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Title: Neural substrates of emotion: Impact of cocaine dependence

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NEURAL SUBSTRATES OF EMOTION: IMPACT OF COCAINE DEPENDENCE

SPECIFIC AIMS

Over one million individuals in the United States meet criteria for cocaine use disorders. Relapse rates are highest among cocaine-dependent (CD) populations. Social stress is a significant risk factor for relapse. Data from human neuroimaging studies suggest that "top-down" prefrontal cortical inhibition of amygdala activity controls emotional responses to social stimuli. A growing literature suggests that hypoactivity in the medial prefrontal cortex coupled with increases in amygdala activity underscore the vulnerability of CD individuals to relapse. Neuroimaging studies of corticolimbic network activity (functional connectivity) have been conducted in CD subjects at rest. Compared with healthy controls, CD subjects exhibited lower corticolimbic connectivity and the degree of corticolimbic uncoupling was associated with time to relapse. Studies measuring corticolimbic connectivity during exposure to a social stress task in CD subjects could provide critical insight into the neurobiologic mechanisms that underscore the sensitivity of CD individuals to social stress. Moreover interventions that improve corticolimbic connectivity in CD subjects may be effective therapeutic strategies for preventing relapse in CD populations. Oxytocin (OT) is an anxiolytic neuropeptide that attenuates amygdala responses to aversive social cues. In order to better understand the neurobiologic mechanisms that control emotion-related behavior in CD populations, we propose a double-blind placebo (PBO) controlled study using blood oxygen level dependent (BOLD) functional magnetic resonance imaging (fMRI) to measure (1) corticolimbic functional connectivity during the Montreal Imaging Stress Task (MIST) and (2) amygdala activity in response to an implicit facial affect recognition paradigm in groups of CD individuals (CD n=80) and healthy non-dependent controls (HC, n=80). Prior to the scanning session, participants will receive either intranasal OT (24 IU) or PBO spray (n=40 per treatment group). The order of the tasks will be counterbalanced.

Specific Aim 1: To determine the impact of cocaine dependence and oxytocin on functional connectivity between corticolimbic brain regions during acute social stress. Psychophysiologic interaction (PPI) analysis using the amygdala as the seed region will be used to assess significant task (stress condition > control condition) x seed interactions. Subjective anxiety and craving data will be collected at baseline and after each run of the MIST.

Hypothesis 1A: The HC-PBO group will exhibit greater functional connectivity between the amygdala and prefrontal cortical regions (orbitofrontal and anterior cingulate cortices) than the CD-PBO group.

Hypothesis 1B: The CD-OT group will exhibit greater functional connectivity between the amygdala and prefrontal cortical regions (orbitofrontal and anterior cingulate cortices) than the CD-PBO group.

Hypothesis 1C: The CD-OT group will report lower subjective anxiety and craving than the CD-PBO group.

Specific Aim 2: Use an implicit facial affect recognition paradigm to determine the impact of cocaine dependence and oxytocin on amygdala activity in response to fearful faces. The BOLD signal measured during neutral faces will be subtracted from the BOLD signal measured during fearful faces.

Hypothesis 2A: The CD-PBO group will exhibit greater amygdala activity in response to fearful faces than HC-PBO group.

Hypothesis 2B: Compared with the CD-PBO group, participants in the CD-OT group will exhibit lower amygdala activity in response to fearful faces.

Currently there are no FDA approved medications for the treatment of cocaine dependence. Identifying the neurobiologic substrates of emotion-related behavior in cocaine-dependent individuals may help in the identification of targets for the development of treatment strategies aimed at controlling emotion-related behavior.

RESEARCH STRATEGY

A. SIGNIFICANCE

Cocaine dependence is characterized by compulsive drug-seeking behavior despite the deleterious consequences of repeated drug use. Cocaine dependence wields devastating effects on the individuals' social relationships. Currently over one million individuals in the United States meet criteria for a cocaine use disorder with an estimated 1,800 initiates of cocaine use per day (Substance Abuse and Mental Health Services Administration, 2013). Cocaine use accounts for approximately 40% of illicit drug-related trips to emergency rooms (Substance Abuse and Mental Health Services Administration Drug Abuse Warning Network, 2013). Moreover, relapse rates are high among cocaine-dependent (CD) populations (McKay et al., 1995; McMahon, 2001; Sinha et al., 1999). To date, there are no FDA approved medications for the treatment of cocaine dependence. In addition, the gold-standard behavioral interventions for substance use disorders have had limited success in sustaining behavioral change (Miller et al., 2001). A critical step towards improving treatment outcomes and developing pharmacological interventions for ameliorating relapse is to identify the neurobiologic dysregulation that controls emotion-related behavior in CD individuals. Importantly, social avoidance can impede effective treatment particularly in group settings, a commonly employed treatment modality. Therefore, therapeutic interventions that reduce anxiety, increase trust and promote social affiliation may improve treatment outcomes for CD individuals.

Role of Social Stress in the Development and Maintenance of Substance Use Disorders

A growing literature suggests that social stress plays an important role in the vulnerability to use and abuse substances (Rhodes et al., 1990; Volkow et al., 2011). In support, preclinical studies have found that chronic social stress increases the reinforcing properties of drugs of abuse. For example, cocaine acts as a reinforcer in socially subordinate monkeys but not in socially dominant monkeys (Morgan et al., 2002). In addition, socially isolated rodents readily self-administer cocaine as compared to rodents housed in groups (Schenk et al., 1987). Epidemiological data demonstrate a strong link between childhood maltreatment and the risk for the development of substance use disorders (SUDs) (Felitti et al., 1998). Clinical studies have found that social stress increases drug craving in CD individuals (Sinha et al., 1999; Waldrop et al., 2010). Of note, CD individuals report twice as many daily hassles as healthy controls (Back et al., 2008). In addition, perceived stress in response to interpersonal conflict has been associated with craving and patterns of cocaine use (Waldrop et al., 2007). Consistent with this model, drug and/or alcohol consumption has been positively associated with lack of social support and avoidance coping (Cronkite et al., 1984; DeFrank et al., 1987; Hall et al., 1991; Pohorecky, 1991). Substance dependent individuals often favor drug use over social interactions (American Psychiatric Association, 2013). Importantly, social avoidance can impede effective treatment particularly in group settings. Taken together these studies suggest that social stress is a significant factor in all stages of the addiction cycle.

Oxytocin Modulates Social Behavior

Oxytocin (OT) is a hypothalamic neuropeptide that elicits physiologic events necessary for copulation, parturition and lactation (Carter, 1992; Kendrick et al., 1992). In rodents, central release of OT increases social cognition (Ferguson et al., 2001). Data from preclinical studies also demonstrate that there are pro-social effects of OT. For example, OT increases approach behavior (Witt et al., 1992), and plays an essential role in establishing partner preferences and monogamous pair bonding in prairie voles (Williams et al., 1994). In addition, OT increased the frequency of choices associated with social reward in a study of non-human primates (Chang et al., 2012). Another study of non-human primates found that OT increased attention to faces and eyes and reduced vigilance towards potential social threats (Ebitz et al., 2013). Current perspectives on the effects of OT in the area of human social cognition and behavior; support its role as enhancing sociality. For example, clinical studies have found that intranasal administration of OT increases interpersonal trust (Kosfeld et al., 2005), social recognition memory (Guastella et al., 2008), empathy (Domes, Heinrichs, Michel, et al., 2007; Hurlemann et al., 2010) and generosity (Zak et al., 2007). However, other clinical studies have found that intranasal OT can increase envy (Shamay-Tsoory et al., 2009), ethnocentrism (De Dreu et al., 2011) and impede trust in individuals with borderline personality disorder (Bartz et al., 2011), findings that conflict with the idea that OT has positive effects on human behavior. These inconsistent findings have been hypothesized to result from reduced anxiety, increases in perceptual salience and/or motivational states related to affiliation (Bartz et al., 2011; Striepens et al., 2012). The anxiolytic effects of oxytocin to social stress have been found in healthy

controls (de Oliveira et al., 2012; Heinrichs et al., 2003; Yatzkar, 2010). Of note, another study of healthy controls found no effect of OT on subjective anxiety (Kirsch et al., 2005). A growing literature suggests that the effects of OT on social behavior are modulated by interindividual factors. For example, in a study of non-human primates, OT increased social contact in subordinate males but not in dominant males (Insel et al., 1991). In humans, OT reduced social stress reactivity in individuals with poor coping skills and emotional regulation but not in healthy controls (Cardoso et al., 2012; Quirin et al., 2011). Another study found that men with early parental separation (EPS) had a more robust cortisol response to OT as compared to a group without EPS (Meinlschmidt et al., 2007). In addition, OT attenuated stress induced dysphoria in individuals with borderline personality disorder and anxiety in individuals with social anxiety disorder (Simeon et al., 2011). Thus, OT may be effective for reducing anxiety to a social stressor in individuals with mood and/or psychiatric disorders (Macdonald et al., 2013).

Dysregulation in the Oxytocin System Role in Drug Abuse and Dependence

Chronic drug use appears to alter the OT system. For example, a immunohistochemical analysis of post-mortem brains of alcoholics found a significant reduction in OT immunoreactivity in the hypothalamus (Sivukhina et al., 2006). Further, mothers using cocaine during pregnancy exhibited significantly lower plasma OT levels, as well as greater hostility and depression as compared to control mothers (Light et al., 2004). A preclinical study found that chronic methamphetamine treatment increased OT receptor expression in the amygdala, suggesting that chronic stimulant use attenuates central OT signaling (Zanos et al., 2014).

A growing literature demonstrates protective effects of OT at every stage of the addiction cycle. For example, animal models of drug reinforcement have found that OT administration decreases cocaine-induced hyperactivity and stereotypy (Sarnyai et al., 1991; Sarnyai et al., 1992). In addition, OT inhibits the development of tolerance to repeated cocaine administration (Sarnyai, Biro, et al., 1992) and attenuates self-administration of cocaine (Sarnyai et al., 1994). Central administration of OT also blocks methamphetamine-induced locomotor activity and reduces methamphetamine-induced reinstatement. In addition, OT attenuates both drug and cue-induced reinstatement of cocaine-seeking behavior (Carson et al., 2010; Morales-Rivera et al., 2014). Oxytocin has also been shown to inhibit stress-induced glutamate release and to interfere with dopamine-driven object oriented reward (McGregor et al., 2012; Qi et al., 2008; Qi et al., 2009). As such, OT could influence addiction by interfering with stress-induced relapse or by decreasing rewarding drug effects. In agreement with these findings, an emerging clinical literature suggests that increasing central OT attenuates the symptoms of drug withdrawal (Cui et al., 2001; Winstock et al., 2009). For example, intranasal administration of OT attenuated craving and anxiety in marijuana-dependent individuals exposed to the Trier Social Stress Test (Trier) (McRae-Clark et al., 2013). Of note, intranasal OT improves fear recognition in healthy controls, a finding that is particularly interesting as CD individuals exhibit deficits in fear recognition (Fischer-Shofty et al., 2010; Kemmis et al., 2007). **Taken together, these preclinical and clinical data suggest an important role of OT in addictions and point to the OT system as a potential neurobiologic target for therapeutic development.**

Corticolimbic Coupling Regulates Emotional Responses to Social Stimuli

Corticolimbic brain regions including the amygdala and regions of the prefrontal cortex play important roles in arousal and appraisal of social stimuli (LeDoux, 2000; Phan et al., 2002). First, studies of non-human primates have found dense reciprocal projections between the amygdala and the prefrontal cortex (Aggleton et al., 1980; Carmichael et al., 1995; Ghashghaei et al., 2007; Price, 2005). Human neuroimaging studies have found that cues evoking sad, fearful and happy emotions increase activity in the amygdala (Blair et al., 1999; Breiter et al., 1996; Phillips et al., 2001). Thus, amygdala activity in response to a variety of emotional states suggests that it is a critical substrate involved in arousal and attention to social stimuli regardless of the valence. Tasks that yield affective states also increase activity in the ventrolateral prefrontal cortex (vlPFC), ventromedial prefrontal cortex (vmPFC), orbitofrontal cortex (OFC) and subgenual anterior cingulate cortex (subgenACC) (Dougherty et al., 1999; O'Doherty et al., 2001; Shin et al., 2000). Functional connectivity between corticolimbic brain regions was measured in subjects that were exposed to the Trier and a no-stress group. Compared with the no-stress group, subjects that were exposed to the Trier exhibited significantly greater coupling between the amygdala and the OFC (Veer, 2010). In general, data from human neuroimaging studies suggest that suppression and/or reappraisal of negative affect is mediated by activation of prefrontal cortical brain regions and deactivation of the amygdala (Beauregard et al., 2001; Levesque et al., 2004; Ochsner et al., 2002; Ohira et al., 2006). For example,

during cognitive reappraisal of aversive stimuli the magnitude of functional connectivity between the amygdala and the OFC was inversely associated with the degree of negative affect attenuation (Banks et al., 2007). **Thus, top-down prefrontal cortical control of the amygdala (corticolimbic coupling) appears to play a preeminent role in regulating emotional responses to social stimuli (FIGURE 1A).**

Corticolimbic Uncoupling & Social Stress: Role in Cocaine Dependence

Data from human neuroimaging studies demonstrates dysregulation in the corticolimbic circuitry of CD individuals. For example, CD individuals exhibit altered corticolimbic activity in response to triggers of relapse (Childress et al., 1999; Grant et al., 1996; Kilts et al., 2001; Sinha et al., 2005). In addition, compared with healthy controls, CD individuals exhibited significantly less activity in the vmPFC and subgenACC during tasks that require the processing of both negative and positive affect (Goldstein et al., 2011; Wexler et al., 2001). Functional connectivity between corticolimbic brain regions has been studied in CD individuals at rest. Compared with healthy controls, CD individuals exhibited significantly less functional connectivity between corticolimbic brain regions (Gu et al., 2010). Moreover, time to relapse was associated with attenuated connectivity between the amygdala and the vmPFC (McHugh et al., 2014). Of note, cortico-amygdala uncoupling has also been found in patients with depression and anxiety disorders (Kim et al., 2009; Pezawas et al., 2005). Thus, corticolimbic uncoupling may be a significant neurobiologic factor that underscores the sensitivity of CD individuals to interpersonal stress and relapse. **Thus, these studies suggest that cocaine dependence is associated with alterations in functional connectivity between corticolimbic brain regions (Figure 1B). In the proposed study, we will explore functional connectivity between corticolimbic brain regions in CD individuals and healthy controls.**

Oxytocin Alters Corticolimbic Connectivity

In general, data from neuroimaging studies of healthy controls, suggest that intranasal OT increases functional connectivity between corticolimbic brain regions. For example, intranasal OT increases functional connectivity between the amygdala and the mPFC of healthy controls at rest (Sripada et al., 2013). A recent neuroimaging study examined the effects of OT on corticolimbic connectivity in patients with generalized social anxiety disorder at rest. Oxytocin increased functional connectivity between the left and right amygdala with the ACC and mPFC. In addition, under the placebo condition higher social anxiety was associated with lower corticolimbic connectivity. Moreover, higher social anxiety was associated with a greater increase in corticolimbic connectivity by OT (Dodhia et al., 2014). Thus, these data support our hypotheses and suggest that corticolimbic network activity is a critical neural substrate in the anxiolytic effects of OT. Of note, intranasal OT also affects amygdala connectivity with the salience network, a finding that has been associated with OTs effects on empathy and trust (Riem et al., 2011; Riem et al., 2012; Seeley et al., 2007; Striepens et al., 2012). Interestingly, intranasal OT can attenuate functional connectivity between the amygdala and brain regions responsible for regulating autonomic and endocrine manifestations of fear and anxiety (Kirsch et al., 2005). **These studies suggest that intranasal OT enhances amygdala connectivity with prefrontal cortical brain regions (Figure 1C). In the proposed study, we will explore the effect of OT on functional connectivity between corticolimbic regions and its relationship to anxiety and craving in CD individuals and controls.**

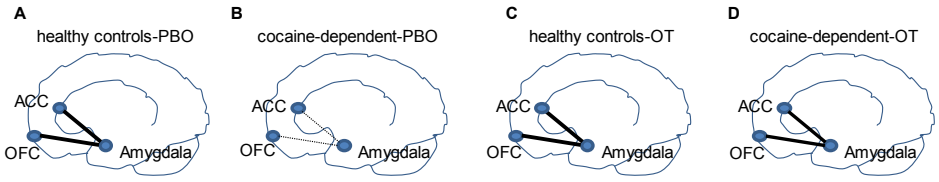


Figure 1. Schematic overview of Specific Aim 1. Corticolimbic functional connectivity during social stress in **(A)** healthy controls treated with placebo (PBO) **(B)** cocaine-dependent individuals treated with PBO **(C)** healthy controls treated with oxytocin (OT) and **(D)** cocaine-dependent individuals treated with OT. Lines represent the magnitude of functional connectivity between the amygdala and orbital frontal cortex (OFC) and anterior cingulate cortex (ACC).
Hypothesis 1A: A > B
Hypothesis 1B: B < D

Evidence Linking Cocaine Dependence with a Loss of Bottom-Up Control: Role of the Amygdala

The ability to recognize emotional cues from faces is a necessary component of social interactions (Blair, 2003). To date, the impact of cocaine use on facial affect processing remains unclear. For example, clinical studies of CD subjects found no difference between CD and healthy controls in the ability to recognize the emotional expressions of faces (Fox et al., 2011; Hulka et al., 2013). Another study found that compared with healthy controls, recreational cocaine users exhibited deficits in the ability to accurately recognize faces depicting

fear (Kemmis et al., 2007). Of note, patients with anxiety disorders also exhibit deficits fear recognition accuracy (Poljac et al., 2011). The amygdala plays a critical role in processing socially salient information from faces, including fear (Adolphs et al., 1994; LeDoux, 2000; Ochsner et al., 2009). The amygdala provides low level bottom-up regulation of the prefrontal cortex both directly through efferent projections to cortical sensory regions and indirectly through brain arousal systems including the locus coeruleus that project to regions of the mPFC (Aston-Jones et al., 1996; Holland et al., 1999; McDonald, 1998; Turner et al., 1980). Accumulating evidence suggest that cocaine use is associated with functional and structural alterations in the amygdala that underscore the anxiogenic effects of drug withdrawal and relapse (Erb et al., 2000; Franklin et al., 2002; Makris et al., 2004; Rudoy et al., 2009; Zhou et al., 2003). Human neuroimaging studies have found amygdala activation in response to faces depicting fear even when the faces are presented outside of the subjects' conscious awareness and have been linked with arousal systems that are independent of mPFC activity (Critchley et al., 2000; Morris et al., 1998; Morris et al., 1999; Whalen et al., 1998). Patients with anxiety disorders exhibit hyperactive amygdala activity in response to implicit faces depicting fear (Rauch et al., 2000; Straube et al., 2004). Thus, elevated bottom-up drive anxiety disorders may also play an important in the deficits in social cognition that have been found in CD subjects.

The amygdala appears to be a critical target for oxytocin's effects on social behavior. Oxytocin receptors have been localized to the amygdala of rodents and humans (Boccia et al., 2013; Insel et al., 1992; Veinante et al., 1997). Data from human neuroimaging studies demonstrate that intranasal OT attenuates amygdala responses to both aversive and positive social cues (Domes, Heinrichs, Glascher, et al., 2007; Kirsch et al., 2005; Labuschagne et al., 2010; Petrovic et al., 2008; Riem et al., 2012; Wittfoth-Schardt et al., 2012). While the exact mechanism mediating OT's effect is unclear, it has been hypothesized that OT receptor activation of GABAergic interneurons in the amygdala plays an important role in the inhibitory effects of OT on amygdala responding to social cues (Ehrlich et al., 2009; Huber et al., 2005). Intranasal OT improves fear recognition in healthy controls, findings that are particularly interesting given the deficits in fear processing that have been found in CD subjects (Fischer-Shofty et al., 2010). **Taken together these data suggest that altered amygdala reactivity is a significant factor in the emotional dysregulation associated with cocaine dependence.**

B. INNOVATION

- The proposed project will be the first to use both social evaluative feedback and an uncontrollable cognitive challenge to study stress-related brain activity in cocaine-dependent individuals.
- This is the first clinical investigation of oxytocin on social stress-related brain activity in cocaine-dependent individuals.
- The proposed studies are the first to examine the impact of social stress on brain activity at a network level.
- Drug addiction has typically been modelled as dysregulation in "top-down" control. The use of an implicit facial affect recognition paradigm will allow us to assess addiction as dysregulation in "bottom-up" drive.

C. APPROACH

C1. Research Team

C1a. Overview

We have assembled a multidisciplinary team with unique and complementary skills in addictions research, neuroimaging and clinical studies of oxytocin. The project will be led by Dr. McRae-Clark. Kathleen Brady, M.D., Ph.D., Karen Hartwell M.D., Julianne Flanagan, PhD and Jane Joseph Ph.D. are Co-Investigators (Co-Is) on the project. Dr. Joseph will oversee the neuroimaging data acquisition and analysis. Drs. Hartwell and Brady are clinician scientists and will oversee the safety of the research participants. Each of the Co-Is will be involved in data interpretation and manuscript development.

C1b. Experience with Addictions Research

The proposed project will be directed by Dr. Aimee McRae-Clark. Dr. McRae-Clark is a Professor in the Department of Psychiatry and a productive clinical researcher with independent NIH-funding focused on clinical trials and human laboratory work with drug-dependent individuals. As such, she has extensive experience in conducting clinical laboratory studies examining stress induction. Dr. McRae-Clark will oversee the day-to-day operations of the project including direct supervision of the research staff as well as coordinate data analysis and presentation.

Dr. Flanagan is a licensed clinical psychologist with over 10 years of experience conducting research on substance use disorders (SUD) and commonly co-occurring conditions such as posttraumatic stress disorder (PTSD). Her area of expertise has expanded to include numerous studies examining clinical applications of oxytocin and other pharmacological interventions for SUD and co-occurring conditions. Dr. Flanagan is an Assistant Professor in the Department of Psychiatry and Behavioral Sciences at the Medical University of South Carolina.

C1c. Experience with OT Administration

Dr. McRae-Clark has extensive experience with clinical studies that have involved administering intranasal OT to (1) marijuana-dependent individuals (McRae-Clark et al., 2013) (2) CD individuals (P50 DA016511) (3) individuals with PTSD (R21 MH099619; U.S. Department of Defense, Sub-Award 804-237) and (4) healthy controls (R21 MH099619). To date, OT has been administered to over 150 research participants with no adverse or serious adverse events reported.

C1d. Experience with Neuroimaging Paradigms Data Acquisition and Analysis

Dr. Joseph has 15 years of experience in human neuroimaging studies of facial affect recognition and functional connectivity analysis (R01 HD052724, R21/R33 MH086958) (Joseph et al., 2009; Joseph et al., 2012). Drs. Moran-Santa Maria and Joseph are currently Co-Is on the neuroimaging component of Dr. Brady's study "Oxytocin and Cocaine Dependence" (P50 DA016511). Drs. Brady and Hartwell also have experience in using BOLD fMRI to assess brain activity in individuals with SUDs (Hartwell et al., 2011; Hartwell et al., 2013; Prisciandaro et al., 2013).

C2. Preliminary Studies

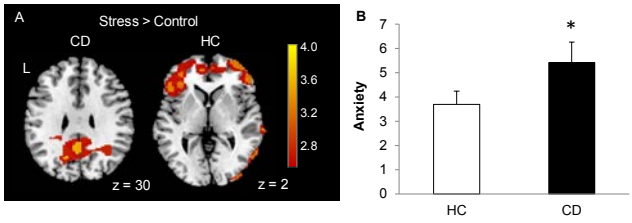


Figure 2. (A) Areas exhibiting significant task-dependent (stress > control) functional connectivity with the left amygdala during the MIST in the CD group bilateral posterior cingulate MNI coordinates (±2, -54, 30) Brodmann area (BA 23); and the HC group bilateral OFC (±40, 58, 2) Brodmann area (BA 10). Z statistic images were thresholded using clusters determined by Z>2.3 and a corrected cluster significance of p=0.05 **(B)** Peak anxiety to the MIST. Values are group means (+) se * Denotes significant difference from HC (p<0.05).

C2a. Evidence for Corticolimbic Uncoupling in CD Individuals

Corticolimbic coupling during the MIST was measured in CD and HC using a psychophysiological interaction (PPI) analysis. Using the left amygdala as the seed region, significant task x seed interactions were observed in both groups. In the CD group the left amygdala exhibited significantly greater functional connectivity with the posterior cingulate cortex during the stress condition than the control condition (Figure 2A). In the HC group, the left amygdala exhibited significantly greater functional connectivity with the OFC during the stress condition than the control condition. Cocaine-dependent subjects reported significantly greater anxiety in response to the MIST than the HC subjects (Figure 2B). Thus, these preliminary findings support our hypotheses and the feasibility of using PPI to assess functional connectivity associated with a social stress task in CD and HC subjects.

C2b.Oxytocin Attenuates Anxiety in Marijuana-Dependent Individuals

In a double blind placebo controlled study, we investigated the impact of intranasal OT administration on subjective responses to the Trier Social Stress Task in marijuana-dependent individuals. Compared with subjects who received placebo, subjects that received OT reported significantly lower subjective anxiety (p=0.05) and drug craving (p<0.05) (McRae-Clark et al., 2013).

C2c. Evidence for Hyperactive Amygdala Responses to Social Stress in CD Subjects

In a pilot study, we used the MIST to compare social stress-related brain activity between CD individuals (n=13) and healthy controls (HC; n=15). Using a block design BOLD fMRI data were collected during three runs of the MIST. During social stress (stress condition-control condition) CD subjects exhibited significantly greater activity in the left amygdala than HC subjects (Figure 3A). In contrast, during social stress the HC subjects did not exhibit significantly greater activity than the CD subjects in any brain region. During mental arithmetic (control condition-rest condition), the HC subjects exhibited significantly greater activity in the anterior cingulate cortex than the CD

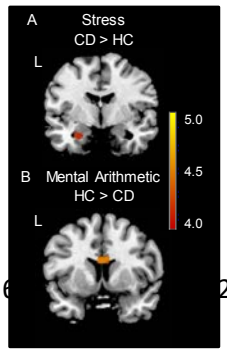


Figure 3. Areas exhibiting significant group differences during stress and mental arithmetic during the MIST. **(A)** Cocaine-dependent (CD) subjects exhibited significantly greater activity in the left amygdala (MNI: -26, -4, -22) than healthy controls (HC). **(B)** HC subjects exhibited significantly greater activity in bilateral anterior cingulate cortices (MNI; 0, 8, 22) than CD participants in response to mental arithmetic. Z statistic images were thresholded using clusters determined by Z>2.3 and a corrected cluster significance of p=0.05.

subjects (Figure 3B). In contrast, during mental arithmetic the CD subjects did not exhibit significantly greater activity than the HC group in any brain region. Thus, these preliminary data from the MIST demonstrate that CD subjects exhibited significantly greater amygdala responses to social stress and significantly lower prefrontal cortical responses to a cognitive challenge compared with HC. In addition, these data demonstrate the research teams' experience in using the MIST to study the neural correlates of social stress in CD individuals and healthy controls.

C3. Design and Methods of Current Proposal

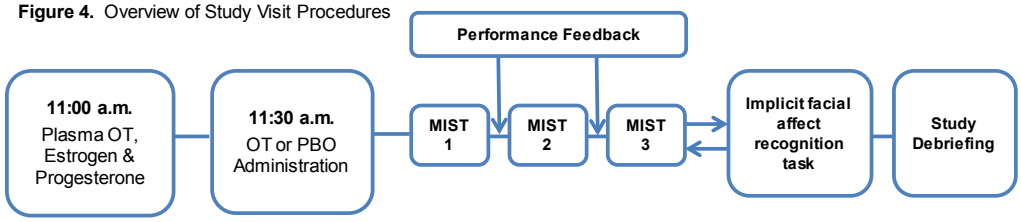
C3a. Overview

The primary objective of this proposal is to identifying the neurobiologic substrates of emotion-related behavior in CD individuals. After the initial screening and assessment visit, an fMRI study visit will be scheduled (Figure 4). The study will use a double-blind placebo-controlled design. Prior to the scan, participants will receive either intranasal OT (24 IU) or a placebo (PBO) spray. During the scan, BOLD fMRI data will be acquired while the participants perform a series of stressful and non-stressful mental arithmetic challenges (Dedovic et al., 2005). Performance feedback will be provided by the MIST software in real-time and also by the investigator. Participants will complete three runs of the MIST. Subjective responses (anxiety and craving) will be collected at baseline and immediately after each run of the MIST. Participants will also complete an implicit facial affect recognition task. Since the amygdala may habituate to the stress tasks, the order of the tasks will be counterbalanced. At the end of the study visit, subjects will be debriefed, compensated and discharged.

C3b. Participants

Subjects will be men and women of all racial and ethnic groups aged 18-65. A total of 160 study participants will be recruited: (1) cocaine-dependent individuals (CD, n=80); and (2) healthy control subjects free of major Axis I diagnoses (HC, n=80). Gender differences in the neural correlates of stress have been found in fMRI studies of HC and CD individuals (Li et al., 2005; Potenza et al., 2012), and in amygdala responses to fearful faces following intranasal OT administration (Domes, Heinrichs, Glascher, et al., 2007; Domes et al., 2010). Based on our experience in recruiting CD subjects for clinical studies, we expect that 15-20% of the subjects will be female. Differential recruitment will be used as needed to ensure that 15-20% of the participants in each study group are female. Groups will also be matched on age, smoking status, and education. On a monthly basis, we will assess the status of each group in terms of core variables and tailor recruitment accordingly. This has worked well in all of our previous clinical research studies.

Figure 4. Overview of Study Visit Procedures



C3c. Recruitment and Retention

We anticipate screening (by telephone or in person) about 350 potential subjects. We will recruit "healthy adults" and "cocaine users" through multiple sources, including advertisements in local media via television, newspaper, internet and word of mouth among subjects. Secondary recruitment sites will include (1) patients referred from the MUSC Center for Drug and Alcohol Programs (CDAP); (2) individuals presenting to the Charleston Center for inpatient or outpatient treatment; and (3) The Ralph H. Johnson VA Medical Center (4) Patients of MUSC who agreed to be contacted for research studies.

Contingency management will be used to encourage drug abstinence and study retention (Petry et al., 2002; Petry et al., 2001). Participants who present to MUSC's Addiction Sciences Division for their scheduled study visit and provide a clean urine drug screen will draw from a bowl containing 250 chips that are assigned a certain value. 230 chips denote a small amount ($1.00), 18 chips denote a larger amount ($10.00), one chip denotes $50.00 and one chip denotes a jumbo amount ($100.00). Participants who arrive at the ASD with a clean urine drug screen be allowed five draws. Participants who arrive at the ASD with a clean urine drug screen after being rescheduled will be allowed three draws.

C3d. Procedures

Individuals will be screened for eligibility over the telephone by a trained research assistant during which

major inclusion/exclusion criteria will be assessed. Individuals who appear eligible will be invited for an in-person interview with a member of the research team. Prior to any study procedures, the individual will sign an IRB-approved informed consent form. Following consent, a battery of standardized assessments will be delivered (described below). A general medical history and physical exam will also be performed to ensure that the subject is eligible to participate. The exam will also include a metal screening questionnaire that will be reviewed by clinical staff. If a patient is ineligible to participate, he or she will be given a referral for medical care and/or an appropriate treatment program.

C3e. Assessments

Screening and Diagnostic Instruments

Quick Screen: This assessment will quickly determine whether an individual meets study inclusion or exclusion criteria. The instrument is designed to assess for substance dependence and obvious psychiatric, medical, and logistic exclusions.

Mini-International Neuropsychiatric Interview (MINI), Version 7: The MINI is a brief structured interview that was designed to assess DSM 5 diagnoses using a series of questions in dichotomous format (yes/no). Earlier studies have found that the MINI is similar in sensitivity, specificity, and inter-rater reliability to other more lengthy diagnostic interviews, such as the SCID-I/P (Sheehan et al., 1998).

Childhood Trauma Questionnaire (CTQ): Since childhood trauma has been shown to affect corticolimbic brain activity (Dannlowski et al., 2012) and corticolimbic brain activity following OT administration (Fan et al., 2014) we will use the CTQ to assess childhood trauma exposure in each of the study participants (D. P. Bernstein et al., 1994; D. P. Bernstein, Fink, L.A., 1998). The CTQ is a 25-item self-report questionnaire used to assess the extent to which individuals have childhood abuse and neglect. Subjects answer each question using a 5-point Likert scale ranging from (1) never true to 5 (very often true). The reliability and validity of the CTQ have been tested in both healthy and substance-dependent populations (D. P. Bernstein et al., 1994; D. P. Bernstein, Fink, L.A., 1998; Carpenter et al., 2007; Hyman et al., 2008; Hyman et al., 2007).

Within Session Rating Scale: A modification of the Within Session Rating Scale (Childress et al., 1986) will be used to assess craving and mood during the procedures. This scale is anchored with the adjectival modifiers ("not at all and extremely"). There are four items assessing domains of craving (want/need/craving/ability to resist). For the market value item, the individual will be asked to name the dollar amount they would be willing to pay for cocaine if they could have it "now". Other items include anxiety and mood. These data will be collected prior to treatment, prior to MIST1, and immediately after each run of the MIST.

Menstrual History Diary: Drug craving mood and affect have been associated with menstrual cycle phase and ovarian hormone status (Kirschbaum et al., 1999; Sinha et al., 2007). Subjects will be asked to estimate the timing of their cycle for the 90-days prior to study entry and to track their cycle during study participation. **Daily Hassles Scale:** The Daily Hassles Scale consists of a list of 117 irritating, frustrating or distressing events that characterize everyday interactions with the environment. Subjects rate intensity for the past month. The Daily Hassles Scale is positively correlated with adaptational outcomes and is a good predictor of psychological symptoms (Kanner et al., 1981). Data from the daily hassles will be used to explore the relationship between stress related corticolimbic brain activity and sensitivity to daily hassles.

Metal Screening Questionnaire (MSQ): The MSQ is a list of 18 "yes or no" questions regarding pacemakers, shrapnel, bullets, implants, tattoos, hairpieces, insulin pumps, cochlear implants, staples and metal clips, false eyes, nerve stimulators, dental bridges, replacement valves, IUDs, dentures, trans-dermal nicotine patches, surgery, metal in the eyes, claustrophobia, and current pregnancy. In addition the MSQ asks "have you ever been shot at or received treated for metal in your eyes?" The questionnaire is anchored by "Is there any possibility of metal, metal pieces, or metal implants in your body?" Each study participant will be asked to fill-out the MSQ at the assessment visit and the clinician will review the MSQ with the participant during the physical exam.

Minnesota Nicotine Withdrawal Scale (MNWS): Nicotine withdrawal could have a significant impact on our study outcomes. The MNWS is a 15-item self-report scale of behaviors associated with nicotine withdrawal (Hughes et al., 1986). Subjects answer each question using a 5-point Likert scale ranging from (0) none to 4 (severe). Smokers will be asked to fill-out the questionnaire both before (prior to medication administration) and after the scanning sessions. Group differences in the severity of nicotine withdrawal may be used as covariates in the analysis.

The Connor-Davidson Resilience Scale (CD-RISC): The CD-RISC is a 25-item self-report measure that assesses the ability to cope with stress and adversity (Connor et al., 2003). Participants will be asked to complete the CD-RISC at the assessment visit.

Interpersonal Support Evaluation List Short Form (ISEL-SF): The ISEL-SF is a 12-item self-report form that measures perceived availability of four domains of social support (belonging, self-esteem, appraisal and tangible)(Cohen, S et al., 1985). Individuals rate each item using a four-point scale ranging from definitely false (1) to definitely true (4). Participants will be asked to complete the ISEL-SF at the assessment visit.

Beck Depression Inventory- 2nd Addition (BDI-II): The BDI-II is a 21-item self-report instrument intended to assess the existence and severity of symptoms of depression (Beck et al., 1996).

State-Trait Anxiety Inventory for Adults (STAI): The STAI is a commonly used measure of trait and state anxiety. It can be used in clinical settings to diagnose anxiety and to distinguish it from depressive syndromes (Speilberger, et al., 1983).

Conflict Tactics Scale- Short Form: This is the most widely used instrument for measuring intimate partner violence (Straus & Douglas, 2004).

Drug Abuse Screening Test (DAST): The DAST-10 yields a quantitative index of the degree of consequences related to drug abuse. The instrument takes approximately 5 minutes to administer and may be given in either a self-report or interview format (Skinner, 1982).

Brief COPE: The Brief COPE is a self-completed questionnaire measuring coping strategies. It comprises 14 subscales for which psychometric properties are described (Carver, 1997).

UPPS-P Impulsive Behavior Scale: The UPPS-P was designed to measure impulsivity across dimensions of the Five Factor Model of personality. It is 45-itme Likert-type scale (Lyman et al., 2006)

Levenson Self-Report Psychopathy Scale (LSRPS): The LSRPS (Levenson et al., 1995) is a 26-item self-report measure designed to capture two features of psychopathy, including a callous and unemotional personality style, and a tendency towards impulsive antisociality. Items are rated on a Likert scale from 1 ("strongly disagree") to 4 ("strongly agree").

Urine Pregnancy Test: Female participants of childbearing potential will be asked to provide a urine sample which will be tested for the presence of human chorionic gonadotropin (hCG) using the QuickVue One-Step urine hCG pregnancy test (Quidel Corporation, San Diego, CA). The test provides a qualitative measure of hCG in urine (≥ 25 mIU/mL; 99% sensitivity; 99% specificity). The urine pregnancy test will be performed at screening and on the day of the study visit, prior to the urine drug screen and breathalyzer test. If the pregnancy test is positive, the subject will be excluded and no further testing will take place.

Blood Sample Collection and Assays: Baseline blood samples for estradiol, progesterone and OT will be collected from each participant 15-30 minutes prior to the OT/PBO administration. Blood will be collected in tubes containing EDTA. Tubes will be centrifuged at 1500 rpm at 4˚C. Plasma will be stored at −70˚C. Determination of OT will be performed using a commercial ELISA kit (Enzo Life Sciences). Intra-assay coefficients of variation for OT average ~3-5% and inter-assay variation is typically less than 10%.

Substance-Related Instruments

Form 90: The Form 90 (Miller, 1996), an assessment instrument commonly used in addiction studies, is similar in concept to the Time Line Follow-back (Sobell & Sobell, 1992). This is a calendar-based instrument designed to assess daily substance consumption. Study participants will be asked to estimate the amount of substance consumed with the aid of visual cues designed to accurately quantify consumption. Cocaine will be recorded in dollar value as well as quantity in order to standardize for different types of cocaine use (crack, IV, nasal, etc.). The data will be summarized in three ways: (a) percent of abstinent days (i.e., no use); (b) amount of use per day and (c) days since last use.

Urine Drug Screening: Drug screens will be performed using the On Track Test Cup® (Roche Diagnostics), an in vitro diagnostic test for the qualitative detection of drug or drug metabolite in the urine. The On Track Test Cup profile (cut off) consists of amphetamines (1000ng/ml), cocaine (300 ng/ml), THC (50 ng/ml), morphine (300 ng/ml), and benzodiazepines (200 ng/ml). Results will be used to ascertain abstinence prior to initiation of test session and to substantiate self-report at the screening visit

Breathalyzer: To ascertain abstinence from alcohol during the study period, subjects will have their breath sampled for the presence of alcohol (Alco-Sensor III, Intoximeters Inc., St. Louis, MO). The Alco-Sensor III can accurately detect breath alcohol levels between .000-.400 BAC.

C3f. Remote screening visit alternative. Patients may also complete the initial visit remotely if warranted. In this case, subjects will be electronically consented using Doxy.me. The informed consent will be emailed to the participant prior to the video call. The patient will have the opportunity to ask questions on the call and will electronically sign the document. A signed copy will be emailed to the participant. The M.I.N.I interview and medical history then be conducted, also via Doxy.me. If clinically indicated, patients may have a physical exam at the scanning visit. Blood pressure will be checked prior to medication administration. Patients will be advised to find a private location during these procedures to protect privacy and confidentiality. Patients will be sent survey links to complete self-report questionnaires. They will receive their ClinCard at the scanning visit. Screen fails will be sent a ClinCard in the mail which will not be loaded with payment until they confirm they have received it.

Session Procedures

Study Visit: Participants will be instructed to arrive at MUSC's Addiction Sciences Division (ASD) on the morning of the study visit. Participants will be informed that they will be expected to remain abstinent from cocaine and other drugs for the three-day period prior to the study visit in order to minimize the impact of recent drug/alcohol use on brain activity and subjective responses to the MIST. Participants will be asked to avoid caffeinated beverages on the morning of the study visit since caffeine may introduce variability in stress reactivity. If a participant is nicotine-dependent (s)he will be provided with a nicotine patch. Upon arriving at the ASD, the participant will be breathalyzed and will provide a urine sample, which will be tested for the presence of cocaine, opiates, barbiturates, benzodiazepines, and stimulants; if female, a urine pregnancy test will also be administered prior to the drug test. If the pregnancy and urine drug tests are negative, with the exception of marijuana, the session will proceed. In the event a participant tests positive for drugs or alcohol, the study visit will be rescheduled. The participant will be escorted to the Clinical Neurobiology Laboratory at the Institute of Psychiatry for a blood draw. Participants will then be escorted to the on-campus Center for Biomedical Imaging (CBI) facility by approved study personnel.

Medication Administration

Intranasal OT and matching PBO (saline spray) will be compounded by MUSC's Investigational Drug Services (IDS) which has extensive experience in extemporaneous OT preparation and quality control monitoring. Randomization will be done by IDS, who will keep a record of the blind. The record will be available should unblinding be required. To achieve balance in sample size with respect to gender, smoking status, and age,a block randomized design with randomly varying block sizes will be used. OT or PBO sprays will be administered at the CBI and under the supervision of the study staff approximately 45-minutes prior to the scanning session. First the participant will be asked to blow their nose. The vial will be primed to ensure that each puff contains OT or placebo spray and not air. The participant will be instructed to exhale through their nose and then spray into one nostril while inhaling. Nostrils will be alternated and the participant will be asked to repeat the procedure for each nostril. Participants will self-administer nasal spray in each nostril until atotal of 24 IU is reached. The number of puffs will vary depending on the strength of the oxytocin; IDS provides this information. This dose and timing of administration were selected based on similar fMRI studies demonstrating BOLD signal changes in the amygdala 45-50 minutes post-administration (Domes, Heinrichs, Glascher, et al., 2007; Domes et al., 2010; Kirsch et al., 2005; Labuschagne et al., 2010).

MRI Data Acquisition

All MRI data will be aquired on a Siemens Trio 3T scanner (Siemens Medical, Erlangen, Germany). , participants will be placed in the mock scanner to acclimate to the scanning environment. If the mock scanner is not available on the test day, participants will proceed directly to the actual scan. Participants will be screened for metal using a handheld metal detector. Study personnel will position subjects on the scanner bed with foam padding placed around their head to prevent motion. Participants will wear earplugs/headphones and the task will be projected on a wide screen located at the end of the scanner bore and viewed via a back-projected mirror that will be mounted on 12-channel head coil. Participants will use a non-ferrous optical hand pad to submit their answers to the arithmetic task. The hand pad will be connected via an optical cable to a computer outside

the scanner room. Their ability to view the projection screen and use of the hand pad will be assessed prior to scanning. During initial scanner tuning, localizing, and structural scanning, participants will be shown "relaxing" images (i.e., 20 scenic pictures, each displayed for 30 seconds). A high resolution T1-weighted MPRAGE anatomical scan (TR = 8.1 ms, TE = 3.7 ms, flip angle = 8°, field of view = 256 mm, 1.0 mm) covering the entire brain and positioned using a sagittal scout image will be acquired for co-registration and normalization of functional images. T2*-weighted gradient echo EPI images will be acquired with the following parameters: TR = 2500 ms, TE = 27 ms, flip angle = 77º, 40 axial slices (FOV = 224 x 224 mm, thickness = 3.5 mm voxels with 0.5 mm gap, in interleaved order. The scanning planes will be oriented parallel to the anterior commissure–posterior commissure line.

The Montreal Imaging Stress Task

The study will use a block design of three, six-minute runs separated by two-minutes of rest for feedback, for a total of 24-minutes. During each run, participants will be exposed to 40-second blocks of three different conditions (rest, control, and stress). Prior to the task, a research assistant will meet with each participant and described the parameters of the task. The 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 stress condition, the participants will be asked to perform the math task as quickly and accurately as possible. They will be given immediate feedback about their performance and will be able to see the performance level of an "average" person through a performance bar that will be located at the top of the screen. A strict time limit will be enforced througout the stress condition. The participants will be told that the average person would answer about 85% of the questions correctly, while in reality, the program's algorithm limits the participants' peformance rate to between 35-45%. At the end of runs one and two, the participants will be given negative feedback from the investigator and will be urged to improve their performance so that their data may be included in the study. To minimize the effects of scanner drift, the beginning condition will be counterbalanced between participants. However, the sequence of conditions will be constant (i.e. control condition will follow the rest condition and the stress condition will follow the control condition).

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 (Critchley et al., 2000). Emotional adult faces will be selected from a variety of sources are standardized in size and enclosed in the same oval surround (Ekman, 1976). Dr. Joseph (Co-I) 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

Subjects will be paid with Greenphire debit cards or cash for each completed study visit as follows: $75 for completing baseline assessments to see if you meet study requirements ($25 for the physical exam and $50 for the interview) and $150 for completing the scanning session. The total amount that they may receive for participating in the study if they complete all study visits is a maximum of $225. If subjects choose not to complete the study, they will be compensated for the part(s) they have completed. Subjects who come to their

scheduled study visit and provide a clean urine drug screen the first time they're scheduled, they will draw 7 chips from a bowl containing 250 chips that are assigned a certain value. Most chips are worth $1.00, some are worth $10.00, one chip is worth $50.00 and one chip is worth $100.00. If their visit has to be re-scheduled for any reason, they will be allowed to draw 3 chips.

Payments that subjects receive from MUSC for participating in a research study are considered taxable income per IRS regulations. Payment types may include, but are not limited to: checks, cash, gift certificates/cards, personal property, and other items of value. If the total amount of payment subjects receive from MUSC reaches or exceeds $600.00 in a calendar year, they will be issued a Form 1099. Participants will complete a W-9, either in person or via Doxy.me.

C3f. Statistical Methods

Specific Aim 1: To determine the impact of cocaine dependence and oxytocin on functional connectivity between corticolimbic brain regions during acute social stress.

Hypothesis 1A: The HC-PBO group will exhibit greater functional connectivity between the amygdala and prefrontal cortical regions (orbitofrontal and anterior cingulate cortices) than the CD-PBO group.

Hypothesis 1B: The CD-OT group will exhibit greater functional connectivity between the amygdala and prefrontal cortical regions (orbitofrontal and anterior cingulate cortices) than the CD-PBO group.

Preprocessing: Post-acquisition preprocessing and statistical analysis of all of the neuroimaging data will be performed using FEAT (FMRI Expert Analysis Tool) Version 5.98, part of FSL (FMRIB's Software Library, www.fmrib.ox.ac.uk/fsl). Functional magnetic resonance imaging (fMRI) blood oxygen level dependent (BOLD) imaging data will be acquired using a standard multi-slice gradient echo planar imaging sequence (TR=2200 ms, TE=35 ms, 64 x 64 matrix, 3 mm isotropic voxels, 271 volumes). Data will be preprocessed using scripting tools from FMRI Expert Analysis Tool (FEAT) Version 5.98, part of FMRIB's Software Library (FSL;www.fmrib.ox.ac.uk/fsl). First non-brain signal will be removed using FSL's BET brain extraction. Scans will be corrected for motion using FSL's linear registration and scans will be spatially smoothed using a Gaussian kernel of 8 mm FWHM. Since head motion can impact functional connectivity analyses, participants with head motion exceeding 0.2 mm will be excluded from the analyses (Power et al., 2012). The "fsl_motion_correction" scripting tool will be used to identify and scrub motion artifacts from each participant's data (Power et al., 2013). Scans will be spatially co-registered with a standardized anatomical template (Montreal Neurological Institute) using a 12 parameter affine transformation (Rorden et al., 2000). Analysis: Functional connectivity will be measured using a PPI based approach (Friston et al., 1997). A customized square wave form representing the task (1 = stress condition and -1 = control condition) 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 left amygdala using the MNI coordinates (x, y, z = -22, 0, -22). The transformation parameters described above will also be applied to the mask. For each participant and each run of the MIST, the mean corrected and high pass filtered time series of the BOLD signal in the left 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 left 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 associated with Specific Aim 1 explore increases in amygdala - prefrontal cortical connectivity in response to social stress, 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 2^{nd}-level random effects analysis. Unpaired t-tests will be used to test for the following group differences (Hypothesis 1A: CD-PBO vs. HC-PBO) and (Hypothesis 1B: CD-OT vs. CD-PBO). Analyses will be conducted using FMRIBs Local Analysis of Mixed Effects (FLAME 1). Additional analyses comparing (HC-OT vs. CD-OT) will be conducted using an unpaired t-test and applying the appropriate corrections for multiple tests. We will also conduct an exploratory gender analysis between the CD and HC women in the four groups (Domes, Heinrichs, Glascher, et al., 2007; Domes et al., 2010; Potenza et al., 2012). We anticipate that 15-20% of the subjects in each group will be female (n=6/8 per group). We

acknowledge that we may be underpowered to conduct these tests. Since there may be important group differences in regional brain activity during the MIST, a secondary whole brain analysis will be conducted using the contrast of interest (stress-control). Analyses will be conducted using FMRIBs Local Analysis of Mixed Effects (FLAME 1). All group level results will be thresholded at Z>2.3 using a corrected cluster threshold of p=0.05.

Hypothesis 1C: The CD-OT group will report lower subjective anxiety and craving than the CD-PBO group.

Analysis: A linear mixed effects model containing all serially measured time points will be used to assess the effects of OT versus placebo on each dependent variable. Restricted maximum likelihood (REML; Patterson and Thompson, 1971) methods will be used to estimate the fixed effects and variance components, and baseline values will be used as covariates in the regression models. The dependent variables anxiety and craving will be modeled separately. Within each family of dependent measures, the Type I error rate will be controlled using a post-hoc Bonferroni correction. The statistical analyses will be conducted using SAS 9.3 software (SAS Institute Inc., Cary, NC, USA).

Specific Aim 2: Use an implicit facial affect recognition paradigm to determine the impact of cocaine dependence and oxytocin on amygdala activity in response to fearful faces.

Hypothesis 2A: The CD-PBO group will exhibit greater amygdala activity in response to fearful faces than HC-PBO group.

Hypothesis 2B: Compared with the CD-PBO group, participants in the CD-OT group will exhibit lower amygdala activity in response to fearful faces.

Data Analysis: 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 (Hypothesis 2A: CD-PBO vs. HC-PBO) and (Hypothesis 2B: CD-PBO vs. CD-OT). Since human neuroimaging studies have found that other brain regions play a role in implicit emotional processing, including the right amygdala and insula (Davidson et al., 1999; Shi et al., 2013), secondary analyses will also include these regions. In addition, OT may impact amygdala responding to happy faces (Domes, Heinrichs, Glascher, et al., 2007), 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).

Power and Sample Size

Specific Aim 1

Power and sample size estimates for Hypotheses 1A and 1B were based on pilot data from the MIST (see Preliminary Studies). Based on these data we expect a between group difference (CD-PBO vs. HC-PBO) in the PPI beta estimate of OFC-amygdala connectivity of Δ=3.6 with a RMSE=4.0 (d = 0.9) (Cohen, 1988). Assuming an effect similar to the pilot data, the total effective sample size would be 40 subjects per group to provide 80% power for a between groups study design with a type I error rate of 0.05.

Power and sample size estimates for Hypothesis 1C were based on data from a double-blind placebo controlled pilot study of OT on stress reactivity to the Trier Social Stress Task in CD subjects. Based on these data we expect a between group difference in cocaine craving following the MIST of Δ=8.24 with a RMSE=11.03

(d=0.75) (Cohen, 1988) and a subjective anxiety difference of Δ=1.07 with a RMSE=2.20 (d=0.49). As in the oxytocin pilot study, participants will be assessed at five time points (m), with an anticipated intraclass correlation coefficient of 0.78 for both craving and anxiety outcomes (ICC=0.78 & 0.79, respectively). The design effect, which accounts for the multiple correlated observation within each subject, is estimated to be 4.12 (DE= [1+(5-1)0.78]) (Donner and Klar, 2000). Assuming independent observations and an effect similar to the pilot data for the anxiety response (d=0.48), the total effective sample size would be 140 subjects (35 CD and 35 controls per treatment arm) to provide 80% power with a type I error rate of 0.05. In a clustered setting, the sample size is $N_{effective}$ = nm/DE where n is the number of participants required when m replications within participants are performed after accounting for the design effect (DE). For the study at hand, the total number of participants required is therefore estimated to be N=135 (=140*4/4.12), As such, our proposed sample size of 160 subjects (40 CD and 40 HC per treatment arm) should provide sufficient statistical power to detect the planned anxiety and craving responses to the MIST. To account for and estimated attrition rate of 45%, we will enroll 210 participants in order to complete 160 scans.

Specific Aim 2

Power and sample size estimates were based on the literature. Neuroimaging studies of patients with PTSD and trauma matched controls have found significant group differences in the BOLD signal change in the amygdala during implicit facial affect processing. Based on data from these studies the appropriate effect size for our study ranges between 1.2-1.4 (Felmingham et al., 2010; Rauch et al., 2000). To detect an effect size (f) of 1.2 with a type 1 error protection level of .05 and n´=19 will have power (1-ß) of .95 (Cohen, 1988). As such, our proposed 40 participants per group should provide sufficient statistical power to detect group differences in the BOLD signal change in the amygdala during implicit facial affect processing.

C3g. Design Considerations

Education: Individual variability in cognitive skills (mental arithmetic) may impact the perception of the task as stressful or non-stressful. However, the MIST program includes an algorithm that ensures that the performance of each individual remains below 50%. In addition, groups will be matched by years of education. Trauma Loads/Recall Bias: Childhood trauma has been shown to affect corticolimbic responses to aversive social cues and to OT administration (Dannlowski et al., 2012; Fan et al., 2014). Study participants will complete the CTQ. Scores on the CTQ may be used as covariates in the analyses. Since the CTQ is a retrospective self-report assessment it is subject to recall bias. However, the psychometric properties of the CTQ are well established in healthy controls and cocaine-dependent populations. Sex: Sex differences have been found in stress-related corticolimbic brain activity in CD subjects and in amygdala responding in healthy controls (Domes, Heinrichs, Glascher, et al., 2007; Domes et al., 2010; Potenza et al., 2012). In our experience in recruiting CD subjects for similar research studies we anticipate that only 12-15% of the CD participants will be female, and therefore do not anticipated gender effects from washing out any potential group differences. Abstinence and Years of Use: Frequency and recency of drug use have been shown to affect drug craving and neuroregulatory stress systems (Fox et al., 2005). Subjects will be asked to report their patterns of drug use in the 90 days prior to the study visit. If there are significant group differences in these factors, they will be used as covariates in the analyses. Ovarian Hormones: Menstrual cycle phase, estrogen and progesterone levels can have a significant impact on drug seeking and craving (Evans et al., 2002; Feltenstein et al., 2007). In addition, estrogen and progesterone levels may impact OT effects in corticolimbic brain regions (Choleris et al., 2008; Gimpl et al., 2002). In order to determine the impact of these variables on study outcomes, , participants will be asked to complete a menstrual history diary and plasma estrogen and progesterone samples will be analyzed.

C3h. Data Management

The REDCap Study Database Version 4, supported by the South Carolina Clinical and Translational Research Institute (SCTR) at MUSC, will be used to capture data directly into an online database (Harris et al., 2009). Auditing will occur quarterly by comparing a random sample of 10% of participant's original datasheets to the values entered for those individuals in all data files. All of the neuroimaging data are automatically transferred to servers managed by CBI. Data will be stored on CBI password protected servers which receive daily data backups.

C3i.Operational Plan and Research Timetable

The first four months of the grant will be used for obtaining regulatory approvals, training and in-servicing CTRC staff and database creation. We have on-going trials involving CD and healthy control populations, thus we have trained personnel and an active recruitment network. We anticipate no issues with study recruitment beginning by the fifth month of the first year and continuing throughout the ninth month of the fifth year allowing for a total of 53 months. At a recruitment rate of approximately three participants per month, we should have no difficulty in completing the study in this time frame. Data analysis and manuscript preparation will take place during the last three months of the fifth year when recruitment has been completed.

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, aged 18-65. We will scan 160 study participants including (1) cocaine-dependent (CD) individuals (n=80) and (2) healthy control (HC) subjects free of major Axis I diagnoses (n=80). Inclusion/exclusion criteria are listed below:

General Inclusion/Exclusion Criteria

Inclusion Criteria

1. Age 18-65.
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. Subjects must consent to remain abstinent from all drugs of abuse (except nicotine) for the three-day period immediately prior to the study visit.
4. Subjects must consent to random assignment.
5. Subjects must have a negative breathalyzer, urine drug screen at the study visit.
6. Subjects must consent to the study visit which includes an outpatient visit to the ASD and completing one functional magnetic resonance imaging (fMRI) scanning session.

Exclusion Criteria

1. Subjects with evidence of or a history of significant hematological, endocrine, cardiovascular, pulmonary, renal, gastrointestinal, or neurological disease including diabetes.
2. Subjects with a history of or current psychotic disorder or bipolar affective disorder.
3. Subjects with current major depressive disorder or post-traumatic stress disorder.
4. Subjects taking any psychotropic medications, including SSRI's or other antidepressants, opiates or opiate antagonists. Subjects taking trazodone or non-benzodiazepene hypnotics for sleep will be included.
5. Women who are pregnant, nursing or of childbearing potential and not practicing an effective means of birth control.
6. Subjects who have a BMI that would proclude them from fitting comfortably in the scanner.
7. Persons with ferrous metal implants or pacemaker.
8. Subjects that are claustrophobic.
9. Subjects with significant psychiatric or medical problems that would impair participation or limit ability to complete the scanning session.
10. Subjects that require maintenance or acute treatment with any psychoactive medication including anti-seizure medications which could potentially interfere with fMRI data acquisition.

Group – Specific Inclusion/Exclusion Criteria

Individuals with Cocaine Dependence

Inclusion Criteria

1. Subjects must meet DSM-5 criteria for current (three months prior to study visit) moderate to severe cocaine use disorder. Individuals may meet criteria for marijuana use disorder, but they must not meet criteria for substance use disorder for any other substance (except nicotine and marijuana) within the 60 days prior to study participation. Due to the high comorbidity of alcohol and cocaine use disorder individuals with alcohol use disorder will be included in the study if they do not require medically supervised detoxification.

Exclusion Criteria

1. Subjects meeting DSM-5 criteria for substance use disorder (other than nicotine, cocaine, marijuana or alcohol) within the 60 days prior to study participation.

Healthy Controls

Inclusion Criteria

1. As above.

Exclusion Criteria

1. Subjects meeting DSM-5 criteria for current or lifetime moderate to severe substance use disorder on any drugs of abuse (except nicotine and marijuana).
2. Subjects meeting DSM-5 criteria for marijuana use disorder within the last year.
3. Subjects with a current (past month) major depression or post-traumatic stress disorder.

1.2 Sources of Materials

Research materials obtained from indiviudual 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, cocaine-dependent individuals could experience cocaine craving, therefore cocaine 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 (Bruins et al., 1992; Fehm-Wolfsdorf et al., 1988). 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 (MacDonald et al., 2011). To date, our research group has adminsted intranasal oxytocin to over 150 cocaine-dependent individuals 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

Medical records will not be reviewed to identify potential study participants. We will recruit “healthy adults” and “cocaine users” through multiple sources, including advertisements in local media via television, newspaper,

the internet and word of mouth among subjects. Flyers and advertisement will be not be used until they have been reviewed and approved by the MUSC Institutional Review Board (IRB). Secondary recruitment sites for participants will include (1) patients referred from the MUSC Center for Drug and Alcohol Programs (CDAP), and (2) individuals presenting to the Charleston Center for inpatient or outpatient treatment. Informed consent (IC) will be collected at the study research offices, in a private and interruption-free environment. The study PI, a Co-I, or other qualified study staff will obtain informed IC. The IC form includes a detailed description of the study procedures, risks and also statements regarding participants' rights to withdraw from the study at any time without consequences. The IC form will specifically review the potential for psychological distresss, 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 (Dr. McRae-Clark). Any questions pertaing 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

All study participants will be closely monitored for psychiatric and medical stability. The research team includes two board certified psychiatrists (Drs. Brady and Hartwell) who are available to monitor study participants for psychiatric stability. Dr. McRae-Clark and an on-call physician will be 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 victims of rape and other forms of violence, individuals with posttraumatic stress disorder, substance-dependent individuals, healthy controls, and work on large-scale studies asking questions about similar topics. There are well-established protocols at our site for emergency psychiatric evaluation, crisis intervention and/or psychiatric hospitalization for suicidal, homicidal, psychotic or other acutely distressed subjects. Immediately on detection of these needs, the assessor will page a psychiatrist to review the subject's situation. In addition, all study personnel will be trained to assess suicide risk. The Mini-International Neuropsychiatric Interview will be used to assess suicidal ideation and intent. If ideation and intent are identified, the PI and a psychiatrist will be notified immediately. The psychiatrist will personally evaluate the subject and, arrange for hospitalization if necessary. 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, study psychiatrists 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 substance-dependent populations.

All sessions will be conducted under the supervision of experienced personnel. The research team and all of the study staff will have succesfully 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 investigators 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 Addiction Sciences Division, in an office that is locked when not in use. Blood samples will be stored in the IOP's Clinical Neurobiology Laboratory. 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,Co-I's, and study staff 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.

The IOP Clinical Neurobiology Laboratory staff have extensive experience in venipuncture, thus any pain and bruising that occur as a result of the venipuncture are likely to be mild and transient. The instrumentation used for physiological recordings, meets all safety standards for non-invasive recordings and AC-powered devices are located out of the study participants' reach. 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.

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. Study participants will complete the metal screening questionnaire at each scanning visit. 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 taught about the potential side effects of oxytocin. Pregnancy tests will be performed at the study visit for women of childbearing potential. The pregnancy test will occur before the participants receive the intranasal spray. 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 reprocussions.

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 drug craving and relapse. Currently there are no FDA approved medications for the treatment of cocaine dependence. An investigation of oxytocin's effects on subjective anxiety and craving in cocaine-dependent individuals may provide important information that can guide treatment for future patients with cocaine dependence. 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 cocaine and other substance use disorders. 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)

This section is based on the recommendations found in the National Institute on Drug Abuse (NIDA) "Guidelines for Developing a Data and Safety Monitoring Plan" (www.drugabuse.gov/funding/dsmbsop.html). A detailed DSMP will be developed and approved by NIH program staff prior to study initiation.

5.1 Summary of the Protocol

The primary objective of this proposal is to identifying the neurobiologic substrates of emotion related behavior in cocaine-dependent populations. Groups of cocaine-dependent and healthy non-dependent controls will receive either intranasal oxytocin (24 IU) or placebo spray prior to participating in a single fMRI scanning session. During the scanning session participants will complete the Montreal Imaging Stress Task and an implicit facial affect recognition task. The primary outcomes of interest are (1) functional connectivity between the amygdala and prefrontal cortical brain regions during the stress condition of the MIST; (2) subjective anxiety and craving responses to the MIST and (3) the BOLD signal in the amygdala in response to implicit fearful faces.

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 research assistants directly into a computer using standard database software using REDCap. Neuroimaging data will be stored and analyzed on the CBI's password protected server. The data analysis plan is outlined in the Data Analysis Plan section.

5.4 Quality Assurance

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

5.5 Regulatory Issues

We currently have an active IND from the FDA for the use of oxytocin in cocaine-dependent and healthy controls with a history of childhood adversity who are free of Axis I diagnoses (IND 109,726). 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 by the PI and yearly by the IRB. Any significant actions taken by the local IRB and protocol changes will be relayed to NIDA. 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 clinicians (Co-Is). 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 clinicians (Co-Is).

5.9 Trial Efficacy

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

5.10 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 stress and fear in individuals with cocaine dependence.

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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