Title: Oxytocin in Cocaine Dependence

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SPECIFIC AIMS

Previous work conducted by the MUSC SCOR identified sex/gender differences in preclinical and clinical models of cocaine cue and stress reactivity that may contribute to gender differences in relapse. The proposed project will build on this work by exploring potential mechanisms for these sex/gender differences. Specifically, several studies have indicated that hypothalamic neuropeptides mediate behavioral responding to stress as well as play a role in neuroadaptations that occur as a consequence of long-term cocaine use. The hypothalamic neuropeptide oxytocin promotes trust, social bonding, and calmness. Given these effects, oxytocin may have a therapeutic role in ameliorating the negative affect commonly observed prior to relapse in cocaine-dependent individuals. As estrogen appears to enhance oxytocin's effects, women may have an enhanced response to oxytocin. The specific aims of the proposed project are:

Specific Aim 1: Using a human laboratory paradigm, determine the impact of oxytocin on stress-induced craving in cocaine-dependent women and men.
Preclinical and clinical investigations have found that the neuropeptide oxytocin exerts anxiolytic effects in stress paradigms. However, little is known about the potential anxiolytic effects of oxytocin in cocaine-dependent individuals, or if a gender differential in response exists.

Hypothesis 1a: Intranasal administration of oxytocin will attenuate drug craving response and stress reactivity to a psychosocial stress task in cocaine-dependent individuals compared to placebo administration.

Hypothesis 1b: The ameliorative effects of oxytocin on drug craving response and stress reactivity will be more pronounced in cocaine-dependent women as compared to cocaine-dependent men.

Exploratory Hypothesis 1c: The stress and craving reduction induced by oxytocin in women will be related to estrogen and progesterone levels.

Specific Aim 2: Using a neuroimaging cue paradigm, explore the impact of oxytocin on neural correlates and subjective responses of cocaine cue reactivity in cocaine-dependent men and women.
Presentation of cocaine-paired cues elicits a stress response in cocaine-dependent individuals. Neuroimaging studies indicate substantial overlap in the corticostriatal limbic circuitry responsible for cue and stress-primed drug craving. Oxytocin receptors have been localized to these same regions and preclinical studies have shown that oxytocin attenuates the acute reinforcing properties of psychostimulants. Despite these findings, little is known about the role of oxytocin in modulating the affective response to cocaine-paired cues and associated neural activity in cocaine-dependent men and women and whether there are gender differences in this modulation.

Hypothesis 2a: Intranasal administration of oxytocin will decrease subjective ratings of cue-induced cocaine craving in cocaine-dependent men and women compared to placebo administration.

Hypothesis 2b: Intranasal administration of oxytocin will decrease functional activity in the anterior cingulate cortex, amygdala, striatum, and prefrontal cortex compared to placebo administration.

Exploratory Hypothesis 2c: The attenuation of functional activity in the anterior cingulate cortex, amygdala, striatum and prefrontal cortex following oxytocin administration will be greater in cocaine-dependent women as compared to cocaine-dependent men.

Exploratory Hypothesis 2d: Oxytocin will modulate resting-state and task-related functional connectivity among sub-networks implicated in reward and motivation (nucleus accumbens, and orbitofrontal cortex),

memory and learning (amygdala and hippocampus), and cognitive control (prefrontal cortex and anterior cingulate cortex). Oxytocin may increase connectivity between reward/motivation and cognitive control sub-networks in craving states as an index of top-down regulation.

In summary, the proposed project will provide important data on the effect of oxytocin on stress and craving in cocaine-dependent individuals, explore the neural circuitry involved in response to oxytocin administration, and investigate whether sex/gender differences in response exist.

RESEARCH STRATEGY

A. Significance

Gender differences in cocaine use disorders. A growing body of literature indicates important gender differences in the symptomatology, course, and treatment of addictive disorders, including cocaine dependence. Approximately 38% of the 5.3 million individuals in the United States who reported cocaine use in 2008 were women (SAMSHA, 2009); despite this lower prevalence of use, there is evidence that women may actually have an increased vulnerability to the development and deleterious consequences of cocaine dependence. Women meet criteria for drug dependence more quickly and enter treatment programs earlier as compared to men (Brecht et al., 2004; Cotto et al., 2010; Hernandez-Avila et al., 2004; McCance-Katz, 1999; Ridenour et al., 2005; Westermeyer & Boedicker, 2000). Cocaine-dependent women also have greater psychiatric, medical, social/family, and employment problems (Brady & Randall, 1999; Elman et al., 2001; Najavits & Lester, 2008; Wong et al., 2002) and are more likely to attribute relapse to negative emotional states and interpersonal conflict than men (Connors et al., 1998; Terry-McElrath et al., 2009). These findings suggest gender differences in cocaine use and dependence which may have important treatment implications.

Stress and substance use. Multiple theories of addiction postulate that stress plays an important role in vulnerability and motivation to use and abuse substances (Brady & Sinha, 2005; Higgins & Marlatt, 1975; Koob & Le Moal, 2001; Russell & Mehrabian, 1975). Generally, these models suggest that drug use may begin as an attempt to modulate tension or distress in some cases. Over time, dependence can develop, and motivation for drug use may include treatment of withdrawal symptoms and response to conditioned cues as well as stress relief and mood enhancement. Thus, vulnerability to drug dependence and relapse may be a result of negative reinforcement/relief from stress or positive reinforcement/mood enhancement. Consistent with this model, clinical studies have shown drug or alcohol consumption to be positively associated with subjective reports of stress, lack of support, and avoidance coping (Cronkite & Moos, 1984; Hall, Havassy, & Wasserman, 1991; McMahon, 2001; Pohorecky, 1991). Data from our previous SCOR work also suggests that cocaine use can be related to stress and negative life events (Waldrop et al., 2007a; Waldrop et al., 2007b).

Stress and craving. Considerable empirical literature exists demonstrating that internal mood states related to stress and negative emotion can elicit craving in a laboratory setting, even in the absence of drug cues (Childress et al., 1994; Cooney et al., 1997; McRae-Clark et al., 2011; Sinha et al., 1999). In cocaine-dependent individuals, studies show that personalized imagery of stress situations as well as exposure to drug-related cues produce significant increases in cocaine craving, heart rate, cortisol, and subjective anxiety (Fox et al., 2008; Sinha et al., 1999; Sinha et al., 2000). Of note, our SCOR data suggests that there may be important gender differences in subjective ratings of stress and craving in response to cocaine cues and interpersonal stressors. Following presentation of cocaine-related cues, peak subjective ratings of stress and craving were correlated in both cocaine-dependent men and women. In response to a social stressor, subjective ratings of peak stress and craving were correlated in cocaine-dependent women; however, a significant correlation was not present in cocaine-dependent men (Waldrop et al., 2010). These findings suggest that stress related to interpersonal triggers is more highly associated with drug craving in women as compared to men. Importantly, several studies have found stress/mood-elicited craving predicted relapse to substance use (Cooney et al., 1997; Killen & Fortmann, 1997; Sinha et al., 2006). We have also recently reported that subjective stress and craving response to a pharmacological stressor (corticotrophin releasing hormone) predicts relapse to cocaine use (Back et al., 2010). Thus, it appears that provocation of subjective

stress not only elicits craving, but it may also independently predict relapse, supporting the targeting of stress-activated pathways for relapse medication development (Winhusen & Somoza, 2001).

Stress and cue reactivity. Cue-reactivity paradigms are commonly used in systematic investigations of craving (Drummond et al., 1990). The presentation of cocaine-related cues may also induce stress responses. Data from the previous funding period demonstrated increased subjective feelings of stress in cocaine-dependent individuals following cue presentation (Waldrop et al., 2010). This is congruent with other reports of increased anxiety and negative emotion following cocaine-cue exposure (Fox et al., 2008; Sinha et al., 2000). Further, cocaine-cue videos produced significantly greater brain activity in cocaine-dependent individuals than healthy controls in regions that were activated in response to sad videos. Thus, drug-paired cues may recruit brain regions responsible for the processing of negative affective stimuli (Wexler et al., 2001).

Oxytocin and stress. Oxytocin is a nine amino acid neuropeptide with peripheral endocrine and central neural actions that is synthesized in the paraventricular and supraoptic nuclei of the hypothalamus. Peripherally, oxytocin elicits physiologic events necessary for copulation, parturition, and lactation (Ludwig & Leng, 2006). Central release of oxytocin within limbic nuclei has been implicated in "pro-social" behaviors and may also have anxiolytic effects. For example, several preclinical studies have shown that oxytocin increases approach behavior in rats (Witt et al., 1992), and is essential for partner preferences and monogamous pair bonding in prairie voles (Williams et al., 1994). Central administration of oxytocin receptor antagonists prevents pair bonding in female prairie voles without any significant impact on mating. In fact, neonatal nurturing promotes long-term plasticity within the limbic circuitry, while childhood maltreatment has been associated with low urinary oxytocin levels (Francis et al., 2002; Fries et al., 2005). Intranasal administration of oxytocin attenuates anticipatory anxiety, subjective stress, and hypothalamic-pituitary-adrenal (HPA) axis activation to a psychosocial laboratory stress task and increases trust (de Oliveira et al., 2011; Heinrichs et al., 2003; Kosfeld et al., 2005). In addition, intranasal oxytocin attenuates circulating cortisol levels in men with a history of childhood maltreatment (Meinlschmidt & Heim, 2007). In agreement, neuroimaging studies demonstrate that intranasal administration of oxytocin attenuates amygdala activation following stressful and fearful stimuli (Baumgartner et al., 2008; Domes et al., 2007; Kirsch et al., 2005). Taken together, these studies demonstrate that oxytocin may play a potential therapeutic role in ameliorating stress and anxiety.

Oxytocin and substance use disorders. Both preclinical and clinical studies suggest that oxytocin may attenuate the effects of both acute and long-term drug use. Molecular studies have localized oxytocin receptors to the mesolimbic dopamine reward circuit, including the amygdala, nucleus accumbens, and ventral tegmental area (VTA) (Vaccari et al., 1998). Similar to drugs of abuse, oxytocin infusion directly into the VTA promotes dopamine release in the nucleus accumbens (Melis et al., 2007). Systemic administration of oxytocin promotes conditioned place preference in rodents, indicating that oxytocin is rewarding (Liberzon et al., 1997). Behavioral studies utilizing animal models of drug reinforcement demonstrate that oxytocin dose-dependently decreases cocaine-induced hyperactivity and stereotypy (Sarnyai et al., 1991; Sarnyai et al., 1992). Interestingly, chronic administration of oxytocin reduces dopamine release in the nucleus accumbens, indicating that oxytocin may be involved in the plasticity that occurs within the reward circuit as a function of repeated drug use (Kovacs et al., 1998). In addition, oxytocin inhibits the development of tolerance to repeated cocaine administration (Sarnyai et al., 1992) and may also attenuate self-administration of cocaine (Sarnyai & Kovacs, 1994). Oxytocin administration has been shown to block methamphetamine-induced locomotor activity and reduce methamphetamine reinstatement (Carson et al., 2010). These preclinical findings implicate oxytocin in the acute effects of stimulants, as well as the long term behavioral consequences of repeated use.

Limited clinical research has examined the effects of oxytocin in drug-dependent populations. Immunohistochemical analysis of post-mortem brains from alcoholics indicate a significant reduction in oxytocin immunoreactivity in the hypothalamus (Sivukhina et al., 2006). Further, mothers using cocaine during pregnancy exhibit significantly lower plasma oxytocin levels, as well as greater hostility and depression as compared to control mothers (Light et al., 2004). Based on a positive preclinical study in which increasing oxytocin levels were hypothesized to moderate the effects of lithium-attenuated cannabinoid withdrawal in rats

(Cui et al., 2001), a trial of lithium on marijuana withdrawal symptoms demonstrated positive outcomes, including decreased anxiety, lending support to this hypothesis (Winstock et al., 2009). These clinical findings, coupled with preclinical reports, *suggest that oxytocin may decrease some symptoms associated with drug dependence such as negative affect, withdrawal, and tolerance.* Our group has recently completed a pilot investigation of oxytocin administration prior to a social stressor task in marijuana-dependent participants. Oxytocin-treated individuals, as compared to placebo, demonstrated a significant trend towards reduced craving and cortisol response (see Preliminary Data). *The proposed study will be the first controlled examination of the effect of oxytocin on stress or cue-induced craving in cocaine-dependent individuals.*

Neural correlates of drug craving, sex differences, and potential role of oxytocin. Compulsive drug-seeking in response to stress and drug cues may be a function of disruption in the circuitry responsible for maintaining executive control and response inhibition in response to emotional evocative stimuli (Baler & Volkow, 2006). In support, neuroimaging studies of cocaine-dependent individuals have implicated prefrontal, limbic and striatal regions in both cue and stress-primed drug craving. For example, cocaine cues (videos and guided imagery) increase activity in limbic regions including the amygdala and anterior cingulate cortex of cocaine-dependent individuals but not healthy controls (Childress et al., 1999; Wexler et al., 2001; Garavan et al., 2000). Kilts and colleagues found that activity in the ventral striatum of cocaine-dependent men was significantly greater during cocaine-cue exposure than during exposure to neutral cues (Kilts, et al., 2001). In contrast, stress attenuated activity in frontal, limbic/paralimbic regions and increased activity in the dorsal striatum of cocaine-dependent individuals (Sinha et al., 2005).

Significant sexual dimorphism in frontal-limbic-striatal structure, function, and neurochemistry are well documented (Cahill, 2006). Therefore, it is not surprising that there are sex differences in the neural correlates of drug craving. For example, stressful imagery produced greater activity in the left anterior and right posterior cingulate, left insula, and inferior medial cortices of cocaine-dependent women as compared to cocaine-dependent men (Li et al., 2005). On the other hand, cocaine-dependent women exposed to guided imagery of cocaine use exhibited significantly less activity in the amygdala, insula, orbitofrontal and ventral cingulate cortices as compared to cocaine-dependent men (Kilts et al., 2004).

Interestingly, oxytocin receptors appear to be widely distributed within frontal-striatal-limbic regions. *In situ* hybridization studies have localized oxytocin receptor mRNA in the hypothalamus, rostral regions of the frontal cortex, bed nucleus of the stria terminalis, anterior cingulate, dorsal striatum, and the amygdala (Yoshimura et al., 1996; Yoshimura et al., 1993). In addition, oxytocin administration attenuates amygdala activity to emotional evocative faces (Domes et al., 2007). Of note, the reduction in amygdala activity was observed in response to both aversive and happy faces, indicating that oxytocin may attenuate reactivity to both aversive and appetitive stimuli.

Functional Connectivity in Cocaine Users. Measures of resting state functional connectivity have been employed in neuroimaging studies to evaluate the strength of communication within networks encoding functions ranging from vision and somatosensory perception to motivation and executive function (Fox & Raichle, 2007; Fox et al., 2005). Recently, measures of resting state functional connectivity have been applied to substance-dependent populations. The strength of connectivity within regions in the reward circuit was compared between healthy controls and cocaine users. Greater functional connectivity was found between the ventral striatum and orbitofrontal cortex of the cocaine group compared to healthy controls (Wilcox et al., 2011). In contrast, Gu and colleagues found that the strength of connectivity between the ventral tegmental area, thalamus/lentiform nucleus/ventral striatum, medial prefrontal cortex, and amygdala was significantly lower in cocaine-dependent individuals as compared to controls (Gu et al., 2010). Tomasi and colleagues found that cocaine abusers exhibited significantly lower connectivity between the midbrain, thalamus, rostral cingulate cortex, and cerebellum (Tomasi et al., 2010). This disparity in findings indicates the need for additional studies assessing functional connectivity in cocaine-dependent individuals. Moreover, the strength of functional connectivity during exposure to cocaine cues has not been explored.

Impact of estrogen and progesterone on reward, craving, and response to oxytocin. Estrogen and progesterone levels fluctuate throughout the menstrual cycle. Progesterone levels peak seven to twelve days after ovulation marking the middle of the luteal phase; estrogen levels dominate throughout the cycle but peak six to ten days after the start of the menstrual cycle, marking the mid-follicular phase (Evans et al., 2002). In addition to sex differences in brain structure, function, and neurochemistry, circulating gonadal hormones play a critical role in mediating the subjective response to the drug itself, cues, and stress. Preclinical studies have localized estrogen and progesterone receptors to brain regions responsible for mediating the physiological and behavioral response to stress and cues (Alves et al., 1998; Cushing & Kramer, 2005; Laflamme et al., 1998). Preclinical studies conducted by MUSC SCOR investigators demonstrated that cocaine seeking is significantly attenuated during the proestrus phase of the estrous cycle when progesterone levels are highest (Feltenstein & See, 2007; Kippin et al., 2005). In a recent study of treatment-seeking cocaine-dependent women, subjective craving following exposure to a laboratory stressor was significantly lower in women with elevated progesterone compared to women with low progesterone (Sinha, et al., 2007). It has also been demonstrated that ovarian hormone levels can impact the response to smoked cocaine with the positive or "rewarding" effects of cocaine significantly lower during the luteal phase compared to the follicular phase (Evans et al., 2002; Sofuoglu et al., 1999). Taken together, these studies indicate that during the follicular phase when estrogen levels are elevated, the positive and rewarding properties of stimulants peak, while increases in progesterone during the luteal phase may be decrease craving and/or drug effect.

Surprisingly few studies have examined the impact of ovarian hormones on the neural correlates of drug craving; however; neuroimaging studies of healthy women tested at different phases of their menstrual cycle are intriguing. For example, Goldstein and colleagues compared brain activity of healthy women exposed to images depicting negative affective stimuli during both the early and late follicular/mid-cycle phase of their menstrual cycle. Activity within the limbic regions was significantly greater during early as compared to late follicular phase (Goldstein et al., 2005). In addition, during the late follicular phase, the magnitude of activity in the amygdala was correlated with arousal, a pattern not observed early in the cycle when both progesterone and estrogen are low. While these data suggest that estrogen may attenuate limbic reactivity to emotional evocative stimuli in healthy subjects, the role of progesterone is unclear since subjects were not tested during the luteal phase of their cycle. Dreher and colleagues measured the limbic response to uncertain anticipated monetary reward in healthy women during the mid-follicular and mid-luteal phases of their menstrual cycle. Anticipation of uncertain rewards produced significantly greater activity in the amygdala and orbitofrontal cortex during the follicular phase than the luteal phase of their cycle (Dreher et al., 2007), suggesting that estrogen may enhance limbic responding to predictors of reward**.**

Studies have indicated that the ability of oxytocin to modulate social behaviors, such as attachment and aggression, is dependent upon gonadal hormone receptors localized the within limbic circuitry. Estrogen promotes oxytocin release and is required for the synthesis of oxytocin receptors in the amygdala (Lim & Young, 2006). Animal studies have also demonstrated greater oxytocin release in females compared to males in response to threat (De Kloet et al., 1986; McCarthy, 1995). In one clinical study, oxytocin or placebo spray was administered to couples engaged in a ten-minute conflict discussion of finances, education, or leisure time (Ditzen et al., 2009). While oxytocin significantly increased positive behaviors (eye contact and emotional self-disclosure) relative to negative behavior (contempt and defensiveness) compared with the placebo, no significant gender differences were found. However, a trend towards an accelerated reduction in salivary cortisol levels following the task was observed in the oxytocin-treated women, but not in the men. As stated previously, cocaine-dependent women are more likely to report negative affect and stress prior to relapse as compared to men. Combined with the evidence that estrogen enhances the effects of oxytocin, a more robust anxiolytic/anticraving response to oxytocin in drug-dependent women as compared to men might be predicted. This hypothesis will be tested in the proposed project.

Genetic Testing: The role of neuropeptides in mediating the affective and behavioral responses to stress and cues is well documented. However, there is still significant individual variability in the OT system. For example, exogenous OT increases trust and bonding others have found that OT reduces trust and social bonding in individuals with borderline personality disorder (Simeon, et al., 2011:Bartz, et al., 2011). In addition, while one study found that low levels of plasma OT were associated with symptom severity in women with

major depression, another study found no correlation (Ozsoy, et al., 2009; Parker, et al., 2010). Thus, understanding how individual variability in the OT system impacts the affective responses to stress particularly in cocaine-dependent individuals could have a significant impact on the development of effective treatment strategies targeted at specific neuropeptide systems. An emerging literature suggests that genetic polymorphisms in neuropeptide receptors play an important role risk and resilience to psychopathology (Brune, 2012). For example, polymorphic variation in the OT receptor gene (rs2254298) has been associated with an increased risk for depression and anxiety disorders. Therefore we plan to examine genes associated with the neuropeptide system that regulate the corticolimbic brain activity. Blood will be collected from consenting participants for genetic testing. Our group has previously collaborated with Dr. Ananda Amstadter at Virginia Commonwealth University (VCU), whose research group has been actively investigating genetic variations associated with addiction. Briefly, whole blood will be collected in EDTA containing vacutainers and stored at -70º at the Institution of Psychiatry Laboratory. De-identified samples will be shipped on dry ice to Dr. Amstadter's Laboratory at VCU. The DNA will be extracted and assayed using standardized commercially available kits.

Summary. Stress is an important predictor of relapse, and targeting stress-activated pathways may lead to therapeutic advancements in the treatment of substance use disorders. Oxytocin has been shown to promote trust, social bonding, and calmness; however, its potential effects have not been explored in cocaine-dependent individuals. Oxytocin receptors have been localized to brain regions that are activated by drug-paired cues and preclinical studies have shown that oxytocin attenuates the acute and long-term behavioral effects of psychostimulants. However, little is known about the role of oxytocin in mediating the affective response to cocaine-paired cues and associated neural activity in cocaine-dependent men and women. This project is a direct evolution from our previous SCOR-supported research. **Our work has progressed from characterizing sex/gender differences in response to social stressors and cocaine cues in cocaine-dependent men and women, to our on-going work evaluating whether stress potentiates cue-induced craving and the impact of hormones on this response. The proposed study will investigate the role of oxytocin in the sex/gender differences in stress response and craving in cocaine-dependent individuals and preliminarily explore its therapeutic potential.**

B. Innovation

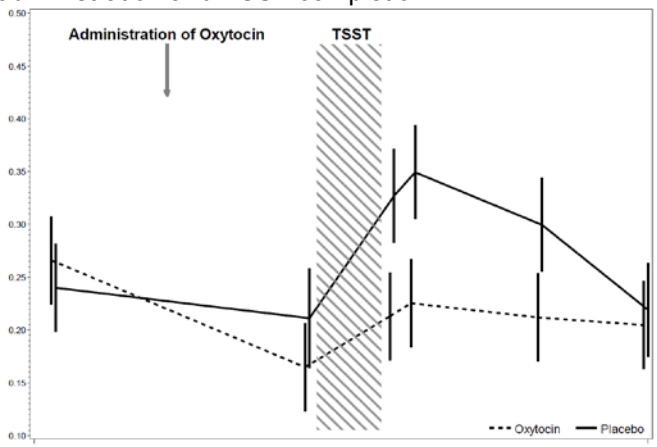
The proposed project will be the **first clinical investigation of oxytocin in cocaine-dependent individuals**. It has been suggested that oxytocin may be useful therapeutically in addiction treatment (Carson et al., 2010); however, limited clinical research with oxytocin in addicted populations has been conducted. Further, although a strong preclinical literature supports the role of ovarian hormones in cocaine craving and relapse, **little is known about the impact of ovarian hormones on the neural correlates of drug craving**. The methodology proposed also incorporates novel technologies and analytical techniques. **The use of neuroimaging will allow identification of the neural circuitry involved in oxytocin-mediated affective response to cocaine-paired cues**, and exploration of sex/gender differences in activation. Inclusion of functional connectivity analyses will allow further elucidation of the sub-networks involved in cocaine-related reward and motivation. Further, the proposed project is innovative as it is **extends an animal model of a potential therapeutic intervention (oxytocin) to a human laboratory setting**. Therefore, this project will further the ability to directly translate findings from an animal model to an ecologically valid test of stress and relapse in cocaine-dependent humans, and explore the impact of hormonal status on response in this model.

C. Approach

C.1. Preliminary Studies. Below, a brief overview of some of the major findings from our previous funding periods is provided. Additionally, preliminary data of direct relevance to the proposed project is presented.

C.1.a. Progress report. In our initial SCOR project, we explored sex/gender differences in response to a social stressor and drug cues in cocaine-dependent individuals. As discussed above, we found gender differences in the relationships between subjective ratings of craving across the tasks suggesting that social stressors may be more closely associated with relapse for cocaine-dependent women as compared to men (Waldrop et al., 2010). Further, greater dysregulation in the HPA-axis response to corticotropin-releasing hormone (CRH) was observed in cocaine-dependent women as compared to cocaine-dependent men or healthy men and women (Brady et al., 2009). Subjective response to CRH and drug cues in the laboratory setting correlated with relapse to cocaine use (Back et al., 2010). We additionally investigated the impact of early life trauma and current life stressors, and how these factors may contribute to stress response, craving, and cocaine use (Back et al., 2008; DeSantis et al., 2011; Moran-Santa Maria et al., 2010; Waldrop et al., 2007). A complete listing of publications resulting from our previous work can be found in Appendix 4. Taken together, our findings suggest that the HPA axis in women may be more sensitive to the toxic effects of cocaine or early life trauma as compared to men. Cocaine-dependent women have a greater physiologic and subjective response to stress and the magnitude of this response may be related to the potential for relapse. This suggests that the stress response is particularly important in understanding sex/gender differences in drug dependence and other psychiatric disorders and is also an important target in developing both psychotherapeutic and pharmacotherapeutic interventions for cocaine-dependent women.

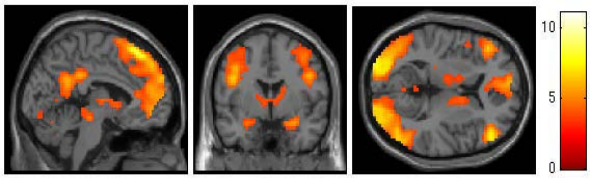
Figure 1. Change in cortisol following oxytocin administration and TSST completion.



C.1.b. Oxytocin Administration. In a paradigm similar to what will be used in the proposed project, we administered oxytocin or placebo nasal spray to 16 marijuana-dependent individuals. As shown in **Figure 1**, oxytocin treatment prior to completion of the Trier Social Stress Task (TSST) resulted in an attenuated cortisol response as compared to placebo (p=0.066). A decrease in craving with oxytocin administration compared to placebo was also observed (p=0.025). These pilot results suggest that oxytocin may be useful for reducing stress response and craving in drug-dependent populations. An open-label pilot study involving oxytocin administration in cocaine-dependent participants is ongoing. To date, we have administered oxytocin to five cocaine dependent individuals, and the drug has been well-tolerated.

C.1.c. Examination of neural correlates of cocaine craving. We are currently conducting a neuroimaging study (R01DA023188-S2; PI: Brady) in which we have examined response to cocaine cues in 39 cocaine-dependent participants using fMRI. In this sample, the presentation of cocaine cues, relative to neutral objects, was associated with a widespread pattern of activation including left occipital cortex, left superior frontal gyrus, right dorsolateral prefrontal cortex, anterior cingulate, bilateral hippocampus, right amygdala, posterior cingulate cortex, and left orbitofrontal cortex (see **Figure 2**). Subjective craving was significantly higher following cocaine images blocks (M = 1.66, SD = 0.89) versus neutral object blocks (M = 0.58, SD = 0.61; p < 0.001), demonstrating our ability to elicit craving with our scanning paradigm.

Figure 2. SPM map of blood oxygen level dependent (BOLD) response to cocaine cues minus neutral objects using a voxelwise threshold of z = 3.09 and a corrected cluster threshold of p < 0.05.



C.1.d. Functional Connectivity in Cocaine Users. We recently completed a preliminary study using a graph theory-based approach to functional connectivity with the Brain Connectivity Toolbox (BCT; Rubinov & Sporns, 2011) to examine changes in functional network structure when cocaine-users (n=37) were viewing cocaine-

related pictures compared to rest. **Figure 3** shows the modular structure based on the group-averaged correlation matrix. In the rest condition, 4 modules emerged (color coded in **Figure 3**), but in the cocaine condition, there was significant reorganization of the modules relative to rest. The blur condition was identical to rest whereas the object condition was different from both the rest and cocaine conditions. Notably, in the cocaine condition there was greater integration across brain regions as evidenced by the emergence of only 3 modules. In addition, the diversity coefficient (which is a measure of functional integration) was greater in the cocaine than the rest condition for all regions that changed module membership in the cocaine condition (**Figure 3**-cocaine; all p's < .05) except for the right middle occipital gyrus (region 52). Regions that became more integrated with the fronto-parietal module (green) included the insula, posterior cingulate, auditory cortex, and dorsal visual regions. We speculate that this reorganization reflects heightened attention to and salience of the cocaine stimuli given the characterization of fronto-parietal cortex as a dorsal attention network (Fox et al., 2006) and insula-cingulate connections as part of a salience network (Menon & Uddin, 2010). There was also increased connectivity/integration of ventral visual regions with temporal and ventro-medial prefrontal regions which may reflect strong engagement of memory systems related to the cocaine images (Childress et al., 1999). Although some typical reward-related regions (caudate, putamen; blue module) were dissociated from the other modules in the cocaine condition, this also occurred when participants were viewing objects. The other changes in module membership in **Figure 4** were specific to the cocaine condition. Although this is a preliminary analysis, it demonstrates the feasibility of conducting graph theory-based functional connectivity analysis for the proposed project. In addition, regions that changed module membership in the cocaine condition included some of the same regions that have oxytocin receptors (e.g., amygdala, anterior/posterior cingulate, and striatum). Finally, Olaf Sporns, a major developer of the BCT, has agreed to be consultant on this project (see Letter of Support). He is currently integrating network-based statistics (Zalesky et al., 2010) into the BCT to allow for group comparisons of functional networks.

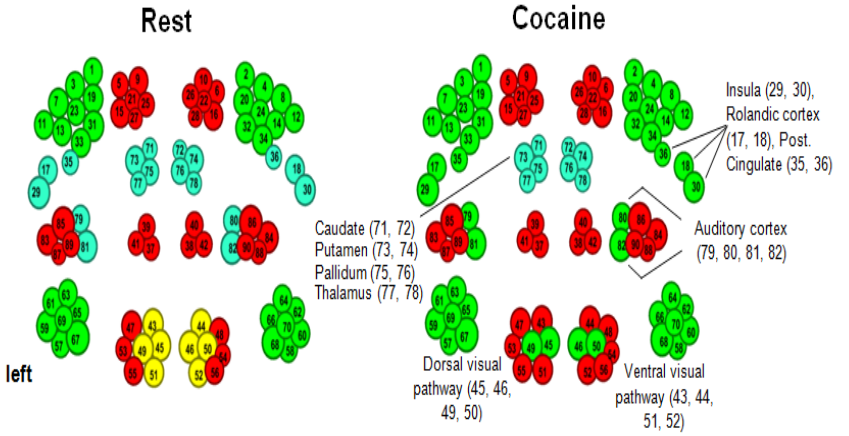
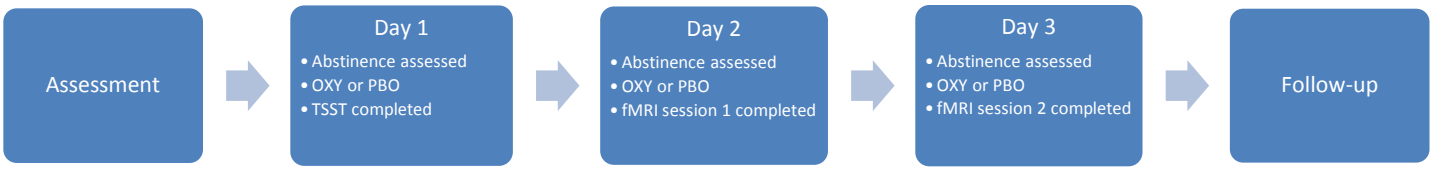


Figure 3. Results of the modularity partition based on the average correlation matrix among 90 regions in the rest (left) and cocaine (right) conditions of a cue-induced craving fMRI study of 37 cocaine users. Numbers indicate the region as specified in the AAL atlas and size of each node represents its average strength (i.e. sum of positive connections). Modules in the resting state include fronto-parietal (green), ventral-visual/temporal/orbitofrontal (red), dorsal visual (yellow) and basal ganglia/thalamus/auditory/insula (blue) sub-networks. Regions that reorganized when viewing cocaine images are noted by labels in the cocaine condition.

C.1.e. Summary. As demonstrated by the work above, the research team has experience with stress-induction studies (Brady, McRae-Clark, Moran-Santa Maria), cue-reactivity research (Brady, McRae-Clark), administration of oxytocin (McRae-Clark), neuroimaging (Brady, Joseph, Moran-Santa Maria), functional connectivity analyses (Sporns, Joseph), and recruitment and retention of cocaine-dependent individuals for participation in laboratory-based studies (Brady, McRae-Clark). Hence, the study team has the necessary skills to ensure the successful completion of the proposed study.

C.2. Research Design and Methods. In this project, the impact of acute pre-treatment with oxytocin (40 IU) or placebo on the subjective and physiologic response to the Trier Social Stress Task (TSST) in men and women with cocaine-dependence will be investigated. Additionally, participants will complete two functional magnetic resonance imaging (fMRI) scans in the presence of cocaine-related cues. Prior to each scan, participants will receive either oxytocin (OXY) or placebo (PBO) in a counter-balanced, double-blind fashion. A schematic of study procedures is shown below.



C.2.a. Participants. A total of 152 participants (76 cocaine-dependent women and 76 cocaine-dependent men), aged 18 or older, will be recruited over a 51-month period. Additional inclusion criteria include consent to random assignment and ability to read and provide informed consent. Exclusion criteria include women who are pregnant, nursing, or plan to become pregnant during the course of the study; women who have had a complete hysterectomy, are post-menopausal, are receiving hormone replacement or hormonal contraceptive therapy,; having a history of or current major psychiatric or medical disorder; requiring concomitant medication that could affect neuroendocrine measures; and being currently dependent on other substances, with the exception of nicotine, alcohol and marijuana. A detailed list of inclusion/exclusion criteria can be found in Human Subjects.

C.2.b. Recruitment. Participants will be recruited primarily through media advertisements, as we have had good results using this method in previous studies. We are using a similar recruitment strategy for our ongoing SCOR protocol. In addition to recruiting through advertisements, our research group also has established relationships with clinical programs that screen individuals for research or treatment program participation. In particular primary clinical sources which could be used for recruitment include individuals presenting to the Charleston Center for inpatient or outpatient treatment and individuals from the Ralph H. Johnson VA Medical Center. For patients involved in clinical programs, participation in this protocol will be arranged so as not to interfere with treatment participation. Chart review may also be conducted to identify potential subjects, although only subjects who have agreed to be contacted about research will be contacted. During the previous SCOR funding period, we consented over 700 cocaine-dependent research participants. Respondent-Driven Sampling" (RDS), will be used to enhance recruitment of the sample (Heckathorn, 1997). The RDS sampling methodology is based on recruiting the eligible friends and acquaintances of each participant so that the sample "snowballs". Each eligible participant who completes an interview will be given coupons to pass on to other potential participants. The coupons will have a unique code linked to the person who passes them out. A referral will be instructed to call the site offices for screening and, if eligible, an appointment for further evaluation. If a referral completes the screening process and is eligible for the study, the participant who referred the person can redeem the coupon for $20 and 5 fishbowl picks **.**

C.2.c. Procedures.

C.2.c.1. Screening/Assessment. Individuals will be screened for eligibility over the telephone by a trained research assistant. Major inclusion/exclusion criteria will be assessed during the telephone interview; if an individual seems potentially eligible, (s)he will be invited for an in-person interview with 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). At the time of this interview, a general medical history and physical exam will be performed to insure that the subject is eligible to participate. If a patient is ineligible to participate, (s)he will be given a referral for medical care and/or an appropriate treatment program.

***a. 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 (M.I.N.I.):** The M.I.N.I. is a brief structured interview that was designed to assess DSM-IV diagnoses using a series of questions in dichotomous format (yes/no). Earlier studies have found that the M.I.N.I. is similar in sensitivity, specificity, and inter-rater reliability to other more lengthy diagnostic interviews, such as

the SCID-I/P (Sheehan & Lecrubier, 2003; Sheehan et al., 1998). **Structured Clinical Interview for DSM-IV (SCID-I/P):** The SCID-P is a structured diagnostic interview that assesses each of the criteria for DSM-IV diagnoses. The alcohol and drug use disorder modules will be to thoroughly assess both current and lifetime diagnostic status for abuse and dependence. It has excellent inter-rater and test-retest reliability (First et al., 2002). **Menstrual History Diary:** Participants 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. Participants rate both frequency and intensity for the past month. The Daily Hassles Scale is positively correlated with adaptational ability and is a good predictor of psychological symptoms (Kanner et al., 1981). **Adverse Childhood Experiences (ACE) Questionnaire:** The modified ACE questionnaire assesses childhood maltreatment and exposure to household dysfunction. It has been utilized to examine the relationship between adverse childhood experiences and health risk behavior in adulthood (Felitti et al., 1998). In addition to providing data to assess the impact of early childhood trauma on cocaine use, utilization of the ACE will allow for potential cross-SCOR collaborations as it also being employed by the UCLA, Yale and Harvard SCORs. **Childhood Trauma Questionnaire (CTQ)**: The CTQ is a 25-item self-report questionnaire used to assess the extent to which individuals have experienced five domains of childhood abuse and neglect (sexual abuse, physical abuse, emotional abuse, emotional neglect and physical neglect). Subjects answer each question using a 5-point Likert scale ranging from (1) never true to 5 (very often true). Severity scores for each domain can range from 5 (no history of abuse/neglect) to 25 (severe abuse/neglect). The questionnaire also includes a minimization/denial scale. The reliability and validity of the CTQ are excellent (D. P. Bernstein, et al., 1997; D. P. Bernstein, Fink, L.A., 1998). The **Pittsburg Sleep Quality Index** will be used to assess sleep patterns (Buysse, et al., 1989). The **Quick Inventory of Depressive Symptoms** (Rush, et al., 2003) will be used to measure depression and a short form anxiety scale from the PROMIS© item bank will be used to measure anxiety. The **Life Events Occurrence Scale** (Maciejewski,, et al.) will be used to inventory adult trauma. A brief survey from the National Health and Nutrition Examination Survey (CDC, 2000) will be used to rate quality of life, and a body map will be used to rate areas of pain. A brief smoking history will also be obtained.

b. Substance-Related Instruments. **Form 90:** The Form 90 (Miller, 1996), an assessment instrument commonly used in substance use 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.). Alcohol, nicotine, and other drug use will also be tracked. For cocaine use, 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) amount used per using day **Inventory of Drug-Taking Situations (IDTS)** (Annis et al., 1997): This instrument measures typical drug using situations based on Marlatt and Gordon's (1985) eight category taxonomy. This instrument contains eight subscales mapping frequency of use in eight distinct types of situations including use during negative situations (unpleasant emotions) and positive situations (social situations). Although initially calculated to assess alcohol use, this scale has been modified for use with drug abusers and has demonstrated sensitivity to gender differences (Ross et al., 1994). Participants will also be asked to provide a urine sample to be tested for drugs of abuse.

c. Progesterone and Estrogen Tracking. We will explore the impact of progesterone and estrogen levels on cocaine craving, stress, and oxytocin effects. Because our hypotheses are related to ovarian hormone levels rather than menstrual cycle phase, direct measurement of hormone levels at the time of testing is the most appropriate measure to address our hypotheses (Becker et al., 2005). As there is significant individual variability in progesterone and estrogen levels across the menstrual cycle, we will measure progesterone and estrogen at the initial assessment and each of the three study visits. We expect that these four samples will provide a profile of hormone levels for each study participant. Since we are interested in having a range of hormone levels for comparison, we will test 50% of participants during the luteal phase when progesterone

levels are high, and 50% of participants during the follicular phase when progesterone levels are low. Cocaine-dependent women have normal 28-day ovulatory cycles (Evans et al., 2002, Evans & Foltin, 2006; Fox et al., 2008); therefore, we will be able to approximate the menstrual cycle phase by taking a menstrual cycle history during the initial assessment and will schedule the study visits accordingly. Direct ovarian hormone measurement will allow us to utilize all participants' data with absolute progesterone and estrogen levels as continuous measures.

C.2.c.2. Session Procedures.

a. Session Preparation. 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 test sessions in order to minimize the impact of recent drug/alcohol use on stress and cue reactivity. Participants will be asked to come into the office to provide a urine sample once during the three days prior to test sessions to encourage abstinence and session completion. Participants will be instructed to arrive at the Addiction Sciences Division (ASD) on each test day, and to avoid caffeinated beverages on the test day since caffeine can introduce variability to the heart rate response. If the individual is nicotine-dependent, (s)he will be provided with nicotine patches throughout their clinic stay. Upon arriving at the ASD, the participant will be breathalyzed and will provide a urine sample, which will be tested for the presence of amphetamines, d-methamphetamine, cocaine, opioids, THC and benzodiazepines; if female, a urine pregnancy test will also be administered. If the pregnancy and urine drug tests are negative, with the possible exception of THC, the session will proceed. In the event a participant tests positive, the laboratory visit will be rescheduled. A blood pressure cuff will be placed on the subject's arm, and baseline subjective measures, physiologic measures (blood pressure and heart rate), and salivary progesterone, estradiol, and cortisol will be collected.

b. TSST. On Day 1, participants will be served lunch and be seated in the ASD testing room where they will be allowed to read or watch television until the TSST testing procedures begin. Forty minutes prior to the TSST, participants will be randomly assigned to receive study medication (intranasal oxytocin or placebo; see Medication Administration). Two more baseline assessments of subjective, hormonal (cortisol), and physiologic measures (blood pressure and heart rate) will be collected, one 20 minutes pre-task and the second5 minutes pre-task.

1. Medication Administration. Participants will be administered 40 IUs of oxytocin nasal spray or matching placebo at 40 minutes prior to study tasks. This dose was selected based on previous studies that have used similar doses of oxytocin (Ditzen et al., 2009; Heinrichs et al., 2003; Kubazansky et al., 2009), as well as our own previous pilot work (see Preliminary Data). Timing of administration is also based on our pilot work and previous studies showing central activity of oxytocin 40 minutes after intranasal administration (Heinrichs et al., 2009). Intranasal oxytocin and matching placebo will be compounded by a MUSC Investigational Drug Service. Randomization will be done by the MUSC Investigational Drug Service, who will keep a record of the blind and be available should unblinding be required. To achieve balance in sample size with respect to gender, a block randomized design with randomly varying block sizes will be used.

2. TSST. The TSST is a standardized psychological stress challenge which has been used extensively in research studies. A meta-analysis supports its utility for evoking a HPA axis stress response in a laboratory setting (Dickerson & Kemeny, 2004). It has induced a robust and reliable physiological stress response in cocaine, marijuana, and alcohol-dependent individuals in our previous studies (McRae et al., 2006; McRae-Clark et al., 2011; Waldrop et al., 2010). When the TSST begins, The participant will be told that (s)he will give a speech and perform an arithmetic task. The topic of the speech will be why (s)he should be hired for a particular job (the individual's "dream job"). The participant will deliver the speech as though speaking to a group of potential employers. The experimenter then tells the participant that (s)he has 5 minutes to prepare the speech, and starts the countdown clock (placed in view of the individual). The experimenter leaves the room to allow the participant to prepare. After 5 minutes, three individuals unfamiliar to the participant (the audience) enter the room and are seated; the individual will be instructed by one audience member to stand

and begin his/her prepared speech (without notes). The speech will be delivered for 5 minutes. If the individual pauses, (s)he will be instructed to continue. At the end of the speech task, the individual will be instructed to serially subtract 13 from 1,022 as quickly and accurately as possible. The mental math recitation will continue for 5 minutes, at the end of which time, the spokesperson will instruct the individual to stop and be seated, and the audience leaves the room . Immediately following the task, a salivary sample will be collected to assay cortisol and physiological (blood pressure and heart rate) and subjective measures (CCQ-Brief, Within Session Rating Scale, obtained. Measurements will also be obtained 10- (CCQ-Brief, Within Session Rating Scale, STAI cortisol, physiological), 30-(CCQ-Brief, Within Session Rating Scale, cortisol, physiological), and 60-minute post-task(CCQ-Brief, Within Session Rating Scale, STAI cortisol, physiological).

3. Assessments. All baseline and within-session measurements have been previously used by our research group. Wherever possible, the same assessment instruments will be used in Project IV to foster cross-study analysis and comparison (i.e., psychiatric assessment, trauma history). . **Urine Drug Screening:** Drug screens will be performed using the *iCup*® DX Drug Screen Cup, an in vitro diagnostic test for the qualitative detection of drug or drug metabolite in the urine. The test profile consists of amphetamines, d-methamphetamine, cocaine, opioids, , THC and benzodiazepines. Results will be used to ascertain abstinence prior to initiation of test sessions and to substantiate self-reports. **Breathalyzer:** Participants will have their breath sampled for the presence of alcohol (Alco-Sensor III, Intoximeters Inc., St. Louis, MO). Self Report Measures. *Within Session Rating Scale*: A modification of the Within Session Rating Scale (Childress, McLellan, & O'Brien, 1986a) will be used to assess craving and mood during the procedure. This 100 mm visual analogue scale is anchored with the adjectival modifiers ("not at all, mildly, moderately, and extremely"). The scale includes items assessing anxiety, stress, anger, irritability, and depressed mood. *State-Trait Inventory (STAI):* The STAI is a 20-item self-report scale (Spielberger, 1983) employing a Likert-scale format with four responses per item (1-4). Ten of the STAI items measure feelings of stress and anxiety, while the remaining ten items measure feelings of relaxation. The STAI has good psychometric properties. *Cocaine Craving Questionnaire-Brief (CCQ-Brief)*: The CCQ-Brief is a 10-item scale derived from the Cocaine Craving Questionnaire (Tiffany et al., 1993) and demonstrated to be a valid and reliable measurement of current cocaine craving (Sussner et al., 2006). Physiological Measures. Heart rate and blood pressure will be measured using an intermittently inflatable cuff as indices of physiological arousal during the test session. Hormonal Measures. *Cortisol:* Cortisol will also be measured in unstimulated passive saliva using the Salimetrics expanded range, high sensitivity salivary cortisol enzyme immunoassay kit. This kit has a lower sensitivity level of <0.003 μg/dL. The correlation between salivary and serum cortisol has been shown to be high ($r=0.91$, $p<0.0001$). Cortisol is commonly measured as an indication of stress response in human laboratory studies. *Progesterone:* Progesterone will be measured from a salivary sample tested using the Salimetrics salivary progesterone enzyme immunoassay kit. The lower limit of sensitivity is 5 pg/ml, and the correlation between serum and saliva is highly significant ($r=0.80$, $p<0.0001$). *Estradiol:* Estradiol will be measured from a salivary sample tested using the Salimetrics 17β-estradiol enzyme immunoassay kit collected in EDTA-tubes and processed using a chemiluminescent immunoassay. The lower limit of sensitivity is 0.1 pg/ml, and correlation with serum is high ($r=0.80$, $p<0.001$).
4. Discharge. Following the 60-minute assessment, participants will be given instruction regarding scanning appointments on days 2 and 3. A member of the research staff will be available to discuss management of any craving urges if craving remains elevated at the end of study procedures.

c. fMRI Sessions. On Days 2 and 3, participants will be asked to arrive at the ASD. Upon arrival, the participant will be breathalyzed and will provide a urine sample, which will be tested for substances and pregnancy (if applicable). If the pregnancy and urine drug tests are negative, with the possible exception of THC, the session will proceed. In the event that a participant tests positive, the scanning session will be rescheduled. Baseline subjective measures, physiologic measures (blood pressure and heart rate), and

salivary progesterone, estradiol, and cortisol will be collected. Individuals will then be escorted to the Center for Advanced Imaging Research (CAIR; see letter of support) by approved study personnel where MRI scans will be performed.

1. Medication Administration. At the beginning of the session, participants will be screened for metal using a handheld metal detector, and oxytocin (40IU) or placebo will be intranasally administered 40 minutes prior to cue exposure procedures. Although the pharmacokinetics of intranasal oxytocin are not fully understood (MacDonald et al., 2011), given the relatively short half-life of systemically administered oxytocin (approximately six minutes), we would not anticipate carry-over effects from repeated once daily dosing. Further, the order of administration will counterbalanced to obviate order effect; half of the participants will receive oxytocin prior to the first fMRI session and half will receive placebo.

2. Image Acquisition Parameters. A 3 Tesla Siemens Trio magnetic resonance imaging scanner equipped with a 12-channel head coil for whole-brain imaging will be used for data acquisition. Participants' heads will be stabilized using foam padding between the head and the head coil. 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. 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 saggital scout image will be acquired for co-registration and normalization of functional images. The scanning planes will be oriented parallel to the anterior commissure–posterior commissure line.

3. Scanning Procedures. Prior to entering the scanner, subjective measures, physiologic measures (heart rate and blood pressure) and salivary cortisol will be collected. Participants will be given earplugs/headphones to protect their hearing and an optical hand pad rating device to allow them to respond to study tasks. Their ability to view a nearby projection screen (via a back-projected mirror mounted to their head coil) and correctly make ratings with the hand pad will be assessed prior to scanning. During initial scanner tuning, localizing, and structural scanning, participants will be shown relaxation images (i.e., 20 scenic pictures, each displayed for 30 seconds). Next participants will be asked to look at a cross-hair for 6-minutes while resting state connectivity data are collected. Next, the cocaine cue exposure paradigm will be administered. The paradigm to be used was adapted from a well-studied alcohol-cue exposure paradigm (George et al., 2001; Myrick et al., 2008), and is currently being utilized in an ongoing imaging study being conducted by the research team (R01DA023188-S1; PI: Brady; see Preliminary Data). The paradigm consists of pictures of cocaine and related objects (e.g., crack pipe), neutral objects (e.g., furniture), and visual control images that match the cocaine pictures in color and hue, but lack object recognition. Stimulus presentation occurs over six 90-second epochs. Each epoch contains three 24-second blocks (cocaine images, neutral objects, control images), containing five pictures displayed for 4.8 seconds each, and one 24 second rest block (i.e., cross-hair). Blocks, and stimuli within blocks, are presented in pseudorandom order. During the task, participants will be asked to rate their stress and craving after each block using their hand pad; ratings range from zero ("none") to four ("severe"). Salivary cortisol and subjective measures will be collected immediately following the task, and 30 and 60 minutes post task.

4. Discharge. Following data collection, the participants will be debriefed, and scheduled for follow-up appointments. As on Day 1, a member of the research staff will be available to discuss management of any craving urges if craving remains elevated at the end of study procedures.

C.2.c.3. Follow-Up Procedures. Participants will return to the research clinic once weekly for the two weeks following completion of scanning procedures. At each visit, a urine drug screen will be obtained and TLFB data on cocaine and other drug use will be obtained. Participants will have had a brief period of monitored abstinence during testing procedures (3 days prior to testing, 3 days during testing); measurement of relapse to cocaine use after early abstinence in a clinical population provides homology and integration with Project III, which is assessing similar outcomes in a preclinical model. At each follow-up visit, measures of daily stress will be obtained using the 10-item Perceived Stress Scale (PSS; Cohen et al., 1983), and subjects will be asked to

specifically assess possible precipitants of use when cocaine use is reported. The PSS has been used previously in cocaine dependent adults (Fox et al., 2009; Hyman et al., 2007), and has demonstrated test-retest reliability. The menstrual history diary will be updated at each visit. Following study completion, referrals to treatment programs will be provided to all participants. At the first follow-up visit, blood will be collected from consenting participants for genetic testing.

C.2.c.4. Subject Compensation. Participants will receive $25 for completing screening assessments and procedures ($10 for the history and physical and $15 for the interview/paperwork), and $25 if they present once during the three days prior to testing. Participants will receive $75 for completing the TSST, $100 for the first scanning session, and $125 for the second scanning session. Patients will receive study session payment at the end of Day 3. Additionally, "fishbowl" contingency management will be used to encourage abstinence and study retention (Petry et al., 2001; Petry et al., 2002). Participants who present to the clinic and provide a clean urine drug screen will receive chances to draw from a bowl containing 250 chips that are assigned a certain value (230 chips denote $1, 18 chips denote $10, one chip denotes $50, and one chip denotes $100). Participants will be allowed three draws on day one, five draws on day two, and seven draws on day three. We have used this procedure successfully in previous trials requiring similar periods of abstinence in cocaine-dependent outpatients (i.e., presenting to the initial laboratory session with a negative UDS and maintaining abstinence for three days during study procedures), and have had a completion rate of approximately 80%. Participants will also be compensated $20 for completing each follow-up visit (two visits total). The fishbowl method will also be used to encourage attendance at follow-up visits; participants will be allowed nine draws at the first follow-up appointment and 13 draws at the final visit. Participants may be paid in gift cards, cash, or money orders. To facilitate female participation, financial assistance for childcare may be provided if necessary.

C.2.c.5. Data Management. The REDCap Study Database Version 4, supported by the MUSC CTSA, 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. fMRI data are automatically transferred to servers managed by CAIR. Task data will be stored on CAIR computers which receive daily data backups.

C.2.d. Statistical Methods. The hypotheses associated with Aim 1 are interested in the effect of oxytocin on cocaine craving and stress in response to the TSST. It is hypothesized that cocaine-dependent participants receiving oxytocin will demonstrate greater attenuation of stress and craving response to TSST as compared to those receiving matched placebo. A linear mixed effects model containing all serially measured time points will be used to assess the effects of oxytocin 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. To test the hypothesis that women will experience greater ameliorative effects of oxytocin on stress and craving response following the TSST as compared to males, similar models will examine the differential (interaction) in the scores reported between men and women by treatment group (oxytocin vs. placebo). Additionally, as an exploratory analysis, the strength of the effects of oxytocin in women will be assessed with differences in estrogen and menstrual cycle phase as independent variables. In a subgroup analysis of women entered into the study, linear mixed effects models will be developed to assess the association of estrogen and menstrual phase (independently) on the stress and craving response to the TSST. Non-linear associations will also be explored using cubic spline models (Marrie et al., 2009). Within each family of dependent measures, the Type I error rate will be controlled using a post-hoc Bonferroni correction.

The hypotheses associated with Aim 2 are concerned with the impact of oxytocin on cocaine cue reactivity. It is hypothesized that oxytocin will decrease cue-induced cocaine craving in cocaine-dependent men and women compared with placebo administration. Post-acquisition preprocessing and statistical analysis will be performed using Matlab 7.8 (Mathworks, Sherborn, MA0 with Statistical Parametric Mapping software 8

(SPM 8, The Wellcome Department of Cognitive Neurology, London). All volumes within a task run will be realigned to the first volume. After realignment (including adjustment for sampling errors), scans with greater than one mm in three axes or one degree in three orientations will be excluded from group analyses. Following realignment, images will be stereotactically normalized into a standard space with a resolution of 2x2x2 mm voxels using the Montreal Neurological Institute (MNI) EPI template in SPM8. Subsequently, the data will be smoothed with a 8x8x8 mm Gaussian kernel and will be high-pass filtered (cue exposure paradigm cut-off period = 288s). Following preprocessing, within-task data from individual participants will be analyzed with a fixed-effects general linear model (GLM), with cocaine cue exposure activity modeled as a box-car function convolved with the standard canonical hemodynamic response function. 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. First-level analysis will be conducted and contrast maps will be generated. For the cue exposure paradigm, the main contrast of interest is the cocaine pictures vs. neutral objects contrast. Following first-level analysis, subject-specific contrasts will be entered into second-level, random-effects analyses. Areas of brain associated with group (oxytocin vs. placebo) and sex (women vs. men) differences on these contrasts will be identified using independent samples t-tests within a GLM framework. The combined group parameter maps will be threshold using a significance level of $p<0.05$, corrected for multiple comparisons.

For Exploratory Hypothesis 2d, a graph-theory approach to functional connectivity will be used. The BCT (Rubinov & Sporns, 2011) includes metrics specifically designed for functional brain networks, which are fully connected with weighted and signed connections. The metrics combine both positive and negative weights, but weight the positive connections more heavily. Within each of 116 anatomical regions of the AAL atlas, the average intensity value at each time point will be extracted for each subject. The time series (shifted by one TR) will be segmented into four separate time series, one for each condition (cocaine images, blur, objects, rest) (Fair et al., 2007; Just et al., 2004). Correlation matrices will then be computed for each subject and each condition and submitted to the BCT. For each subject and each condition, several BCT metrics will be computed (see Rubinov & Sporns, 2011 for more details). Network measures include *modularity* and *number of modules*. Node-specific measures include *diversity coefficient*, *within-module weights*, *within-module clustering coefficient*, and *strength.* We will also compute 20 random networks for each subject that preserve the weight and strength properties of the cocaine network (following Rubinov & Sporns, 2011), since that is the network of most interest. The same metrics that are computed for the cocaine, object, blur, and rest networks will be computed for the 20 random networks, then averaged to provide a single metric for the random network. In addition, a group-average correlation matrix will be computed (Pearson r-values from individual participants are Fisher z-transformed prior to averaging) for each of the four conditions and subject groups to obtain a modularity partition for the group, which will be visualized using Gephi software (Bastian, Heymann, & Jacomy, 2009), as in Figure 4. Network and node-based metrics will be submitted to mixed ANOVAs (one ANOVA for each metric and each node in the case of node-based metrics) with gender and treatment as between-subjects variables and condition (cocaine, object, blur, rest, random) as the repeated factor. Most metrics allow for comparison with the random networks except for strength and weight metrics because those are preserved in random networks and will be identical to the cocaine condition.

C.2.e. Power and Sample Size. *Aim 1.* It is hypothesized that oxytocin will decrease ratings of subjective stress and cocaine craving following the TSST relative to placebo. Pilot data suggests an expected between group mean differences in total cocaine craving (CCQ) following the TSST of $\Delta=8.24$ with a RMSE=11.03 (d=0.75) (Cohen, 1988) and a stress rating (VAS) difference of $\Delta=1.07$ with a RMSE=2.20 (d=0.49). As in the oxytocin pilot study, participants will be assessed at four post TSST time points (m), with an anticipated intraclass correlation coefficient of 0.78 for both craving and stress outcomes (ICC=0.78 & 0.79, respectively). The design effect, which accounts for the multiple correlated observation within each subject, is estimated to be 3.35 (DE= [1+(4-1)0.78]) (Donner and Klar, 2000). Assuming independent observations and an effect similar to the pilot data for the stress response (d=0.48), the total effective sample size would be 140 subjects (35 men and 35 women 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=118 (=140*3.35/4), which will be rounded to 120 to provide balance with respect to gender. Given an expected retention rate of approximately 80%, we will recruit 152 participants to obtain a final sample size of 120. This sample size will provide adequate power (80%) to detect the planned stress response, and more than adequate (98%) to detect the planned craving response to the TSST. In addition, the sample size of 120 participants will also provide 80% power to detect a 1 standard deviation differential in treatment effect between cocaine-dependent men and women for the craving response to the TSST. *Aim 2.* Existing fMRI studies involving cocaine cue exposure have included between 6 (Maas et al., 1998) and 17 (Garavan et al., 2000) participants per group, and have reliably found activation in brain regions associated with cocaine craving (e.g., anterior cingulate, amygdala). However, Mumford & Nichols (2008) simulation study suggested that 23 participants per group may be necessary to obtain 80% power for a generic fMRI task design featuring 10, 15-second task block and 10, 15-second rest blocks. As such, our proposed 60 completer participants per group should provide adequate statistical power.

c.2.f. Design Considerations. *Choice of experimental groups*. Inclusion of control participants was considered; however, craving is a main outcome measure of the proposed study, and it would not be expected that control participants would have a craving response to either the TSST or cocaine cues. Therefore, it was agreed that resources in this project would be best spent on including sufficient numbers of participants with cocaine dependence, as a demonstrated reduction in craving with oxytocin in this population may have important clinical implications. *Potential modifiers of cerebral blood flow.* Sex differences in hematocrit levels and baseline cerebral blood flow may impact the BOLD signal (Kastrup et al., 1997; Levin, et al., 2001). However, the present group comparisons will be based on changes in activation relative to control conditions (e.g., cocaine versus object activation differences for oxytocin versus placebo); therefore, even if baseline levels of blood flow are different across groups, the present analyses rely on fractional changes in fMRI signal relative to control and baseline conditions. Abstinence from cocaine has been shown to alter regional cerebral blood flow (Kosten et al., 1998; Volkow et al., 1988). If any statistical group differences are found, abstinence (days since last use) will be included as a covariate in the analysis.

c.2.g. Operational Plan and Research Timetable. The first three months of the grant period will be used to obtain regulatory approvals and database creation. Fortunately, because we have ongoing trials involving cocaine-dependent populations, we have trained personnel and an active recruitment network. We also have an active IND for use of oxytocin in cocaine-dependent individuals. We anticipate no issues with study recruitment beginning by the fourth month of the first year, and for a total of 51 months. At a recruitment rate of approximately three participants per month, we should have no difficulty in completing the study in this timeframe. Data analysis and manuscript preparation will take place during the last six months of the fifth year.

c.2.h. Use of Core Unit Services. This project will utilize multiple services coordinated through the Administrative Core (AC), such as biostatistical support (database creation, regular data audits, and statistical analyses), physician assistant services, and financial management. The use of shared personnel to perform these services across research components allows for significant overall cost-savings.

PROTECTION OF HUMAN SUBJECTS

1. RISKS TO THE SUBJECTS

a. Human Subjects Involvement and Characteristics

Admission into the study is open to men and women and to all racial and ethnic groups, age 18-65. 152 participants (76 cocaine-dependent women and 76 cocaine dependent men) will be recruited primarily through internet and newspaper advertisements. Inclusion/exclusion criteria that apply to all subjects are listed below:

1) General Inclusion / Exclusion Criteria

Inclusion Criteria

1. Subjects must be able to provide informed consent and function at an intellectual level sufficient to allow accurate completion of all assessment instruments.
2. Subjects must meet DSM-IV criteria for current cocaine dependence (within the past three months). While individuals may also meet criteria for abuse of other substances, they must not meet criteria for dependence on any other substance (except nicotine) within the last 60 days. Alcohol has been known to affect HPA function (Adinoff et al., 1991), however to enhance recruitment efforts individuals with alcohol dependence or abuse will be included in the study if they do not require medically supervised detoxification. Also, due to the high comorbidity of cocaine and marijuana dependence, and limited evidence that marijuana use affects HPA function, subjects with marijuana dependence will be included.
3. Subjects must consent to remain abstinent from all drugs of abuse (except nicotine) for a three-day period immediately prior to the throughout study procedures.
4. Subjects must consent to random assignment.
5. Subjects must consent to participating in study procedures at the ASD and completion of two fMRI scans.

Exclusion Criteria

1. Women who are pregnant, nursing or of childbearing potential and not practicing an effective means of birth control (not including hormonal contraceptives).
2. Women who are currently taking, or have taken in the past month, oral or other types of hormonal contraceptives or hormone replacement therapies.
3. Women with premenstrual dysphoric disorder who are outside of the follicular phase.
4. Women who have had a complete hysterectomy or are over 50 over one year post-menopausal, as ovarian hormones will be measured in the study.
5. Subjects with evidence of or a history of significant hematological, endocrine, cardiovascular, pulmonary, renal, gastrointestinal, or neurological disease including diabetes, as these conditions may affect physiological/subjective responses. Neurological exclusions include history of stroke, seizure disorders, multiple sclerosis, Parkinson's disease, and Alzheimer's disease.
6. Subjects with Addison's disease, Cushing's disease or other diseases of the adrenal cortex likely to affect hormonal/neuroendocrine status.
7. Subjects with a history of or current psychotic disorder or bipolar affective disorder as these may interfere with subjective measurements.
8. Subjects with current major depressive disorder or post-traumatic stress disorder as these disorders are associated with characteristic changes in stress response.
9. Subjects receiving synthetic glucocorticoid therapy, any exogenous steroid therapy, or treatment with other agents that interfere with hormonal measurements within one month of test session.
10. Subjects taking any mood stabilizers, antipsychotics, benzodiazepines, opiates or opiate antagonists because these may affect test response. Subjects taking SSRI's will be included.
11. Subjects with any acute illness or fever. Individuals who otherwise meet study criteria will be rescheduled for evaluation for participation.
12. Subjects whose height to weight ratio would preclude them from fitting comfortably in the MRI scanner.

13. Subjects who are unwilling or unable to maintain abstinence from alcohol and other drugs of abuse (except nicotine) for three days prior to the stress task procedure.
14. Persons with ferrous metal implants or pacemaker since fMRI will be used.
15. Subjects who are claustrophobic.
16. Subjects with significant psychiatric or medical problems that would impair participation or limit ability to participate in scan.
17. Subjects who require maintenance or acute treatment with any psychoactive medication including anti-seizure medications which could potentially interfere with fMRI.
18. Subjects meeting DSM-IV criteria for substance dependence (other than nicotine, cocaine, alcohol or marijuana) within the past 60 days.

b. Sources of Materials

Research material obtained from individual subjects includes questionnaires and interviews with study personnel, saliva and urine samples, as well as structural and functional MRI scans. To ensure confidentiality, all subject data will be letter/number coded, and only the investigators will have access to the master lists of codes. The research material will be obtained specifically for research purposes. Written research material obtained will be stored in the Addiction Sciences Division, in an office that is locked when not in use. Saliva and urine samples will be stored in the Research Nexus and the Clinical Neurobiology Laboratory. Neuroimaging data will be stored on a secure password protected server maintained by MUSC's Center for Advanced Imaging Research.

c. Potential Risks

Adverse effects associated with systemic oxytocin use in pregnancy may include seizures, mental disturbances, unexpected bleeding or contraction of the uterus. However, several studies have been conducted in humans with intranasal doses between 20 and 60 IU, and no side effects have been reported (Bruins, Hijman, & Van Ree, 1992; Fehm-Wolfsdorf, Bachholz, Born, Voigt, & Fehm, 1988; Pitman, Orr, & Lasko, 1993). A recent 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. Exposure to cocaine and negative emotional cues may produce some craving for cocaine or other discomfort. However, this discomfort is usually brief and subjects will be in the cocaine-free safety of a clinic environment. Since the scanner requires subjects to be motionless in an enclosed environment, there is the possibility of a claustrophobic reaction or anxiety or discomfort secondary to being stationary for 45-60 minutes per scan. Ferrous objects in the body that are undetected could move during scans. This could lead to tissue damage and hemorrhage. For patients who consent to genetic testing, there is the possibility of complications from venipuncture such as mild pain and possible bruising at the needle site.

2. ADEQUACY OF PROTECTION AGAINSTS RISKS

a. Recruitment and Informed Consent

Patients will primarily be recruited through the use of advertisements (internet, newspaper). The study PI, a Co-I, or other qualified study staff will obtain informed consent. The informed consent form includes a detailed description of the study procedures, along with statements regarding participants' rights to withdraw from the procedure at any time without consequences. The informed consent form will be explained to subjects in easy-to-understand language, and subjects will be instructed to read the form carefully prior to signing it. 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.

b. Protections Against Risks

All study participants will be closely monitored for psychiatric and medical stability. Patients in acute distress, including those endorsing suicidal ideation, will be seen by one of the psychiatrists in our division prior to being discharged. If the psychiatrist determines that the subject is in immediate danger, arrangements will be made for inpatient hospitalization at the Center for Drug and Alcohol Programs. Subjects who do not pose an immediate threat to self or others will be referred for services at the Center for Drug and Alcohol Programs, or a community mental health department if appropriate. The instrumentation used for physiological recordings meets all safety standards for non-invasive recordings, and participants are located out of reach of any AC-powered devices in the laboratory. All sessions will be conducted under the supervision of experienced personnel. If crisis intervention is necessary, senior staff will be available to evaluate the subject and provide an intervention or referral. If hospitalization is indicated, the patient will be hospitalized through the Center for Drug and Alcohol Programs at MUSC or an appropriate referral will be made. All subjects will be fully informed that they may withdraw from the experiment at any time without penalty. All subject records will be kept in a locked filing cabinet, and confidentiality of all materials will be maintained. Offices also will be locked at times when not in use. Neuroimaging data are coded and maintained on a password protected server.

To ensure confidentiality, all subject data will be coded by letters and/or numbers, and only the investigators will have access to the master lists of codes. All patient records will be kept in an office that will be locked at times when not in use. The research staff understands the importance of maintaining confidentiality. This method of maintaining confidentiality has been used for several years by our research group and has been effective. All co-investigators and study personnel have completed (or will complete upon hiring) training in Good Research Practices as mandated by the MUSC IRB.

Subjects will be taught about potential side effects of oxytocin, and will be closely followed by psychiatrists, a PharmD, and other members of the research team. Pregnancy tests will be performed on the day of oxytocin administration. Oxytocin administration will occur in a fully staffed clinical environment with emergency medications (i.e., IM diphenhydramine, alprazolam) and equipment available as needed. Adverse events will be monitored throughout the study. If abnormalities in the brain images are found, the subjects will be immediately referred to an appropriate clinical care provider. A careful metal screening history will be taken from each subject to assess the possibility of metal devices and individuals will be screened with a metal detector for the possibility of implanted metal objects since magnetic movement of a metal device or metal object could well result in injury or risk to life. 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.

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

Possible risks to study participants include adverse reactions to oxytocin administration. Benefits include detailed assessment of substance use and referral for treatment. 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. An investigation of oxytocin's effects on stress- and cue-induced craving may provide important information that can guide treatment for future patients with cocaine dependence.

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 drug dependence. The minimal risks of the investigation are considered reasonable in relation to the expected knowledge to be gained.

5. DATA AND SAFETY MONITORING PLAN

This section is based on the recommendations in NIDA's "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.

This application proposes to investigate the effects of oxytocin on stress and cue induced reactivity in cocaine-dependent individuals, and whether gender differences in response exist. The primary outcome of interest is craving and stress response to the interventions. Inclusion/exclusion criteria are outlined above. Power calculations and sample sizes are in the Data Analysis Plan section above.

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. The data analysis plan is outlined in the Data Analysis Plan section.

5.4 Quality Assurance.

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

5.5 Regulatory Issues.

We currently have an active IND from the Food and Drug Administration for the use of oxytocin in cocaine-dependent individuals (IND 109,726). Potential conflicts of interest will be reported using the upcoming NIH rules for disclosure. Adverse Events (AEs)/Serious Adverse Events (SAEs) occurring during the course of the project will be collected, documented, and reported in accordance with protocol and IRB reporting requirements. All research staff involved with adverse event reporting will receive general and protocol specific AE/SAE training including identification, assessment and evaluation, and documentation and reporting. A research assistant will identify any potential adverse events during the course of the study from participant self-report and administration of the visit assessments and procedures. The research assistant will provide information to a study physician, who will be responsible for AE/SAE assessment and evaluation including a determination of seriousness and study relatedness. Any significant actions taken by the local IRB and protocol changes will be relayed to NIDA.

5.6 Definition of AE and SAE.

An Adverse Event (AE) is defined as any untoward medical occurrence in a study subject administered a pharmaceutical product that does not necessarily have a causal relationship with this treatment (ICH GCP). Any unwanted change, physically, psychologically or behaviorally, that occurs in a study participant during the course of the trial is an adverse event. 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 OR

- Requires intervention to prevent one of the above outcomes.

5.7 Documentation and Reporting.
AEs/SAEs are documented and reported as per protocol and IRB requirements. Research staff will identify adverse events 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 are generally documented on AE Logs and AE Case Report Forms (CRFs). Additional relevant AE information if available should be documented in a progress note in the research record as appropriate to allow monitoring and evaluating of the AE. If the AE meets the definition for serious, appropriate SAE protocol specific reporting forms are 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 Institutional Review Board (IRB) within 24 hours and complete the AE report form in conjunction with the PI. The MUSC IRB meets monthly and is located at 165 Cannon Street, Rm. 501, Charleston, SC 29425. Communication with the IRB is through email, memos, official IRB forms, and online reporting. A report will also be sent to the NIH program officer assigned to the project.

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 for forwarding 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 NIDA Medical Safety Officer within two weeks of the initial SAE report.

We will report adverse events to the Medical University of South Carolina (MUSC) Institutional Review Board (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.

5.8 Trial Safety.
The potential risks and benefits and methods to minimize these risks are outlined above. The research staff will report any unexpected AEs or any scores of “severe” on the side-effect symptom rating form or any FDA-defined serious AEs to the PI within 24 hrs so that the PI can decide on the appropriate action. All unexpected AEs will be monitored while they are active to determine if treatment is needed. 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 Drs. Brady or McRae-Clark. All requests by participant’s physicians and other medical providers will be referred directly to Dr. Brady.

5.9 Trial Efficacy.

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

5.10 DSM Plan Administration.
Drs. Brady and McRae-Clark will be responsible for monitoring the study. They will examine (monthly) the outcomes database and CAIR server for missing data, unexpected distributions or responses, and outliers. A DSM report will be filed with the IRB and NIDA on a yearly basis, unless greater than expected problems occur. The report will include participant characteristics, retention and disposition of study participants, quality assurance issues and reports of AEs, significant/unexpected AEs and serious AEs. We will report outcomes at the end of the trial.

5.11 DSM Board.
If directed by NIDA program staff, we will create a Data Safety and Monitoring Board to monitor both the rate and severity of adverse events. This panel will include 3 clinicians with expertise in substance dependence and a statistician.

5.12 Risk Benefit Ratio.
The assessments and questionnaires are non-invasive and have inherently minimal risks. Potential risks of concern are loss of confidentiality, and adverse events to oxytocin. The potential risks of fMRI are minimal and include claustrophobia. As discussed above, our research team will attempt to minimize these risks. Knowledge gained by the proposed study would help fill an important void in research on gender differences in 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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