

Impact of Neurobiological Substrates of Social Stress in
Individuals with Alcohol Use Disorder

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Impact of Oxytocin on Neurobiologic Substrates of Social Stress in Individuals with Alcohol Use Disorder

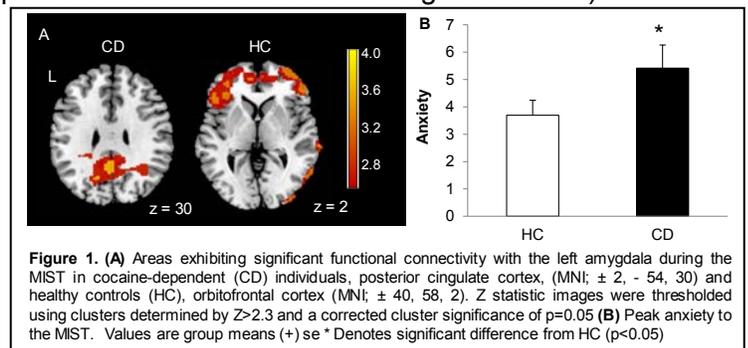
Social stress is an important factor in the motivation to use and abuse alcohol, and social avoidance is a symptom of alcohol dependence [1, 2]. Social avoidance also impedes effective treatment particularly in group settings that are a commonly employed treatment modality. Data from human neuroimaging studies suggest that functional connectivity between the prefrontal cortex and limbic brain regions is important for maintaining affective and behavioral responses to social stimuli while results from clinical and preclinical studies suggest that chronic alcohol exposure produces alterations in corticolimbic brain regions that may underlie compulsive drug seeking behavior and relapse [3-6]. Therefore, therapeutic interventions that increase corticolimbic connectivity may be effective at ameliorating reactivity to social stress and reducing craving in alcohol dependent individuals. The neuropeptide oxytocin (OT) increases social approach, trust, and reduces anxiety to social stress [7-9]. Data from recent studies show that OT administration restored corticolimbic connectivity in individuals with social anxiety disorder and decreased withdrawal symptoms in alcohol dependent individuals [10, 11]. In addition, OT attenuated drug craving and anxiety in marijuana-dependent individuals exposed to a social stress task [12]. In this project, we propose a double-blind placebo controlled pilot study to compare the impact of OT versus placebo on corticolimbic connectivity in alcohol dependent subjects. Blood oxygen level dependent (BOLD) functional magnetic resonance imaging (fMRI) will be used to measure corticolimbic connectivity at rest and during the Montreal Imaging Stress Task (MIST) [13]. Subjective craving and anxiety data will also be collected. Prior to the scanning session, participants will receive either intranasal OT (24 IU) or a placebo (PBO) spray.

Aim 1: Determine the impact of OT on corticolimbic connectivity in alcohol dependent individuals exposed to social stress. Psychophysiological interaction (PPI) analysis using the amygdala as the seed region will be used to assess significant task (stress condition > control condition) x seed interactions. Hypothesis 1A: The OT group will exhibit greater functional connectivity between the amygdala and prefrontal cortical regions (orbitofrontal and anterior cingulate cortices) than the PBO group. Hypothesis 1B: The OT group will report lower subjective anxiety and craving in response to the MIST than the PBO group.

Aim 2: To determine the impact of OT on resting-state functional connectivity in alcohol dependent individuals. Hypothesis 2A: OT will modulate resting-state functional connectivity among sub-networks implicated in reward and motivation (nucleus accumbens, and orbitofrontal cortex), memory and learning (amygdala and hippocampus), and cognitive control (prefrontal cortex and anterior cingulate cortex).

Preliminary Data: Evidence for Corticolimbic Uncoupling During Social Stress in Individuals with SUDs

We examined corticolimbic coupling during the MIST in cocaine-dependent (DSM-4) (n=13) and healthy control (n=15) subjects using a psychophysiological interaction (PPI) analysis. Using the left amygdala as the seed region, significant task x seed interactions were found in both groups (**Figure 1A**). In the cocaine-dependent group, the left amygdala exhibited significantly greater functional connectivity with the posterior cingulate cortex during stress. In the control group, the left amygdala exhibited significantly greater functional connectivity with the orbitofrontal cortex during stress. The MIST produced a significantly greater increase in anxiety in cocaine-dependent individuals than healthy controls (**Figure 1B**). These data provide support and lend feasibility to employing these imaging and stress task procedures in this proposed pilot project.



Approach:

Participants: Twenty-four individuals with AUD will be recruited through the Clinical Intake and Assessment (CIA) Core of the ARC. Screening and basic assessments will be conducted by the CIA Core to determine eligibility and suitability for participation in this pilot project. Each individual will sign an IRB-

approved informed consent form. ***Inclusion criteria*** include: 1) male or female, any race or ethnicity; ages 21-40, 2) able to comprehend English and function at an intellectual level sufficient to provide informed consent and complete the assessments, 3) meet DSM-5 criteria for current alcohol use disorder and reports drinking on average, at least 20 standard alcoholic drinks per week for at least the past three months, 5) not engaged in and does not want treatment for alcohol related problems, 6) lives within a 50 mile radius of the ARC, and 7) able to maintain abstinence from alcohol for the two days prior to the study visit. ***Exclusion criteria*** include: 1) meet DSM-5 criteria for any other psychoactive substance use disorder, 2) psychoactive substance use (except marijuana and nicotine) within the last 30 days, 3) meet DSM-5 criteria for current major depression, panic disorder, obsessive-compulsive disorder, post-traumatic stress syndrome, bipolar affective disorder, schizophrenia, dissociate disorders, eating disorders, and any other psychotic disorder or organic mental disorder, 4) women who are pregnant or breastfeeding, 5) current suicidal ideation or homicidal ideation, 6) taking psychoactive medications or medications known to affect alcohol intake, 8) history of/or current cardiovascular, renal, GI, neurological or endocrine diseases, 9) history of alcohol related illness, 10) SGPT (ALT) or SGOT (AST) levels greater than 2.5 times normal at screening, 11) charges pending for a violent crime, 12) an unstable living situation, 13) presence of ferrous metal in the body, 14) claustrophobia or morbid obesity, and 15) history of head injury with >2 minutes of unconsciousness.

Study Visit Assessments: The Drinking Motives Questionnaire Revised will be used to assess motivation for drinking across four subscales: (1) coping motives; (2) social motives; (3) conformity motives; and (4) enhancement motives [14]. The Form 90 will be used to assess daily alcohol consumption in the 90 days prior to the study visit and the time from last drink [15]. A modification of the Within Session Rating Scale will be used to assess craving and mood [16]. The Childhood Trauma Questionnaire (CTQ) will be used to assess trauma prior to age 18 [24]. To determine MRI safety, the Metal Screening Questionnaire (MSQ) will be filled out by each participant and reviewed by study staff. To assess potential alcohol withdrawal symptoms, the Clinical Institute Withdrawal Assessment of Alcohol Scale, Revised (**CIWA-Ar**) will be administered [30].

Study Visit Procedures: Participants will be asked to arrive at the Addiction Sciences Division on the study visit day. Female participants will have their urine tested for pregnancy. Females who test positive for pregnancy will be excluded. All participants will be tested for drugs of abuse and alcohol. Patients testing positive for drugs, with the exception of marijuana, will be excluded; patients testing positive for alcohol may be re-scheduled. Participants who have not had a physical exam in the Addiction Sciences Division within the last 30 days will have and H&P. Participants will be asked about substance use in the last 90 days and will fill out the Drinking Motives Questionnaire, the CTQ, and the MSQ. They will then be escorted to the scanner at 30 Bee Street.

The study will use a double-blind placebo controlled design. Intranasal OT (n=12) or PBO (n=12) sprays will be administered at 11:30 am, approximately 45 min prior to the scanning session. This dose and timing of OT administration were selected based on the literature [17, 18]. ***MIST Procedure:*** The study will use a block design of three, 6-min runs separated by 2-min of rest for feedback, for a total of 24 min. During each run, participants will be exposed to 40-sec blocks of three different conditions (rest, control, and experimental). Prior to the task, participants will be shown images of what the screen will look like during each condition. The participants will be instructed to relax during the rest condition and focus on the screen. During the control condition, the participants will be asked to answer math problems as accurately as possible but will also be told that their responses will not be recorded. During the experimental condition, the participants will be asked to perform the math task as quickly and accurately as possible. A performance bar located on the screen will allow them to see their performance as compared to an “average” person. The participants will be told that the average person would answer about 85% of the problems correctly; however, the program limits the participants’ performance rate to between 35-45%. A time limit will be enforced throughout the experimental condition. After each run, the participants will be given negative feedback from the investigator. ***BOLD-fMRI Procedure:*** Data will be acquired on a Siemens Trio 3T scanner in MUSC’s Center for Biomedical Imaging. For co-registration and normalization of functional images, a high resolution T1-weighted MPRAGE anatomical image will be acquired with the following parameters: TR= 2100 ms, TE= 4.18 ms, flip angle= 12°, field of view= 256 mm, slice thickness= 1.0 mm. The scanning planes will be oriented parallel to the anterior commissure–posterior commissure line. Participants will be asked to relax and keep their eyes opened and fixed on a cross-hair for 6 min while resting state data are collected. Participants will then complete the MIST. T2*-weighted gradient-echo planar images (EPI) will be acquired with the following parameters: TR= 2000 ms, TE= 27 ms, flip angle= 76°, matrix 64 x 64, field of view= 23 cm, slice thickness= 3.7 mm with no gap, with 36 slices to cover the entire

brain. ***Implicit Facial Affect Recognition Task:*** The amygdala response to emotional faces that are presented outside the focus of attention (i.e. implicit tasks) is significantly greater than that observed during overt (explicit) presentation of the same stimuli [25]. Emotional adult faces will be selected from a variety of sources are standardized in size and enclosed in the same oval surround [26]. Dr. Joseph (MUSC) has developed a corpus of faces for a recent project (National Institute of Mental Health, R21 MH086958-01, “A comparative developmental connectivity study of face processing”) that will be used for the present project. The faces will depict male and female Caucasian, Asian and African Americans expressing three different categories of emotion; fear, anger, and happiness. Neutral faces will also be presented (Figure 5). Because the participants will also be from different ethnic categories, it is important to include a mixture of races. In a block design, participants will view a series of faces (for 27.5 sec) within a block and report on the gender of those faces at the end of the block (for 5 sec). Each block will present 56 faces that depict the same emotion and same gender so there will be 6 pseudorandomly ordered task blocks (3 emotions x 2 genders) and 7 rest blocks (27.5 sec each) that present a crosshair to be fixated. In each task block, each emotional face will be presented for 33 msec. followed by a neutral face mask (from a different individual) for 167 msec. followed by a blank screen for 291 msec. At the end of the block participants will report the gender using two buttons on a response pad. Assignment of face sets to sessions will be counterbalanced across subjects.

Payment to Participants: Participants will receive \$100 for completing the scanning visit.

Data Analyses *MIST:* Post-acquisition preprocessing and statistical analysis of all of imaging data will be performed using FEAT (FMRIB Expert Analysis Tool) Version 5.98, part of FSL (FMRIB's Software Library). Data will be preprocessed using scripting tools from FMRIB Expert Analysis Tool (FEAT). Functional connectivity will be measured using a PPI seed-based approach [19]. A customized square wave-form representing the task and the duration of each condition will be convolved with a double-gamma hemodynamic response function. A mask of the seed region will be made using a 12-mm diameter sphere located in the center of the amygdala using the MNI coordinates (x, y, z = ±22, 0, -22). For each participant, the mean corrected and high pass filtered time series of the BOLD signal in the amygdala will be extracted and used in a single subject whole brain PPI analysis. The PPI model will include (1) the task vector; (2) the time series of the BOLD signal in the amygdala; (3) a term representing the positive task x seed interaction; and (4) a term representing the negative task x seed interaction. The first level analysis will generate contrast images of the parameter estimates for each of the four regressors. Since the hypotheses explore increases in corticolimbic connectivity, the group analysis will focus on the positive interaction term. The contrast images of the parameter estimates of the positive interaction term will be entered into a 2nd level random effects analysis. Unpaired t-tests will be used to test for group differences. All group level results will be thresholded at $Z > 2.3$ using a corrected cluster threshold of $p = 0.05$. A linear mixed effects model containing all serially measured time points will be used to assess the effects of OT versus placebo on alcohol craving and anxiety ***Implicit Facial Affect Recognition Task:*** Following preprocessing (described above), within-task data from individual participants will be analyzed with a fixed-effects general linear model (GLM), with each emotion (described below) modeled as a box-car function convolved with a double-gamma hemodynamic response function. The response period at the end of each block will also be modeled as a separate variable to remove effects of explicit decision making and response selection. The GLM procedure is repeated for each voxel with six movement parameters (3 rotation values in radian and 3 translation values in millimeter) included as covariates to control for the influence of head motion on the data. Following first-level analysis, subject-specific contrasts will be entered into second-level, random-effects analyses. Since the hypotheses associated with Specific Aim 2 explore amygdala activity, we will use a region of interest approach (ROI). The BOLD signal from the left amygdala will be extracted using an anatomical mask generated by a 12-mm diameter sphere located in the center of the left amygdala using the MNI coordinates (x, y, z = -22, 0, -22). The percent signal change between the fearful and neutral conditions (contrast of interest) will be calculated for each participant. Separate random-effects one-way analysis of variance tests (ANOVA) will be used to test for the following group differences (AUD-PBO vs. AUD-OT). Since human neuroimaging studies have found that other brain regions play a role in implicit emotional processing, including the right amygdala and insula [27,28], secondary analyses will also include these regions. In addition, OT may impact amygdala responding to happy faces [29] therefore group differences in amygdala responding in the happy-neutral contrast may be explored in future

analyses. For multiple comparisons that are not part of the hypothesis a priori, the Bonferroni correction will be used. In addition, contrasts of other pertinent baseline characteristics will be performed between groups. If the groups differ significantly on any of these baseline characteristics, the corresponding variables will be used as covariates in the above analyses. The statistical analyses will be conducted using SAS 9.3 software (SAS Institute Inc., Cary, NC, USA).

PROTECTION OF HUMAN SUBJECTS

1. RISKS TO THE SUBJECTS

1.1 Human Subject Involvement and Characteristics

Admission into the study is open to men and women and to all racial and ethnic groups, age 21-40 with alcohol use disorder. Participants will be recruited through the Medical University of South Carolina's (MUSC) Addictions Research Center (ARC) Assessment Core. Inclusion/exclusion criteria are listed below:

General Inclusion/Exclusion Criteria

Inclusion Criteria

1. Age 21-40.
2. Subjects must be able to provide informed consent and function at an intellectual level sufficient to allow accurate completion of all assessment instruments.
3. Meets the DSM-5 criteria for current alcohol use disorder.
4. Reports drinking on average, at least 20 standard alcoholic drinks per week for at least the past three months.
5. Currently not engaged in, and does not want treatment for, alcohol related problems.
6. Lives within 50 miles of the study site.
7. Subjects must consent to random assignment.
8. Able to maintain abstinence for two days (without the aid of detoxification medications) as determined by self-report and breathalyzer measurements. Subjects must also have a negative breathalyzer urine drug screen at the study visit.
9. Subjects must consent to the study visit which includes an outpatient admission to the Addiction Sciences Division and completing one functional magnetic resonance imaging (fMRI) scanning session.

Exclusion Criteria

1. Currently meets DSM-5 criteria for any other psychoactive substance use disorder.
2. Is determined
3. Any psychoactive substance use (except marijuana and nicotine) within the last 30 days by self-report and urine drug screen. For marijuana, no use within the last seven days by verbal report and negative (or decreasing) urine THC levels.
4. Meets DSM-5 criteria for current major depression, panic disorder, obsessive-compulsive disorder, post-traumatic stress syndrome, bipolar affective disorder, schizophrenia, dissociate disorders, eating disorders, and any other psychotic disorder or organic mental disorder.
5. Has current suicidal ideation or homicidal ideation.
6. Has the need for maintenance or acute treatment with any psychoactive medication including anti-seizure medications and medications for ADHD.
7. Is currently taking medication known to affect alcohol intake (e.g., disulfiram, naltrexone, acamprosate, topiramate).
8. Has clinically significant medical problems such as cardiovascular, renal, GI, neurological (e.g. seizure disorder) or endocrine problems that would impair participation or limit medication ingestion.
9. Has past history of alcohol related medical illness such as gastrointestinal bleeding, pancreatitis, peptic ulcer, hepatic cirrhosis or alcoholic hepatitis.
10. Has hepatocellular disease indicated by elevations of SGPT (ALT) or SGOT (AST) greater than 2.5 times normal at screening.
11. Females of childbearing potential who are pregnant (by urine HCG), nursing, or who are not using a reliable form of birth control.

12. Has current charges pending for a violent crime (not including DUI related offenses).
13. Does not have a stable living situation.
14. Presence of ferrous metal in the body, as evidence by metal screening and self-report.
15. Severe claustrophobia or morbid obesity that preclude placement in the MRI scanner.
16. History of head injury with >2 minutes of unconsciousness.

1.2 Sources of Materials

Research materials obtained from individual subjects includes structured clinical interviews, questionnaires, blood samples, urine drug screens, urine pregnancy tests, breathalyzer tests, structural and functional MRI scans.

1.3 Potential Risks

Risks associated with the assessment include the possibility that subjects might be upset by questions related to their substance use and psychiatric history. Risks associated with venipuncture may be mild pain and possible bruising. Under certain conditions, participants may experience psychological discomfort from a positive pregnancy test. There are very few potential risks from fMRI itself. There is no exposure to ionizing radiation and the machine and scanning sequences and gradients are approved by the FDA for routine clinical use. Individuals who are claustrophobic might experience anxiety during the scanning procedures. However, we will pre-select individuals who, in general, do not have this problem. A patient may experience some loud noises during the scanning procedure and there is a mild risk of hearing damage if patients are not given hearing protection. Participants may experience psychological discomfort from undergoing the scanning procedure, such as boredom and fatigue. Ferrous objects in the body that are undetected could move during scans. This could lead to tissue damage and hemorrhage. The MIST is designed to induce a stress response, and therefore subjects are likely to experience some psychological discomfort. In addition, individuals could experience alcohol craving, therefore alcohol use after the study visit is a potential risk. Adverse effects associated with systemic oxytocin use in pregnancy include seizures, mental disturbances, unexpected bleeding or contraction of the uterus. However, several studies have been conducted in men and women who are not pregnant with intranasal doses between 20 and 60 IU, and no side effects have been reported [20, 21]. A review by MacDonald and colleagues (2011) also found no adverse outcomes associated with oxytocin dosages of 18-40 IU for short term use in controlled research settings [22]. To date, our research group has administered intranasal oxytocin to over 170 individuals with substance use disorders and healthy controls with no side effects. These risks are outlined in the informed consent documents.

2. ADEQUACY OF PROTECTION AGAINST RISKS

2.1 Recruitment and Informed Consent

Patients will primarily be recruited through the MUSC ARC Assessment Core through the use of advertisements (internet, newspaper, radio and TV). Medical records will NOT be reviewed to identify potential study subjects. The study PI, a Co-I, or other qualified study staff will obtain informed consent. The informed consent (IC) form includes a detailed description of the study procedures, along with statements regarding participants' rights to withdraw from the procedure at any time without consequences. The IC form will specifically review the potential for psychological distress, risks associated with MRI and oxytocin that may occur as a result of study participation. The IC will also inform individuals that the scanner is located on-campus at a research facility and not at a clinical facility, therefore immediate emergency medical services may not be available. The IC form will be explained to individuals using language that is easy-to-understand and individuals will be instructed to read the form carefully prior to signing it. The IC form will include emergency contact information for the PI. Any questions pertaining to the study or consent will be answered. Potential participants will not be required to make a decision to participate at this initial contact, though that possibility will be available. If individuals wish to discuss study participation with their family and/or significant others, they will be encouraged to do so. Consent will be documented by the signature of the participant on the informed consent agreement, accompanied by the signature of the individual obtaining the consent.

2.2 Protections Against Risks

Dr. Joseph and an on-call physician will be available by pager/cell telephone during the entire study for any questions or emergencies that may arise. Our past research experience suggests that data collection using many of these same assessments and questionnaires can be conducted without undue psychological distress. Efforts will be made to protect the confidential nature of the information collected; however, this

cannot be guaranteed (e.g., subpoena). This experience includes substantial research with individuals with SUDs, healthy controls and work on large-scale studies asking questions about similar topics. At the conclusion of the study visit, each participant will be debriefed and provided with full disclosure about the deception of the MIST. Subjects will be told that the computer makes adjustments to keep the number of correct responses to less than 50% and therefore a good score on the task is impossible. If appropriate, the psychiatrist will personally evaluate the subject. Should any craving or anxiety induced during the experimental manipulation fail to subside within 3-4 hours, a study psychiatrist will be available to arrange either hospitalization through the MUSC CDAP or make an appropriate referral. These protective procedures are in place for all of our on-going research studies of stress reactivity in individuals with SUDs.

All sessions will be conducted under the supervision of experienced personnel. The research team and all of the study staff will have successfully completed the Miami Collaborative IRB Training Initiative (CITI) course and its associated tests in research ethics. To ensure confidentiality, each participant will be assigned a unique identification number and all information will be collected under that number. Identification numbers linked with names will be retained separately from the data files and locked in a different cabinet. Only the investigator will have access to the master lists of codes. No names or personal identifiers will be included in the data files. Files will be stored in the Clinical Neurosciences Division, in an office that is locked when not in use. Structural and functional neuroimaging data will be stored on a secure password protected server maintained by the MUSC Center for Biomedical Imaging (CBI). Only the PI and Co-I's will have access to files on the server. Care will be taken to prevent disclosure of pregnancy tests or any other lab results to anyone other than the study participant.

All of the study staff will be required to complete the CBI MRI safety training class. The course is taught by an AART (American Registry of Radiologic Technologists) registered technician. The staff will be trained about safety in the MRI environment, and how to screen oneself and others. The staff will also have knowledge of safety procedures for entering the scanner facility, safely removing participants from the scanner, when and how to quench the magnet and basic emergency procedures including emergency contact information. Standard operating procedures for emergency situations are located on-site. The technician and the PI are authorized to operate the equipment and will be present throughout the scanning sessions. Although there are no known risks of MRI scanning to a developing fetus at 3.0 T, the possibility that risks could be discovered in the future cannot be ignored. Urine pregnancy tests will be used to exclude pregnant women from study participation. A careful metal screening history will be taken from each subject to assess the possibility of metal devices/implants and will be reviewed by the PI, MRI technician and/or clinical staff who have had extensive training and experience with MRI safety. If the screening yields information that raises a question of safety, the subject will be asked to provide the appropriate documentation (i.e. film) before they are allowed to participate. In addition, participants will be asked to empty their pockets and will be screened with a hand-held ferromagnetic-detector wand. Subjects will wear earplugs and sound-dampening headphones to decrease the intensity of the scanner noise. Prior exposure to pictures of the scanner, getting into the scanner and seeing others in the scanner often reduces psychological discomfort or identifies people for whom scanning is not appropriate. If abnormalities in the brain images are found, the subjects will be referred to an appropriate clinical care provider.

Subjects will be informed about the potential symptoms of alcohol withdrawal and advised to call for medical assistance if they experience severe symptoms. The CIWA-Ar will be administered prior to procedures on the study visit day [23].

Subjects will be taught about the potential side effects of oxytocin. Pregnancy tests will be performed at the screening visit and again at the study visit. The pregnancy test will occur before the participants receive the intranasal spray. Urine pregnancy tests are routinely used in all of our clinical research studies. In the event that a pregnancy test is positive, the results will be disclosed to the participant by the PI or Co-I and if necessary the participant will be given an appropriate referral for counseling. Oxytocin administration will occur in a fully staffed clinical environment with emergency medications (i.e., IM diphenhydramine, alprazolam) and equipment available as needed. All subjects will be informed at the onset that they may terminate study participation at any time without repercussions.

3. POTENTIAL BENEFITS OF THE PROPOSED RESEARCH TO THE SUBJECT AND OTHERS

There are no guarantees of specific benefits to individual study participants. However, potential benefits include a detailed psychiatric and substance use assessment and referral for treatment if requested. In addition, subjects may benefit from the realization that, through their study participation, they are helping to

advance our state of knowledge regarding the neurobiologic factors that underscore stress induced alcohol craving and relapse. An investigation of oxytocin's effects on subjective anxiety and alcohol craving in may provide important information that can guide treatment individuals with alcohol use disorder. While the benefits to the individual patient are minimal, the minimal risks are reasonable in relation to the benefits to be gained from the investigation.

4. IMPORTANCE OF THE KNOWLEDGE TO BE GAINED

This study may provide important information that can improve treatment for future patients with alcohol use disorder. The minimal risks of the investigation are considered reasonable in relation to the expected knowledge to be gained.

5. DATA AND SAFETY MONITORING PLAN (DSMP)

5.1 Summary of the Protocol

The primary objective of this proposal is to identifying the neurobiologic substrates of social stress in individuals with alcohol use disorder. Individuals with alcohol use disorder will receive either intranasal oxytocin (24 IU) or a placebo spray prior to participating in a single fMRI scanning session. During the scanning session participants will complete the Montreal Imaging Stress Task, a Facial Affect Recognition Task, and a resting state scan. The primary outcomes of interest are (1) corticolimbic functional connectivity at rest; (2) corticolimbic functional connectivity during the MIST and (3) subjective anxiety and craving responses to the MIST.

5.2 Trial Management

The study will be managed from the Addiction Sciences Division within the Department of Psychiatry and Behavioral Sciences at the Medical University of South Carolina. The target population is described above in the inclusion/exclusion criteria.

5.3 Data Management and Analysis

Data will be entered by a research assistant directly into a computer using standard database software using REDCap. Neuroimaging data will be stored and analyzed on the CBI's password protected server.

5.4 Quality Assurance

Quarterly data audits, overseen by the PI will be conducted. Confidentiality protections are outlined above.

5.5 Regulatory Issues

Dr. Moran-Santa Maria was issued an IND from the FDA for the use of (IND 122,040). An FDA amendments has been submitted to transfer her IND to another investigator. Potential conflicts of interest will be reported using NIH guidelines outlined in *"Issuance of the Final Rule - Responsibility of Applicants for Promoting Objectivity in Research for which Public Health Service Funding is Sought and Responsible Prospective Contractors"* for disclosure. All unexpected Adverse Events (AEs) will be reported to the MUSC Committee on Human Research and NIDA within 48-business hours. Serious Adverse Events (SAEs) will be reported within 24-business hours. Follow-up of all unexpected and serious AEs will also be reported to these agencies. All AEs will be reviewed weekly by the PI, bi-annually by the Data Safety and Monitoring Board (DSMB) and yearly by the IRB. Any significant actions taken by the local IRB and protocol changes will be relayed to NIAAAA. AEs and SAEs occurring during the course of the project will be collected, documented, and reported in accordance with the protocol and IRB reporting requirements. All research staff involved with AE reporting will receive general and protocol specific AE/SAE training including identification, assessment, evaluation, documentation, and reporting. The research assistant, study coordinator, or the PI will identify any potential AEs and SAEs during the course of the study. This information will be provided to the study physician, who will be responsible for AE/SAE assessment and evaluation including a determination of seriousness and study relatedness.

5.6 Definition of AE and SAE

An Adverse Event (AE) is defined as any unwanted change, physically, psychologically or behaviorally, that occurs in a study participant during the course of the study that may or may not be related to study participation. A Serious Adverse Event (SAE) is defined as an adverse event that has one of the following outcomes:

- Results in death
- Is life-threatening
- Requires inpatient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability/incapacity
- Is a congenital anomaly/birth defect
- Requires intervention to prevent one of the above outcomes

5.7 Documentation and Reporting

AEs/SAEs will be documented and reported as per protocol and IRB requirements. Research staff will identify AEs and obtain all available information to assess severity, seriousness, study relatedness, expectedness, outcome and the need for change or discontinuation in the study intervention. Adverse events will be documented on AE Logs and AE Case Report Forms (CRFs). Additional relevant AE information if available will be documented in a progress note in the research record as appropriate to allow monitoring and evaluating of the AE. All AEs will be reported to the IRB online as soon as possible, but no later than 10 working days after the investigator first learns of the event. The MUSC IRB AE reporting requirements are as follows: All deaths that occur during the study or 30 days post termination from the study are required to be reported as adverse events even if they are expected or unrelated. Other adverse events are reportable to the MUSC IRB if the AE is unexpected AND related or possibly related AND serious or more prevalent than expected. All three criteria must be met for an AE to be reported to the MUSC IRB. The IRB definition of unexpected is that the AE is not identified in nature, severity or frequency in the current protocol, informed consent, investigator brochure or with other current risk information. The definition of related is that there is a reasonable possibility that the adverse event may have been caused by the drug, device or intervention. Reportable AEs are reviewed by the IRB Chair and reported to the IRB Board at the next meeting. If the AE meets the definition for serious, appropriate SAE protocol specific reporting forms will be completed and disseminated to the appropriate persons and within the designated timeframes as indicated above. For each AE/SAE recorded, the research staff will follow the AE/SAE until resolution, stabilization or until the participant is no longer in the study as stated in the protocol. When a reportable SAE is identified, the research staff will notify the MUSC IRB within 24-hours and complete the AE report form in conjunction with the PI. If complete information is not available when the initial 24-hour SAE report is disseminated, follow-up information will be gathered to enable a complete assessment and outcome of the event. This information may include hospital discharge records, autopsy reports, clinic records, etc. The research staff will attach copies of source documents to the SAE report for review by the PI and study clinician. These source documents will be forwarded to the NIH program officer as appropriate within 2-weeks of the initial SAE report. In addition, the PI will provide a signed, dated SAE summary report, which will be sent to the designated NIH Institutional Medical Safety Officer within two weeks of the initial SAE report.

The MUSC IRB meets monthly and is located on-campus at 165 Cannon Street, Rm. 501, Charleston SC, 29425. Communication with the IRB will be through email, memos, official IRB forms, and online reporting.

5.8 Trial Safety

The potential benefits, risks and methods to minimize risks are outlined above. Protocols for reporting AEs and SAEs are outlined above. All unexpected AEs and SAEs will be monitored until resolved. A detailed summary of all AEs will be prepared weekly by the research staff. Study procedures will follow the FDA's Good Clinical Practice Guidelines (www.fda.gov/oc/gcp). Any outside requests for information or any breaches in confidentiality will be reported to the PI. All requests by participant's physicians and other medical providers will be referred directly to the PI and the study clinician.

5.9 Trial Efficacy

This is not an intervention trial. An interim analysis is not planned at the time.

5.10 DSM Plan Administration

Dr. Joseph will be responsible for monitoring the study. She will examine (monthly) the outcomes database and CBI server for missing data. Dr. Joseph will work with the ARC statistician to monitor unexpected distributions, responses and outliers. A DSM report will be filed with the IRB on a yearly basis, unless greater than expected problems occur. The report will include participant characteristics, retention and disposition of

study participants, quality assurance issues and reports of AEs, significant/unexpected AEs and serious AEs. We will report outcomes at the end of the study.

5.11 DSM Board

If directed by NIAAA program staff, we will create a Data Safety and Monitoring Board to monitor both the rate and severity of AEs. This panel will include three clinicians with expertise in alcohol use disorder and a statistician.

5.12 Risk Benefit Ratio

While the benefits to the individual participant are minimal, the minimal risks are reasonable in relation to the benefits to be gained from the investigation. Potential risks of concern are loss of confidentiality, and adverse events to oxytocin. The assessments and questionnaires are non-invasive and have inherently minimal risks. The potential risks of MRI are minimal. As discussed above, our research team has extensive experience with these study populations, psychiatric assessments, fMRI, oxytocin administration, the MIST and will attempt to minimize these risks. Knowledge gained by the proposed study would help fill an important void in our understanding neurobiologic substrates of social stress in individuals with alcohol use disorder.

6. CLINICALTRIALS.GOV REQUIREMENTS

In accordance with Public Law 110-85, this project will be registered at the ClinicalTrials.gov Protocol Registration System Information Website prior to study initiation.

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