth control pills, skin patches, shots, under-the-skin implants, or vaginal ring), a barrier contraceptive such as a condom with spermicide cream or gel, diaphragms or cervical cap with spermicide cream or gel, an intrauterine device (IUD), tubal ligation or partner vasectomy, or avoiding sexual activity that could cause them to become pregnant. If a female subject indicates she suspects she is pregnant or tests positive on a pregnancy screen, she will be withdrawn from the study.

5. STUDY TREATMENTS

5.1. Allocation to Treatment

Eligible participants will be randomly assigned to administer either Syntocinon® or placebo first, prior to scanning using a computer-generated randomization scheme. Neither the research staff, nor the participants will know the identity of the test and control drugs. On behalf of PharmaWorld, Volkapotheke Roten Ochsen will transfer Syntocinon® into bottles identical to placebo using sterile procedures. To maintain the blind, packaging and labeling of test and control treatments will be identical to maintain the blind.

5.2. Breaking the Blind

The randomization can be broken, if necessary per the physicians caring for the subject (or at the request of the IRB) to ensure subject safety. With the exception of a medical emergency, the blind will only be broken by the PI.

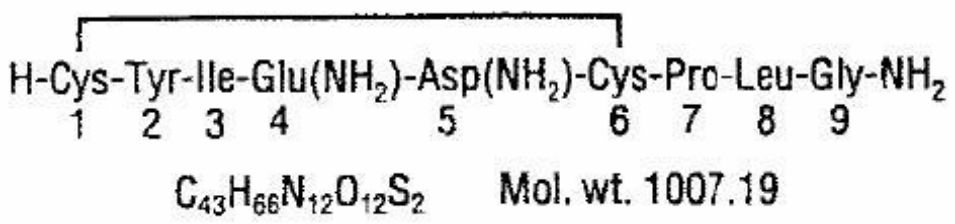
5.3. Drug Supplies

Syntocinon® will be supplied by the international pharmacy, PharmaWorld, located in Zurich, Switzerland. Syntocinon® solution will be contained within 5mL bottles (contain a total of 40 IU of oxytocin). Volkapotheke Roten Ochsen will rebottle the Syntocinon® into bottles identical to those used for the placebo spray that will dispense Syntocinon® at a rate of 4 IU/0.1 mL/puff.

5.3.1. Formulation, Preparation and Dispensing

Syntocinon® (oxytocin) is a synthetic, (1-6) cyclic nonapeptide. Chemically, oxytocin is designated as Glycinamide, L-cysteinyl-L-tyrosyl-L-isoleucyl-L-glutaminyl-L-asparaginyl-L-cysteinyl-L-prolyl- L-leucy1-, cyclic (1-6)-disulfide.

The structural formula is:



Syntocinon® (oxytocin) spray is provided as a sterile solution for intranasal administration. Each 5 mL of solution contains 40 USP or International Units of oxytocin and contains the following inactive ingredients: E 216, E 218 and chlorobutanol hemihydrate as preservatives, excipients to 1 ml.

Formulation of Control Product

A placebo solution (containing the same ingredients as the Syntocinon® spray minus oxytocin) will be placed into identical bottles by Volkapotheke Roten Ochsen and supplied by PharmaWorld.

5.3.2. Administration

Participants will be asked to self-administer 3 puffs of placebo or Syntocinon® per nostril (for Syntocinon®, this corresponds to 4 IU/puff, for a total of 6 puffs/24 IU). Participants will administer placebo and Syntocinon® while seated, inhaling a full spray per nostril, alternating between nostrils, waiting 15 seconds between each puff (total of 6 puffs). Participants will be studied on two separate occasions, approximately 1-2 weeks apart in a double-blind design where the order of Syntocinon® and placebo is randomized across subjects.

5.3.3. Drug Storage and Drug Accountability

5.3.3.1. Supply of Study Drug at the Site

Syntocinon® and placebo will be shipped by the international pharmacy, PharmaWorld, located in Zurich, Switzerland to the University of Utah. Drug shipment will occur once all the required regulatory documentation has been received and the study approved by the University of Utah Institutional Review Board.

5.3.3.2. Storage

Syntocinon® and placebo will be stored at limited access lab at the University of Utah in a refrigerator (2-8°C). If the temperature of the study drug storage exceeds or falls below this range, this will be reported and captured as a deviation.

5.3.3.3. Study Drug Accountability

Subjects will be asked to self-administer study drugs. An accurate and current accounting of the dispensing 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 Drug Accountability Record. The study monitor will verify these documents throughout the course of the study.

5.4. Concomitant medications

Participants who utilize intranasally administered medications that cannot be stopped 48 hours prior to each scanning session will be excluded. Participants reporting current treatment with the following medications will be excluded: antipsychotics, antidepressants, mood stabilizers, psychostimulants and psychostimulant appetite suppressants, testosterone, DHEA, opioid drugs, or treatment with isoniazid, glucocorticoids, centrally active antihypertensive drugs (e.g., clonidine, reserpine), or sedative hypnotic medications (benzodiazepines, barbiturates) within 1 week prior to study enrollment.

6. STUDY PROCEDURES

We will require written informed consent from all study participants prior to entering the study. A table of study activities is given in section 6.8.

6.1. Screening

6.1.1. Demographics

Demographic information (date of birth, gender, ethnicity, race) along with handedness will be recorded at Screening.

6.1.2. Medical History

If the participant meets inclusion criteria, informed consent will be obtained. All relevant medical history, including history of current or past history of neurological or psychiatric illness, will be recorded. Current and past drug use (illicit and prescribed) will also be recorded. Participants will also be asked questions to determine whether they would be fit for fMRI scanning (e.g. if they are claustrophobic, unable to lie still for the duration of the scan, have metal implants). Participants will be asked whether they will be able to self-administer an intranasal medication (including whether they currently are experiencing nasal obstruction, upper-respiratory tract infection or utilize intranasally administered medications that could not be stopped 48 hours prior to each scan). Participants will also be specifically asked whether they have any known allergies to oxytocin or to preservatives used in the spray.

6.2. Intake

1. If the participant has been determined to meet criteria for the study, we will review the study with the participant and obtain informed consent.
2. Participants will be asked to submit to a urine drug screen to ensure no current illicit drug use and asked to give a saliva sample for ethanol screening. Pregnancy tests will also be done on female participants.
3. Participants will undergo a psychiatric interview to verify that participants meet study criteria.
4. Participants will be asked to perform the American National Adult Reading Test (AMNART, v2).
5. Participants will be asked to complete questionnaires at intake to assess trait measures of mood, personality, drug and alcohol use, and attachment that are expected to take approximately 2 to 2.5 hours (see section 6.10).
6. Participants will undergo an effort based task (PR task) designed to assess motivation for monetary incentives.
    - The PR task has been previously described (Chelonis et al 2011). During this task, participants will be instructed to repeatedly press a key in front of them over 5 minutes. They will be informed that if they press the key enough times, they will receive a quarter, and if they continue to press the key they will continue to win quarters (up to $5 dollars).
7. Ask participant to give a saliva sample for genetic testing (see section 6.3.3 regarding saliva analysis).
8. Schedule the participant for their scanning sessions and give them detailed information regarding their scanning day including location and schedule.

6.3. Scanning Day Measurements

6.3.1. Drug, Alcohol, & Pregnancy Screen

Urine will be collected and a drug screen, and if applicable, a pregnancy screen will be performed prior to each scanning session. A saliva ethanol test will also be done. Participants will be asked to refrain from alcohol use 24 hours prior to each visit.

6.3.2. fMRI Scanning

Functional MR images will be acquired on a research dedicated 3 Tesla scanner (Philips) located at the University of Utah. Each of the fMRI tasks described below will be programmed using E-prime software and presented to subjects via a display placed behind the gantry. Subjects will enter the scanner approximately 30 minutes following drug administration so that scanning coincides with peak oxytocin levels [3,21,32,33].

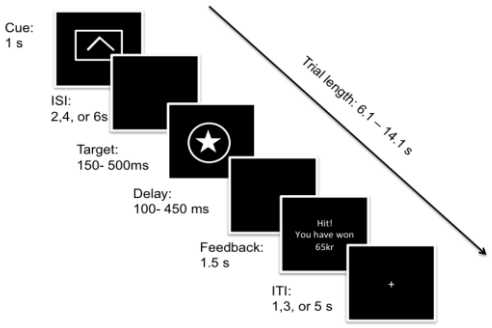


Figure 1. Example of a Single Trial in the MID Task. Adapted from Knutson, 2000.

6.3.2.1. fMRI Tasks

Modified Incentive Delay Tasks

The paradigm to be utilized has been used by our laboratory and others to assess functioning of the motivational circuitry in response to monetary rewards (i.e. Monetary Incentive Delay Task, MID) [34-36]. In this adapted paradigm, subjects will be presented with an incentive cue (shape) which signals the potential to either win money, avoid losing money, or whether no money is at stake (See Figure 1). Subjects can earn/avoid losing money during these conditions by pressing a button when a target box, presented after the incentive cue is on the screen. The reaction time used to define the presentation time for the target will be adjusted to ensure a ~66% success rate. Following the target box, subjects will see a feedback screen which will inform them whether they successfully won money or avoided losing money.

Figure 2. Example of social incentive feedback. Stimuli taken from Rademacher, 2010.

During some of the trials (i.e during the Social Incentive Delay Task, SID), the feedback screen will feature a social incentive (i.e. happy faces if they won or successfully avoided losing money or upset faces if they failed to win or lost money). Previous studies performed by Spreckelmeyer and colleagues (Figure 2) have demonstrated that the brain responds differently to social incentives compared to just monetary rewards [37,38]. During the social reward trials, participants will be told that they will be performing this task on behalf of another (a study member) who will be sitting outside of the scanner observing their performance. When the participant successfully 'wins' they will see a picture of that study member's smiling face and if they fail to avoid losing, they will see a picture of that study member's upset face.

Following the scan, the participant will be asked to provide VAS ratings to determine how they felt about the cues from the SID and MID and the feedback screens to examine their affective reactions.

Hemodynamic Response Function (HRF) Task

This paradigm is used to determine the Hemodynamic Response Function (HRF) which shows how a person's BOLD signal changes over time in response to changes in brain activity which corresponds approximately to changes in blood flow and corresponding oxygen consumption. This is a useful measure to collect to aid in the analysis of fMRI data and can be used to determine whether pharmacological agents have more generalized effects on BOLD activity. In this task, participants will be asked to look at either a fixation cross or a flashing

checkerboard. When the flashing checkerboard is onscreen, participants will be asked to press their thumbs and forefingers together rapidly.

Why-How Task / Photography Judgment Test

This paradigm is used to explore the processing of social cues. Specifically, participants are asked to make judgments about each of the photos (i.e. Photography Judgement Test) by answering a series of yes or no questions paired with a set of photographs featuring facial expressions or intentional actions (Figure 4). This measure will help determine whether oxytocin can influence the basic processing of social information which may in turn interfere with responses to social rewards.

Figure 3. Depiction of the Why/How Task. Image from Spunt, 2014.

Anatomical/Connectivity Scans

Anatomical scans will be acquired on the same 3 Tesla scanner used to collect the functional data. Two sets of anatomical images will be collected: high resolution anatomical scans and a lower resolution anatomical scan acquired in the same slice locations as the functional scans to improve standardization. Anatomical/Connectivity scans may include a high resolution T1-weighted image used for anatomical standardization to a standardized template, resting state, and diffusion tensor imaging to map white matter.

6.3.2.2. Post Scanning Tasks

Delayed Discounting

We will use this brief task to examine the extent to which individuals discount delayed rewards. During the task, participants will be asked to respond to a series of hypothetical choices. In the first series of choices, participants will be asked to imagine a hypothetical scenario where they could receive money. They are then asked to decide between different amounts of money being delivered at various times. For example, would they prefer to receive "$50 now" vs "$100 in a month" or "$50 now" vs "100 in a year". An algorithm will be used to determine the participants' individual discount rate. In the second series of choices, participants will be asked to make similar choices but to imagine that they could be sharing this reward with another person.

Time Perception

Related to the concept of delayed discounting is the ability to correctly perceive time. In order to test this, participants will be asked to click a button when they believe a certain amount of time has passed (e.g. 1 minute). Participants will be explicitly instructed not to count during their performance of the task.

Facial Emotion Recognition Task (FERT)

This task will determine how well participants are able to recognize facial expressions of emotion. This particular ability has been previously demonstrated to be influenced by oxytocin administration. During this task, participants will be told that they will see a series of faces (see Figure 4) with various emotional expressions (e.g. happy, sad, fear, anger). Faces will be displayed on a computer screen and participants will be told to rate the intensity of the emotion the faces is displaying.

Figure 4. Example of FERT Faces. Stimuli taken from NimStim Face Stimulus Set

6.3.3. Saliva Collection

Saliva will be obtained at intake for those participants who allow genetic analyses to be performed on their saliva. These samples will be labeled with the participant's unique ID number and stored in a laboratory freezer until assayed. Saliva will later be processed and analyzed for genetic analyses. Saliva will be collected during the scanning sessions for future testing of hormone levels.

6.4. Scanning Session 1

1. Review procedure with participant, noting any changes in health (such as nasal congestion or upper-respiratory issues) prior to drug administration.
2. Collect urine and saliva for drug and ethanol screening to ensure no acute drug use. A urine pregnancy test will be done for female participants. Record blood pressure and heart rate. Ask participant to give a saliva sample for laboratory testing.
3. Participant will practice the fMRI tasks on a laptop to become acquainted with each of the tasks.
4. Participant will self-administer either placebo or Syntocinon® (blinded to both research staff and participant) over 5 minutes.
5. Record and respond to any adverse experiences. Record blood pressure and heart rate.
6. Ask participant to complete measures designed to assess mood, social perception and anxiety while waiting for scanning (see section 6.10).
7. Participant will be placed into the scanner and scanned under several conditions which may include:
    a. Preliminary scans including collection of anatomical overlay
    b. MID task
    c. SID task
    d. HRF task
    e. Why/How / Photography Judgement Task
    f. Anatomical / connectivity images
8. Ask participant to complete the delayed discounting and FERT.
9. Ask participant to rate faces (e.g. trustworthiness) that may have given as part of the

SID task. Record blood pressure and heart rate. Ask participant to give a saliva sample for laboratory testing.

10. Any adverse events will be recorded and information for the next scanning session will be provided. A phone number will be collected so the participant can be contacted the next day to follow-up on any potential effects.

6.5. Scanning Session 1 Follow-up

1. Call participant and check on any adverse events that may have occurred following the scanning session.
2. Verify schedule for the next scanning session.

6.6. Scanning Session 2

1. Review procedure with participant, noting any changes in health (such as nasal congestion or upper-respiratory issues) prior to drug administration.
2. Collect urine for drug screen and perform urine drug analysis to ensure no acute drug use. Record blood pressure and heart rate. Ask participant to give a saliva sample for laboratory testing.
3. Participant will practice the fMRI tasks on a laptop to remind them how to perform each of the tasks.
4. Participant will self-administer either placebo or Syntocinon® (blinded to both research staff and participant) over 5 minutes.
5. Record and respond to any adverse experiences. Record blood pressure and heart rate.
6. Ask participant to complete several measures designed to assess mood, social perception, and anxiety while waiting for scanning.
7. Participant will be placed into the scanner and scanned under several conditions which may include:
    a. Preliminary scans including collection of anatomical overlay
    b. SID task
    c. MID task
    d. Hemodynamic Response Function
    e. Why/How / Photography Judgement Task
    f. Anatomical/connectivity images
8. Ask participant to complete the delayed discounting, time perception, and FERT.
9. Ask participant to complete measures designed to assess mood, social perception and anxiety (see section 6.10)
10. Ask participant to rate faces (e.g. trustworthiness) that may have been given as part of the SID task. Record blood pressure and heart rate. Ask participant to give a saliva sample for laboratory testing.
11. Any adverse events will be recorded and information for the next scanning session will

be provided. Participants will be asked questions regarding their scanning experience and to guess whether they received oxytocin or placebo during each scan. A phone number will be collected so the participant can be contacted the next day to follow-up on any potential effects.

6.7. Scanning Session 2 Follow-up

1. Call participant and check on any adverse events that may have occurred following the scanning session.

6.8. AUD Follow-up

1. For AUD participants: Participants will be contacted 30, 60, & 90 days following the final scan to assess levels of social support, drinking and well-being (see section 6.11).

6.9. Participant Compensation

Participants will be paid $50 for completing the initial survey battery at intake and up to $5 more depending on how well they do on one of the tasks. Participants will receive $100 for each fMRI scan and up to $40 more per scan depending on how well they perform the MID task. If participants complete both scans the total compensation will be between $250 and $335. The AUD group can earn an additional $5 for completing the 30-day follow-up, $10 for the 60-day follow-up, and $15 for the 90-day follow-up. Compensation will be provided in the form of cash or gift cards. Participants will be paid after the completion of each scan. If for some unforeseen reason any of the sessions are cut short or run longer than anticipated, participants will be paid a prorated amount for the session ($20/hour). Reasonable local transportation costs will be reimbursed. Participants can also request to receive a black and white printed picture of their brain obtained by MRI when they come in for their second fMRI visit.

6.10. Schedule of Activities

Protocol Activity	Screening	Intake	Scanning Session 1	Scanning Session 1 Follow-up	Scanning Session 2	Scanning Session 2 Follow-up
Visit # / Phone call	**Phone call**	**1**	**2**	**Phone call**	**3**	**Phone call**
Screening Questionnaire	X					
Informed Consent		X				
M.I.N.I. Interview		X				
Intake Questionnaires		X				
Intake Tasks		X				
Urine Drug, Ethanol, & Pregnancy Screen		X	X		X	
Syntocinon® or Placebo Administration			X		X	
Scanning Questionnaires			X		X	
fMRI Scanning: Anatomical			X		X	
fMRI Scanning: Tasks			X		X	
Obtain Saliva Samples		X	X		X	
Monitor Heart Rate and Blood Pressure			X		X	
Adverse Event Recording			X	X	X	X

*AUD participants will also be contacted 30,60, & 90 days following their final scan and asked to complete several questionnaires (see section 6.11).

6.11. List of Questionnaires & Surveys

Intake

Mood/Anxiety

- Patient Health Questionnaire (PHQ-9, v3) (2-3 minutes)
- Adverse Childhood Experiences (ACE, v3) (5 minutes)

Personality & Personal History

- NEO-Personality FFI (NEO-FFI, v3) (15 minutes)
- General History (v3) & Women's Health (WHQ, v4) Questionnaires (4 minutes)
- Drug History (v4) and Alcohol Use (AUQ, v4) Questionnaires (5 minutes)
- Readiness Ruler (v2, 1 minute)
- Alcohol Use Disorders Identification Test (AUDIT, v4) (5 minutes)
- Fagerstrom Test (v2, 3 minutes)
- Autism Spectrum Quotient (AQ-10, v3) (5 minutes)

Social and Well Being Measures

- Important People/Social Relationships Index (SRI-IPI, v6) (15-20 minutes)
- MOS Social Support Survey (MOS-SS v3) (1-2 minutes)
- Social Value Orientation (SVO, v3) (5 minutes)
- Emotional Contagion (EC, v4) (5 minutes)
- Anticipatory & Consummatory Interpersonal Pleasure Scale (ACIPS, v3) (7 minutes)
- Dysfunctional Attitudes Scale (DAS-A, v5) (10 minutes)
- Brief Fear of Negative Evaluation (BFNE-II, v3) (5 minutes)
- Interpersonal Reactivity Index (Brief IRI, v3) (5 minutes)
- Toronto Alexithymia Scale (TAS-20, v2) (7 minutes)

Scanning

- Positive and Negative Affect Schedule (PANAS, v3) (5 minutes)
- Inclusion of Other in the Self scale (iOS, v2) (1-2 minutes)
- UCLA Loneliness Scale (UCLA-LS, v3) (2-5 minutes)
- Scanning VAS Scales (v3, 2-3 minutes)
- Drug Effects Questionnaire (DEQ, v3) (3 minutes)

Follow-up (30, 60, 90 days)

- Satisfaction with Life Scale (SWLS, v4) (1-2 minutes)
- Follow-up VAS Scales (v2, 1-2 minutes)
- MOS Social Support Survey (MOS-SS, v3) (1-2 minutes)
- Timeline Followback/Quick Drinking Screen (QDS, v2) (3 minutes)

7. ADVERSE EVENT REPORTING

Adverse Event Definition

An adverse event (AE) is any untoward medical occurrence in a subject participating in an investigational study or protocol regardless of causality assessment. An adverse event can be an unfavorable and unintended sign (including an abnormal laboratory finding), symptom, syndrome or disease associated with or occurring during the use of an investigational product whether or not considered related to the investigational product.

These events may be:

a. *Definitely related:* clearly associated with study drug/treatment

b. *Probably related*: likely associated with study drug/treatment

c. *Possibly related*: may be associated with study drug or other treatment

d. *Unlikely to be related*, or

e. *Definitely not related* to the study drug/treatment

For reporting purposes, an AE should be regarded as definitely or probably related to the regimen if the investigator believes that at least one of following criteria are met:

a. There is a clinically plausible time sequence between onset of the AE and the administration of the study drug or treatment.

b. There is a biologically plausible mechanism for the study drug or treatment causing or contributing to the AE.

c. The AE cannot be attributed solely to concurrent/underlying illness, other drugs, or procedures.

d. A potential alternative cause does not exist.

Serious Adverse Events (SAE): An adverse drug experience occurring at any dose that results in any of the following outcomes:

a. Death

b. A life-threatening adverse drug experience

c. Inpatient hospitalization or prolongation of existing hospitalization

d. A persistent or significant disability &/or incapacity

e. A congenital anomaly or birth defect

Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse drug experience when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. A serious adverse experience includes any experience that is fatal or immediately life threatening, results in a persistent or significant disability/incapacity, requires or prolongs in-patient hospitalization, or is a congenital anomaly, cancer, or overdose.

Other important medical events that may not result in death, not be life-threatening, or not require

hospitalization may be considered a serious adverse experience when, based upon appropriate medical judgment, the event may jeopardize the subject/patient and may require medical or surgical intervention to prevent one of the outcomes listed previously.

Expected adverse events are those adverse events that are listed in the protocol, the Investigator's Brochure (current edition) or in the study informed consent document.

Unexpected adverse events are those that:

a. are not described in the Investigator's Brochure as far as oxytocin is concerned.

b. are not anticipated in the study informed consent. This includes adverse events for which the specificity or severity is not consistent with the description in the informed consent.

Unanticipated problem: Per FDA Procedural Guidance for Clinical Investigators, Sponsors, and IRBs (January 2009), A serious problem that has implications for the conduct of the study (requiring a significant and usually safety-related, change in the protocol such as revising inclusion/exclusion criteria or including a new monitoring requirement, informed consent or investigator's brochure).

Unanticipated problem Reporting: Per 21 CFR 312.66, 312.53 (c)(1)(vii), and 56.108(b)(1), should an Unanticipated problem occur during the investigation, the investigator will promptly report all unanticipated problems involving risks to human subjects or others to IRB/FDA.

The severity or grade of an adverse event may be measured using the following definitions:

Mild: Noticeable to the subject, but does not interfere with subject's expected daily activities, usually does not require additional therapy or intervention, dose reduction, or discontinuation of the study.

Moderate: Interferes with the subject's expected daily activities, may require some additional therapy or intervention but does not require discontinuation of the study.

Severe: Extremely limits the subject's daily activities and may require discontinuation of study therapy, and/or additional treatment or intervention to resolve.

Event reporting: The study will comply with the IRB & FDA reporting requirements and guidelines.

8. DATA ANALYSIS/STATISTICAL METHODS

8.1. Sample Size Determination

Work done by Knutson, Spreckelmeyer and Rademacher, indicate that significant task effects can be observed in the incentive delay task in samples in the range of 15-18 participants [34,35,37,38]. We implemented an fMRI-based power analysis using the fMRIpower software package (fmripower.org). The effect sizes below are expressed in standard deviation units, which is analogous to the Cohen *d* measure. All power calculations are based on ROIs with a p-value threshold of 0.005 for a 1-sided hypothesis test. With 44 subjects, we will have at least 90% power to detect an effect of size 0.77 in the nucleus accumbens, a region known to play a significant role in the processing of rewards [39,40].

8.2. Analysis of Primary Endpoint

8.2.1. fMRI Data Processing.

Preliminary processing. All fMRI data will be submitted to an initial series of preprocessing steps (e.g. slice-timing correction, realignment, artifact checks). The functional data acquired for each subject will be coregistered to the structural image collected and finally warped into standardized space using specialized neuroimaging software (e.g. Statistical Parametric Mapping software [SPM8, Wellcome Department of Cognitive Neurology, University College, London, UK]).

Subject-level analysis. For each task (for both Syntocinon® and Placebo conditions), a general linear model will be constructed with parameters corresponding to the anticipation and outcome of each type of reward at stake, modeling each session separately. Each stimulus type will be convolved with the hemodynamic response function to assess task-related changes in BOLD signal. Contrasts will be constructed to test for BOLD differences in anticipation activity (anticipation of reward vs null) and outcome activity (outcomes for reward trials versus outcomes for null trials) for each reward type. The statistical maps generated by these analyses will then be slightly smoothed and introduced into second level-analyses.

8.2.2. Data Analysis & Anticipated Results.

Analysis of Primary Objective 1 and 2. Examine the effects of oxytocin on the modified monetary and social incentive delay tasks.

Analyses. We will conduct whole-brain voxel-wise analyses with drug (Syntocinon® or Placebo) as a within-subject factor. We will examine the main effects of drug on reward related BOLD activity across the brain during both anticipation and outcome phases.

Anticipated Results. As oxytocin administration has been previously demonstrated to reduce amygdala activity in response to social challenges it is hypothesized that we will see a blunting of amygdala reactivity during the anticipation and attainment of social rewards (particularly for negative social incentives). Oxytocin has also been demonstrated to increase dopaminergic activity within the mesolimbic dopamine system (20) so we may see an enhancement of ventral striatal activity during the anticipation of social rewards; however, as Knutson and collaborators have reported that inducing dopamine release within the ventral striatum by amphetamine can actually reduce the difference in BOLD signal activity seen between the anticipation of reward and the anticipation of null (51), reductions in the BOLD response to anticipation of social reward within the ventral striatum is also possible.

While oxytocin has been predominately noted to affect social processes, there is some data to suggest that oxytocin's effects extend beyond modulating affiliative behaviors. Given this, we hypothesize oxytocin administration will show similar, though perhaps diminished, effects on amygdala and ventral striatal activity in response to the anticipation of monetary rewards as we see in anticipation of social rewards.

Analysis of Primary Objective 3. Compare responses to the modified monetary incentive delay task in participants with AUD versus controls.

Analyses. We will conduct whole-brain voxel-wise analyses with group (AUD or Control) as a within-subject factor. We will examine the main effects of group on reward related BOLD

activity across the brain during both anticipation and outcome phases.

Anticipated Results. It has been observed that individuals with AUD or with a family history of alcoholism show blunted NAc activity during the anticipation of non-drug rewards which may reflect a bias towards alcohol related stimuli. Both the insula and ACING are also implicated in the neurobiology of AUD. Indeed, lower insular activity has been demonstrated to be predictive of relapse and ACING responsivity to the anticipation of monetary reward is inversely related to characteristics associated with AUD including impulsivity. Hence, we expect to observe lower BOLD responses to the anticipation of rewards within the NAc, insula and ACING, in the AUD group compared to controls.

Analysis of Primary Objective 4. Compare the effects of oxytocin on the modified monetary incentive delay task in participants with AUD versus controls.

Analyses. We will conduct whole-brain voxel-wise analyses with drug (Syntocinon® or Placebo) and group (AUD or Control) as within-subject factors. We will examine the main effects of drug on reward related BOLD activity across the brain during both anticipation and outcome phases and compare this activity between groups.

Anticipated Results. We expect the oxytocin intervention to have a larger effect on BOLD responses to the anticipation of social rewards on individuals with AUD for several reasons. First, it is hypothesized that individuals with social impairments, which are common in persons diagnosed with substance dependence, may receive greater benefit from exogenous oxytocin. Second, it is proposed that circulating levels of oxytocin may moderate how a given individual response to exogenous administration of oxytocin. Lower plasma oxytocin levels have been noted in individuals with substance dependence and morphological abnormalities have been noted in the oxytocinergic neurons of individuals with AUD. Finally, though oxytocin is known to impact social reward processing, it also may influence reward activity more generally. To explore the selectivity of oxytocin's effects, I will analyze anticipatory reward activity in regions identified as significant in the group analyses above and compare SID and MID activity. While we expect specific effects of oxytocin on the SID, if oxytocin was shown to influence reward activity irrespective of social content, this would indicate an exciting, broad role of oxytocin in reward in AUD. This would be of great interest to those seeking to utilize oxytocin to treat AUD, where reward dysfunction is known to play a key role.

8.2.3. Potential Pitfalls and Alternative Strategies.

- We will employ a more sensitive technique, called region-of-interest analyses, to detect potential variation in these conditions. Region-of-interest (ROI) analysis involves looking for changes within selected brain areas, instead of looking for changes across the whole brain. As a relatively smaller number of regions will be examined, the overall number of comparisons will be reduced, resulting in an increase in statistical power. Thus, subtle differences between the conditions can be identified. For our purposes, these ROIs would include the ventral striatum, the amygdala and thalamus.
- Humans are not a homogenous group, thus inter-individual variability may pose some difficulty in discerning effects. Variables such as age can have an impact on task parameters. To address this concern, we will (1) perform regression analyses to determine if there is a relationship between these variables and the contrasts of interest and (2)

perform an analysis of covariance (ANCOVA) to control for these variables. The inclusion of covariates in our analyses will increase overall statistical power as variability will be taken into account. In addition, as some groups have reported certain affiliative characteristics to be related to oxytocin measures, we will also be obtaining measures of attachment and sociability that can be used to help explain some of the variation.

8.3. Safety Analysis

Adverse event data will be recorded and reported to the data safety monitor.

9. MONITORING

9.1. Monitoring Plan and Information

To assure adequate protection of the rights of human subjects, per 21 CFR §312.50, 312.53, the study will be monitored to ensure proper informed consent procedures were followed and to ensure the quality and integrity of the data.

9.2. Safety Monitoring

This study will utilize study monitors within the Clinical Trials Office at the University of Utah School of Medicine. This unit specializes in providing clinical investigators with research support services and has experience overseeing projects from Phase I-IV and supporting IND/IDE projects. The monitoring team helps researchers develop and implement monitoring and auditing plans, aids researchers in IRB submission, maintains regulatory files and coordinates with the FDA and NIH when necessary.

Taking into account the objective, purpose, design, complexity, size and endpoints of this study, the following monitoring will occur:

- 100% monitoring of the first two subjects
- 100% monitoring of informed consent documents
- 100% monitoring of protocol deviations
- 100% monitoring of adverse events
- Monitoring of essential documents

Monitoring will occur on an annual basis. Following each visit, the monitor will generate a monitoring report to describe the progress of the study, any visit findings, and identify needed follow up. These reports will be available to all study team members, including other monitors, study management, and data management. All open action items will be reviewed for completion at consecutive monitoring visits. The monitoring report will include, but will not be limited to, the following:

- The date of the monitoring visit, and the individual(s) conducting it
- A list of subject records reviewed (e.g. subject charts, hospital records, lab slips, etc.) as well as case report forms reviewed
- A description of any noncompliance, potential noncompliance, data discrepancies, or other deficiencies identified
- A description of any actions taken, to be taken, or recommended actions, as well as the person responsible for completing action items.

10. DATA HANDLING AND RECORD KEEPING

To ensure confidentiality of patient information, study data will be collected and managed using an encrypted, 21 CFR Part 11 and HIPAA-Compliant electronic database system, REDCap (Research Electronic Data Capture). REDCap is a secure web application designed to support data capture for research studies, providing user-friendly web-based case report forms, real-time data entry validation (e.g. for data types and range checks), audit trails, electronic signatures, and Standard Operating Procedures (SOPs) that protect data confidentiality and integrity. REDCap has been disseminated for use locally and currently supports over 240+ academic/non-profit consortium partners on six continents and over 26,000 research end-users (www.project-redcap.org). The REDCap software was developed by Vanderbilt University and has been obtained and installed for usage at the University of Utah. All access to the data requires individual authentication (a unique username and password) and all data into and out of the server is encrypted, which prevents eavesdropping and tampering with any transmitted data, and is the standard for encrypting communications across all industries, including financial services and healthcare.

10.1. Record Retention

Per 21 CRF §312.62, study records will be retained for at least 2 years after the investigation is discontinued.

10.2. Annual Progress Report

An annual progress report will be submitted to the FDA within 60 days of the anniversary of the date that the IND went into effect per 21 CFR §312.33.

11. ETHICS

11.1. IRB/FDA

Prior to study commencement, an Investigator Initiated Investigational New Drug (IND) request will be submitted to the Food and Drug Administration (FDA), for review and approval. The study will also be reviewed and approved by the Institutional Review Board (IRB, University of Utah, Salt Lake City, UT).

This study will be carried out in compliance with the protocol and the principles of Good Clinical Practice.

Before implementing this study, the protocol, the proposed informed consent form and other information to be provided to subjects, must be reviewed by a properly constituted Institutional Review Board (IRB). Any amendments or administrative changes in the research protocol during the period, for which the IRB approval has already been given, will not be initiated without submission of an amendment for IRB review and approval. Any amendments to the protocol that significantly affect the safety of subjects, scope of the investigation, or the scientific quality of the study will be submitted for FDA review. These requirements for approval will in no way prevent any immediate action from being taken by the investigator in the interests of preserving the safety of all patients included in the trial.

11.2. Protocol Deviations

A protocol deviation (or violation) is defined as any departure from the defined procedures

and treatment plans as outlined in the protocol version submitted and previously approved by the IRB. Protocol deviations have the potential to place participants at risk and can also undermine the scientific integrity of the study thus jeopardizing the justification for the research. Protocol deviations are unplanned and unintentional events.

Because some protocol deviations pose no conceivable threat to participant safety or scientific integrity, reporting is left to the discretion of the PI within the context of the guidelines below. The IRB/FDA will be promptly notified of protocol deviations which are:

- Exceptions to eligibility criteria.
- Intended to eliminate apparent immediate hazard to a research participant
- Harmful (caused harm to participants or others, or place them at increased risk of harm—including physical, psychological, economic, or social harm)
- Possible serious or continued noncompliance

11.3. Subject Information and Consent

The study team member will explain to each subject (or legally authorized representative) the nature of the study, its purpose, the procedures involved, the expected duration, the potential risks and benefits involved and any discomfort it may entail. Each subject will be informed that participation in the study is voluntary and that he/she may withdraw from the study at any time and that withdrawal of consent will not affect his/her subsequent medical treatment or relationship with the treating physician.

This informed consent will be given by means of a standard written statement, written in non-technical language. The subject should read and consider the statement before signing and dating it, and should be given a copy of the signed document. If the subject cannot read or sign the documents, oral presentation may be made or signature given by the subject's legally appointed representative, if witnessed by a person not involved in the study, mentioning that the patient could not read or sign the documents. No patient can enter the study before his/her informed consent has been obtained.

The informed consent form is considered to be part of the protocol, and will be submitted for IRB approval.

12. STUDY DISCONTINUATION CRITERIA

The study team will review all Serious Adverse Events (SAEs) and make recommendations regarding the continuation or discontinuation of the study. The study will be stopped or the protocols modified when there is sufficient evidence of unacceptable side effects of the study drug or placebo. Participants are free to leave the study at any time without any penalty. If the participant chooses to tell the study team why they are leaving the study, the reasons will be kept as part of the study record.

Patients can be discontinued from the study for a variety of reasons including:

- The researcher feels it is not in the best interest of the participant to stay in the study
- The participant does not follow instructions
- The participant becomes ineligible to participate

- The participant's condition changes and they require treatment which is not allowed to be taken during the study
- The study is suspended or cancelled

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