FACTORS THAT AFFECT SOCIAL HIERARCHY AND THE INFLUENCE OF

SOCIAL RANK AND MENSTRUAL CYCLE ON COCAINE SELF-

ADMINISTRATION IN FEMALE CYNOMOLGUS MONKEYS

BY

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TABLE OF CONTENTS

LIST OF ABBREVIATONS

5-CSRT	Five-choice serial reaction time
5-HT	Serotonin
5-HIAA	5-hydroxyindoleacetic acid
$[^{11}C]$DASB	$[^{11}C]$-3-amino-4-(2-dimethylaminomethylphenylthio)benzonitrile
ACC	Anterior cingulate cortex
ACTH	Adrenocorticotropic hormone
ACUC	Animal Care and Use Committee
ADHD	Attention deficit and hyperactivity disorder
AMPH	Amphetamine
ANOVA	Analysis of variance
BP	Break point
Cd	Caudate nucleus
CNS	Central nervous system
CRH	Corticotropin-releasing hormone
CSF	Cerebrospinal fluid
DA	Dopamine
DAT	Dopamine transporter
DEX	Dexamethasone
DVR	Distribution volume ratio
DOPAC	Dihydroxyphenylacetic acid
DRL	Differential reinforcement of low-rates
ES	Estradiol
FDA	Food and drug administration
FI	Fixed-interval
FP	Follicular phase
FR	Fixed-ratio
GR	Glucocorticoid receptor
Hip	Hippocampus
His	High saccharin intake
HPA	Hypothalamus-pituitary-adrenal axis
HR	High responders
HVA	Homovanillic acid
Los	Low saccharin intake
LP	Luteal phase
LR	Low responders
MAD	Mean adjusted delay
MRI	Magnetic resonance imaging
NE	Norepinephrine
NET	Norepinephrine transporter
NHP	Nonhuman primate
NIDA	National Institute on Drug Abuse
NOR	Novel object reactivity
OVX	Ovariectomized

PET	Positron emission tomography
PFC	Prefrontal cortex
PRP	Platelet-rich plasma
Pt	Putamen
ROI	Region of interest
PG	Progesterone
PR	Progressive-ratio
SAMHSA	Substance Abuse and Mental Health Services Administration
SEM	Standard error of the mean
SERT	Serotonin transporter
Tha	Thalamus
WBS	Whole blood serotonin

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Riddick, Natallia V.

FACTORS THAT AFFECT SOCIAL HIERARCHY AND THE INFLUENCE OF
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Dissertation under the direction of
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Accumulating evidence from both preclinical and clinical studies suggests that women may have a greater biological vulnerability to cocaine addiction than males. Studying behavioral, neurochemical, neurobiological or physiological trait and state variables may provide some insight into individual difference leading to high risk for drug abuse. The research described in this dissertation was designed to examine a number of trait and state markers associated with social hierarchy in female nonhuman primates. In addition, the effect of menstrual cycle phase and social rank was examined on acquisition and maintenance of cocaine reinforcement.

Chapter II was aimed at examining physiological, neurochemical, neuropharmacological and behavioral variables that may be associated with social rank. The two measures that correlated with social rank were cerebrospinal fluid concentrations of the 5-HT metabolite 5-HIAA, which were significantly higher in the animals that eventually became subordinate and latency to touch a novel object, which was significantly lower in eventual subordinate monkeys. Age, body weight, locomotor activity in a novel environment, HPA-axis function and peripheral serotonin (5-HT) activity, as measured in blood and central 5-HT activity, as measured with brain imaging,

did not predict social rank and did not change after stable social group formations. These data suggest that levels of central 5-HIAA and measures of novel object reactivity may be trait markers that influence eventual social rank in female macaques.

The studies in Chapter III examined the influence of social rank and menstrual cycle phase on acquisition and maintenance of cocaine self-administration in socially housed female monkeys. Dominant monkeys appeared more sensitive to the reinforcing effects of cocaine, with over 60% acquiring self-administration at the lowest dose (0.001 mg/kg) compared to ~ 20% subordinate monkeys. At higher cocaine doses, there were no differences in rates of acquisition. In maintenance, the peak of the cocaine dose-response curve for dominant monkeys was to the left of those for subordinate monkeys. Finally, menstrual cycle phase did not affect cocaine sensitivity in dominant or subordinate monkeys. These studies are at odds with earlier work in male monkeys and suggest that dominant female monkeys are more vulnerable to cocaine reinforcement than subordinate monkeys. These findings highlight the importance of sex differences in animal models of cocaine abuse.

In conclusion, the research presented in this dissertation further extends our understanding of the neurobiological and behavioral underpinnings of social rank and cocaine reinforcement and extends the work to female monkeys. The findings suggest a gender-specific "vulnerable" phenotype for cocaine reinforcement. Further studies of social rank-related differences in neurobiology and other individual traits are warranted to better understand vulnerability to drug abuse in females which could lead to identification of potential new targets for pharmacological and behavioral therapies of cocaine abuse.

CHAPTER I

INTRODUCTION

I. Drug use epidemiology
    A. Gender specific
    B. Detrimental consequences

II. Cocaine actions
    A. Dopamine component
    B. Serotonin component

III. Animal Models
    A. Subjects
    B. Nonhuman Primates living in social groups
    C. Drug self administration
        D. 1. Schedules of reinforcement
        D. 2. Fixed ratio schedule of reinforcement

IV. Interactions between trait and state variables and cocaine abuse
    A. Predisposing factors to drug abuse
        A. 1. Temperament
            A. 1. a. Behavioral phenotypes
            A. 1. b. Impulsivity and aggression
        B. 1. Dopamine and serotonin system impairment
        C. 1. Environment
            C. 1. a. Social stress

 V. Female sensitivity to cocaine
    A. Reinforcing effects
    B. Subjective effects

VI. Gonadal hormones and behavioral effects of stimulants in females
    A. Menstrual cycle phase effect in humans
        A. 1. Subjective effects
        A. 2. Peak plasma levels
    B. Menstrual cycle/estrus phase effect in animals
        B. 1. Two stages of drug dependence
            B. 1. a. Acquisition
            B. 1. b. Maintenance
    C. Exogenous ovarian hormones treatments in ovariectomized animals
        C. 1. Estrogen
        C. 2. Progesterone

I. Drug Use Epidemiology: Drug abuse has become a major societal problem during the 20th century, costing billions of dollars in economic expenses that include health care, accidents, crime related to substance use and loss of productivity (WHO, 2004). More than half a million emergency department visits in 1999 were due to substance abuse and overall health care related expenses neared $18.9 billion (SAMHSA, 1999). Worldwide loss of productivity is estimated to be nearly $134.2 billion (Miller and Brady, 2004). The trend continues into the early 21st century with the most abused illicit drugs being marijuana, followed by cocaine and pain relievers. In 2002, an estimated 19.1 million Americans age 12 years or older were current illicit drug users, with an estimated 2.0 million persons abusing cocaine (SAMHSA, 2002). By 2006 the number grew to 20.4 million using illicit drugs with 2.4 million specifically abusing cocaine (SAMHSA, 2006). The focus of the research described in this dissertation involves cocaine abuse. At present, there are no FDA-approved medications for cocaine addiction. As a result, NIDA has made the development of novel animal models a primary focus for understanding the neurobiology associated with cocaine addiction in order to develop new treatment agents.

A. Gender Specific Epidemiology: Risk factors for cocaine abuse include age, sex, ethnicity, culture, and environment (Beauvais et al., 1996; Moon et al., 1999). Most of these factors will be discussed in detail later in the chapter. An estimated 6.2% of women and 10.5% of men in the US are current illicit drug abusers (SAMHSA, 2006). For example, the rates of marijuana use for males are about twice as high as the rate for females (8.1 and 4.1%). However, males and females had similar rates of stimulants use (0.5%). Furthermore, women are more likely to be admitted to treatment centers because of opiate or stimulant dependence than males (SAMHSA, 2006). Also, at the time of

their arrest, 17% of women compared to 11% of males reported cocaine as their primary drug of choice (DASIS Report, 2005), and women in the criminal justice system are more likely to report cocaine as their primary drug of use then men who report marijuana as their problem substance (Haas and Peters, 2000). Although as a population cocaine use is less prevalent in women than men, it appears to cause severe health and psychological consequences when abused (Najavits and Lester, 2008). As will be highlighted below, treatment strategies developed for men may not be effective for women. Factors related to social variables, environmental context, and hormones may differentially impact treatment outcome depending on gender.

B. Detrimental consequences of drug abuse in women: The focus of the research in this dissertation will be on female subjects. It is important to fully understand sex differences in drug dependence which requires, therefore, the use of females in basic research. Overall, compared to men, women are thought to have heightened vulnerability to detrimental consequences of substance abuse such as medical, social, physical, and psychological (Greenfield et al., 2003). Women have increased sensitivity to drug cues and greater cocaine intake during binges (Kilts et al., 2004). Despite these factors, women are under-represented in addiction treatments and research primarily due to social stigma associated with drug abusing women (Greenfield et al., 2003). Psychiatric comorbidities are also far more common in women with substance dependence disorder. Women entering drug treatment programs are more likely to be diagnosed with depression and anxiety disorders and their symptoms do not improve as fast as most men following abstinence (Najavits and Lester, 2008; Zilberman et al., 2003a,b).

Two of the more comprehensive studies completed in 1990s, National Comorbidity Survey (NCS) and Epidemiologic Catchment Area Program (ECAP), interviewed approximately 20,000 people of different ages and explored the issue of psychiatric comorbidities in both sexes (Kessler et al., 1994; Regier et al., 1993). ECAP determined that approximately 45% of individuals with alcohol-related disorders and 72% with other drug-related disorders exhibited at least one comorbid psychiatric problem. NCS study confirmed the ECAP findings and extended it to observe that women had elevated rates of affective disorders and anxiety and drug dependence comorbidities were more common that in males.

The most common psychiatric disorders associated with substance abuse are mood and anxiety disorders, ADHD, and antisocial personality disorder. Consistent with previous research, NCS and ECAP also found that women drug abusers tended to have elevated rates of affective and anxiety disorders whereas men tended to have elevated rates of antisocial personality disorders. As a consequence of women being more vulnerable to the psychiatric disease and substance abuse co-occurrences, negative impact is observed on the course of treatment outcome and prognosis for both disorders primarily due to the difficulty of treating them at the same time.

In addition to causing a host of psychiatric issues, cocaine has been implicated in a number of physical disorders such as reproductive dysfunctions, amenorrhea, and possibly infertility (Cregler and Mark, 1986a,b; Mello et al., 1997, 2000, 2004; Potter et al., 1998). Cocaine use during pregnancy is associated with poor outcomes such as premature labor, placental abruption, uterine rupture, cardiac dysrhythmias, hepatic rupture, cerebral ischemia/infarction, and death (Buehler, 1995; Dombrowski et al., 1991;

Iriye et al., 1994; Kuczkowski, 2003; Lampley et al., 1996; Mishra et al., 1995; Moen et al., 1993).

Currently in the United States, cocaine abuse is more prevalent in adult males compared to females possibly because males have more opportunities to try drugs (Van Etten and Anthony, 2001). In women, less opportunity to use drugs may have contributed to initiating drug use at a later age; however, women progress to dependence faster than men, resulting in dependence occurring at approximately the same age in both sexes. This is also true for alcohol, heroin, and marijuana (Haas and Peters, 2000; Piazza et al., 1989a; White et al., 1996).

Consistent with the "gateway" drug theory (Kandel, 1975), both males and females report using marijuana and alcohol significantly earlier in their lives than cocaine. However, the rate of progression from "gateway" drugs to cocaine is faster in females (Haas and Peters, 2000). Women are also more likely to report shorter abstinence periods than men (Kosten et al., 1993; White et al., 1996). Overall, males and females differ in their progression from recreational use to heavy abuse, stay abstinent less and transition from "gateway" drugs to cocaine faster. The existence of this phenomenon has significant treatment implications because the window of opportunity for intervention between different stages of cocaine addiction is narrower in women than it is for men.

II. Mechanisms of action for cocaine: The neurobiology associated with cocaine reinforcement has been investigated extensively. Cocaine acts primarily at monoamine transporters by blocking reuptake of dopamine (DA), serotonin (5-HT), and

norepinephrine (NE) and increasing levels of each neurotransmitter (Heikkila et al., 1979; Hertting et al., 1961; Ritz et al., 1987). Most research has focused on DA, which is believed to be directly related to cocaine reinforcement. In contrast, 5-HT concentrations are believed to be inversely related to cocaine reinforcement (i.e., elevate 5-HT leads to decreases in cocaine reinforcement). The research described in this dissertation focus on 5-HT in female monkeys.

A. Dopamine: Although it affects several neurotransmitter systems, its behavioral and reinforcing effects (S^R) are thought to primarily be mediated by the DA system (Bergman et al., 1989; Di Chiara et al., 1988; Kuhar et al., 1991; Madras et al., 1989; Ritz et al., 1987; Roberts et al., 1977, 1980; Wise, 1984). DA neurons originate in the ventral tegmental area (VTA) and project to nucleus accumbens (mesolimbic pathway) and prefrontal cortical areas (mesocortical pathway) where DA receptors such as D1- and D2-like receptors are abundant and their stimulation is in part responsible for cocaine being one of the most reinforcing drugs of abuse (Volkow et al., 1999; Wise and Rompre, 1989). The subjective effects of cocaine, including euphoria and arousal, are mediated through the mesolimbic pathway that is strongly associated with natural and drug reward (Di Chiara et al., 1988; Wise, 1996; Wise and Rompre, 1989). There is much research investigating the use of DA-selective drugs as potential treatment agents for cocaine abuse (Bergman et al., 1990; Glowa and Wojnicki, 1996; Kimmel et al., 2008; Mello and Negus, 1996; Nader et al., 1999; Negus and Mello, 2003; Woolverton and Balster, 1981; Woolverton and Virus, 1989); however, none have been successful thus far. The difficulty with developing treatments around the DA system stem from the two extremes – DA antagonists can be aversive and compliance is poor and DA agonists have abuse

liability (for review see Lile, 2006) making them less than ideal treatment agents. The same limitations cannot be described for 5-HT treatments.

B. Serotonin: There is also a growing interest in the role of 5-HT in cocaine abuse due to the location of the 5-HT neuronal projections, its ability to modulate DA neuronal function within the three major DA pathways (i.e. mesocortical, mesolimbic, and nigrostriatal), and the ability of several illicit drugs such as cocaine and amphetamine to directly affect the 5-HT system by blocking 5-HT reuptake (reviewed by Alex and Pehek, 2007). 5-HT neurons originate in medial and dorsal raphe nuclei and project throughout the brain including midbrain in which they form direct synaptic contacts with DA somas (Herve et al., 1987; Nedergaard et al., 1988). Thus, 5-HT can regulate DA function via actions on the DA cells or terminals via its receptors (Di Matteo et al., 1999, 2000, 2002). There are at least fourteen known 5-HT receptor subtypes, many of which play a role in mediating 5-HT/DA system interactions (Barnes and Sharp, 1999). Many of the subtypes such as 5-HT1A, 5-HT1B, 5-HT2A, 5-HT3, and 5-HT4 receptors facilitate DA release, while 5-HT2C receptor inhibits tonic and evoked DA release (reviewed by Alex and Pehek, 2007). Therefore, drugs which directly act at serotonin receptors would have different effects on the DA system function based on location and density of the various 5-HT receptor subtypes. As mentioned above, some stimulants such as cocaine bind the 5-HT transporter (SERT) and block neurotransmitter reuptake which results in increases in synaptic 5-HT and consequently enhanced stimulation of the 5-HT postsynaptic receptors. In turn, receptor stimulation would then indirectly modulate the DA system functioning and drug effects associated with it. For example, a tryptophan-rich diet, which elevates 5-HT levels, reduces cocaine self-administration (Carroll et al., 1990). In

addition, 5-HT2C receptor stimulation attenuates cocaine self-administration and cocaine-induced reinstatement in rats by inhibiting central DA (Fletcher et al., 2002). Taken together, the findings suggest that 5-HT system is an important mediator of the DA system and is indirectly involved in modulation of reinforcing effects of drugs of abuse such as cocaine. For this dissertation, several measures of 5-HT function will be examined including peripheral (whole blood 5-HT) and central (metabolites in cerebrospinal fluid and availability of SERT in brain).

III. Animal model to study drug abuse: This dissertation work utilizes female laboratory animals and behavioral pharmacology techniques to study cocaine reinforcement. Hence, this section will describe some animal models used in the drug abuse field specifically highlighting the advantages of using nonhuman primates in women's health studies, followed by a brief description of behavioral techniques central to these studies.

A. Subjects: Although humans make the best model to study drug addiction, it is clearly impossible to control for all the potentially confounding variables such as lifestyle, environment, and diet among others. Therefore, appropriate animal models are utilized to allow investigators to exert experimental control over environmental variables, be able to manipulate single variables and use (if necessary) invasive techniques. Rat self-administration models are the most frequently used model for drug abuse research; however, there are significant biological differences between rodents and primates (Weerts et al., 2007).   Significant differences are found in the relevant receptor systems in the brain, reproductive system, and pharmacokinetic factors that are involved in drug abuse. Therefore, the use of nonhuman primates (NHP), especially macaque monkeys

such as rhesus (*Macaca mulatta*) and cynomolgus (*M. fascicularis*) offer considerable advantages over the rodent model due to neurobiological and neurochemical similarities to humans and long life span which allows for design of longitudinal, within-subject studies. For example, NHP show a greater than 95% gene homology to humans (Hacia et al., 1998) and greater homology in DA, 5-HT, and NE systems (Weerts et al., 2007) than rodents.

Menstrual cycle is a primary variable (independent and dependent) in this dissertation. Consequently, it is important to note that substantial reproductive differences exist among different mammalian species, making some more useful as models to study the effect of gonadal hormones on various disease states, including drug abuse. Human and NHP reproductive cycles are well characterized; however, at present time it is unclear how the 4-day rodent estrus cycle relates to the primate 28-day menstrual cycle. The menstrual cycle of macaques is associated with the same milieu of hormonal changes as observed in women (Appt, 2004; Goodman and Hodgen, 1983; Pohl and Knobil, 1982). The monthly cycle starts with the follicular phase (FP) at the onset of the menstrual period and lasts until ovulation. This phase (day 1-12 of the cycle) is characterized by low and later moderate estrogen (ES) levels and low progesterone (PG) concentrations. The FP is followed by ovulation and then the beginning of the luteal phase (LP; day 17-28 of the cycle) which is characterized by high PG and moderate ES levels. The LP lasts until the onset of the menses (Newman et al, 2006). In contrast, rodents' estrous cycle lasts 3-5 days and each phase only 6 to 57 hours (reviewed in Roth et al., 2004). In addition, human and NHP have the largest ES peak in the FP and a moderate peak in the LP. This means that ES can potentially have an effect on drug

reinforcement in both phases unlike in rodents where the ES peak occurs only in proestrus phase and lasts a very short time. Since menstrual cycle and hormonal variations that accompany it are a major part of this dissertation research, the use of the female cynomolgus monkeys that are so similar in overall hormonal profile and neurobiology to humans will facilitate the translational value of the studies.

B. Social behavior: NHP living in social groups have also been used to study psychiatric diseases, drug abuse, atherosclerosis and the neurobiology of behaviors such as aggression and impulsivity because of their dependence on social relationships and their ability to engage in complex behaviors (Kaplan et al., 1982; Morgan et al., 2002; Shively, 1998). Social rank (i.e. an individual's relative position within a dominance hierarchy or the establishment and maintenance of asymmetrical hierarchical relationships) and kinship are the most prominent organizing features of macaque societies (Kaplan, 1987). In the wild, a female's social rank is based on the rank of her mother. However, in laboratory conditions where the animals are typically not related, macaques form hierarchies based on the outcome of agonistic encounters (Kaplan et al., 1982; Kaplan, 1987). Thus, the study of macaques in captivity allows for the examination of variables that may impact future social rank.

Social rank has been shown to have significant effects on susceptibility and resistance to diseases, indicating a powerful influence of the environment (e.g., Abbott et al., 2003; Kaplan, 2004; Morgan et al., 2002; Sapolsky, 2005; Virgin and Sapolsky, 1997). The study of social rank-health relationship in NHPs has been frequently related to different levels of stress. Physical and psychosocial stressors can both engender feelings of lack of control in subordinate animals, and anticipation of a stressful event (Sapolsky,

1991). When received chronically, stress can increase risks for numerous diseases (Sapolsky, 2005). Dominance hierarchies produce marked inequalities in division of resources (e.g. food, space, access to sexual partners, etc.); thus, an animal's social rank can profoundly affect its quality of life and its health (Kaplan, 1987; Sapolsky, 2005). Among female cynomolgus monkeys, social subordinates received more aggression and are groomed less; subordinate monkeys have increased risk for coronary artery atherosclerosis, poor ovarian function, and adrenal hypersecretion of cortisol (Kaplan et al., 1987; Shively et al., 1986, 1997). In this dissertation, we will focus on a dominance structure of female long-tailed macaques (*Macaca fascicularis).* Their social rank is characterized by a linear hierarchy, where the division of resources is largely skewed towards the more dominant animal (Cawthon Lang, 2006) and social subordination is marked by the highest level of stress, including a high frequency of aggression and intimidation from the dominant monkeys (Kaplan, 1987; Sapolsky, 2005). Although physical aggression (e.g. slaps, grabs, bites) towards more subordinate individuals is present in female macaque's social behaviors, psychological intimidation and harassment (e.g. stares, mouthtreats, gestures) by the higher ranking animal are the more common methods to maintain dominance (Cawthon Lang, 2006; Kaplan, 1987; Sapolsky, 2005).

Studies assessing disease states as a function of social rank have typically examined the effects of "state" variables, that is, characteristics attributable to environmental circumstances. In order to more fully understand the role of the environment, including social rank, on disease states, other investigators have examined potential "trait" variables, that is, pre-existing characteristics that may underlie an individual's likelihood of occupying a high or low social rank. Such information is

critical in understanding the interactions between the individual and the social context. Furthermore, studying individual trait and state variables that predict social group standing or change following rank formation can help uncover neural mechanisms responsible for vulnerability to or protection from reinforcing effects of cocaine and other psychiatric disorders.

For example, behavioral and hormonal markers that predict eventual social rank in monkeys were examined by Morgan and colleagues (2000) in male cynomolgus monkeys. Monkeys' body weights, serum cortisol levels, testosterone levels, and locomotor activity in an open-field apparatus were measured before and after social housing. Body weights correlated significantly with eventual social rank, such that the heavier monkeys tended to occupy more dominant positions than lighter monkeys. Animals that responded with higher levels of locomotion in the open-field apparatus following an acute injection of cocaine (0.01 mg/kg) eventually became subordinate, suggesting another predictive phenotype of social rank. Cortisol and testosterone concentrations failed to correlate with eventual social rank. In a follow-up study from the same laboratory, it was discovered that subordinate monkeys found cocaine reinforcing whereas dominant animals did not, emphasizing the powerful effect of the environment on drug taking behavior (Morgan et al., 2002). While these findings are significant in male cynomolgus monkeys, the relationship between social rank and cocaine reinforcement has not been examined in female monkeys. Coe and colleagues (1978) reported that female squirrel monkeys have a lower stress response characterized by lower cortisol levels than male monkeys. In addition, several studies which will be discussed later in the chapter cite sex differences in drug abuse. Therefore, in this

dissertation, we will extend this work to female subjects and examine several neurobiological, neurochemical, and behavioral variables as predictors of social rank (Chapter II) and the association of these markers and social rank with vulnerability to cocaine abuse (Chapter III).

C. Drug self administration: The development of techniques allowing laboratory animals (i.e. rodents, monkeys, etc.) to self-administer drugs of abuse using a variety of routes has revolutionized the research on behavioral aspects of drug dependence (Griffiths et al., 1979, 1980; Katz, 1990a,b; Mello and Negus, 1996; Pickens, 1977; Schuster and Thompson, 1969; Woolverton and Nader, 1990). The techniques described in these studies are based on the principle of positive reinforcement which is defined as the ability of a stimulus (in this case drug or food) to increase the probability of a behavior (i.e. response) that immediately preceded its presentation (Skinner, 1938). Conversely, the presentation or removal of a stimulus contingent on a response that decreases the probability of that behavior is an example of punishment. The behavior controlled by its consequences is termed operant behavior and the controlling events are termed reinforcers and punishers (Schuster and Thompson, 1969). It has been demonstrated repeatedly that responding can be engendered and maintained above vehicle levels in laboratory animals under conditions in which intravenous (i.v.) injections of cocaine (Goldberg and Kelleher, 1976; Pickens and Thompson, 1968; Woods and Schuster, 1968) are contingent on responding. For the purposes of this dissertation, only schedules of reinforcement relevant to the conditions studied in Chapter III will be discussed in more detail.

Simple schedules of reinforcement are generally classified as ratio schedules (stimulus presentation occurs following completion of a preset number of responses) or interval schedules (stimulus presentation occurs following the first response after a set amount of time has elapsed). While fixed-ratio (FR) schedules generally produce high rates of responding interrupted by brief pauses at the beginning of each ratio (or following a reinforcer delivery), fixed-interval (FI) schedules are typically characterized by low rates of responding with response rates increasing towards the end of the interval and remaining high until reinforcer delivery creating a so-called scalloped response pattern (Woolverton and Nader, 1990).

FR (and FI) schedules are generally used to examine the potency of a given drug as a reinforcer and generate highly reproducible patterns of responding under different conditions (Katz, 1990a,b; Kelleher and Morse, 1968). Generally, under these schedules cocaine-maintained responding is characterized as an "inverted U-shaped" function of dose (Griffiths et al., 1980; Mello and Negus, 1996). The dose-response curve consists of an ascending limb (responding proportionally increased with dose), peak (dose that engenders the highest responding), and descending limb (responding proportionally decreased with dose increasing). Several investigators have suggested that the descending limb is due to aversive, satiating or behavioral disrupting effects of cocaine observed when higher doses are available (Griffiths et al., 1979; Mello and Negus, 1996; Zernig et al., 2004). In this dissertation, an FR schedule of cocaine self administration was used to compare reinforcing effects of cocaine in female cynomolgus monkeys of different social status (i.e. dominant and subordinate). In addition, cocaine dose-response curves were determined in both phases of the menstrual cycle to evaluate differences in the

reinforcing effects of cocaine as a function of the cycle as well as social rank (Chapter III).

IV. Interactions between trait and state variables and cocaine abuse: In order to fully understand the biological factors involved in drug abuse and specifically cocaine, a wide variety of other contributors need to be examined. Temperament differences, genetics, maternal behavior, environmental variables, physiology, and differences in brain neurobiology and neurochemistry have all been mentioned as possible predisposing factors to drug abuse (Kabbaj et al., 2004a,b; Kabbaj and Isgor, 2007). Of relevance for this dissertation are temperament, environmental variables (i.e., social variables), menstrual cycle, and brain neurobiology and neurochemistry.

    A. Predisposing Factors to Drug Abuse

    A.1.Temperament: Temperament is a variable (trait and/or state) with several defined characteristics, which has been described as influencing vulnerability to drug abuse. Higher responses to novelty, locomotor activity in response to a stimulant injection, hyperactive wheel running, and high saccharin preference have been shown in rodents to predict or correlate with increased sensitivity to S^R effects of drugs (Carroll et al., 2002; Mantsch et al., 2001; Piazza et al, 1989, 1990a, 2000; Pierre and Vezina, 1997; Rhodes et al., 2001; Suto et al., 2001). Piazza and colleagues (1989, 2000) were the first to demonstrate a relationship between locomotor response to a novel environment and propensity to self-administer psychostimulants. They categorized the behavioral phenotype of rats based on exploratory behavior in a novel environment as "high-responders" (HR) or "low responders" (LR). When given access to a low dose of

15

amphetamine or cocaine, HR rats acquired self-administration faster than did the LR rats indicating greater vulnerability to S^R effects of psychostimulants. This behavioral phenotype has since been extensively studied in several laboratories and more information has been garnered about the underlying reasons for the differences observed between HR and LR rats and their response to stimulants. Within the CNS, it has been shown that HR rats have lower DA D2 receptor densities in nucleus accumbens compared to LR animals (Hooks et al., 1994). These findings are consistent with both humans (Volkow et al., 1999) and animal (Dalley et al., 2007; Morgan et al., 2002; Nader et al., 2006; Nader and Czoty, 2005) studies showing low D2 receptor availability was associated with higher risk for stimulant abuse.

In addition to neurobiological changes in the DA system, HR rats appear to be less anxious as measured by light-dark box and elevated plus maze tests (Davis et al., 2008). Interestingly, LR rats had lower levels of plasma corticosterone but higher concentrations of corticotropin-releasing hormone (CRH) and higher densities of hippocampal glucocorticoid receptors (GR) following novel environment exposure or placement on the light side of the test box (Dellu et al., 1996; Kabbaj et al., 2000; Koob et al., 1993; Piazza et al., 1991). It is conceivable though that higher corticosterone levels in HR animals potentiating psychostimulant reinforcement and low levels of CRH and GR decrease fear- and anxiety-related behaviors allowing HR rats to engage into novelty-seeking (Liang et al., 1992). Indeed, HR rats were shown to self-administer corticosterone more readily that LR rats (Kabbaj, 2004b; Piazza et al., 1993). These neurological and behavioral characteristics in HR rats may be comparable to high thrill-seeking found in humans which is associated with increased risk for a variety of

16

psychiatric disorders, alcoholism, and drug addiction (Dellu et al., 1996; Zuckerman and Neeb, 1979). Therefore, in combination with decreased D2 receptor levels, emotional reactivity to fear-related events, and low anxiety phenotype make HR behavioral phenotype more vulnerable to S^R effects of psychostimulants.

In another study, Carroll and colleagues (2002) bred rats for high (HiS) and low (LoS) intake of sweet solution of saccharin. During the acquisition of cocaine self-administration in which cocaine was available under an FR schedule of reinforcement, HiS female rats acquired self-administration faster than LoS rats. Interestingly, when allowed to self-administer cocaine under a schedule that assessing reinforcing strength, progressive-ratio (PR) schedule of reinforcement, phenotype-based differences disappeared. This finding suggests that behavioral phenotype may not be uniformly associated with all aspects of drug reinforcement and that differences seen in acquisition may not necessarily persist in maintenance or with other measures of reinforcement. Furthermore, it is of interest to note that a group of rats allowed to acquire heroin self-administration did not show differences based on saccharin phenotype indicating a stimulant-specific effect (Carroll et al., 2002).

Much of the research with humans is in concordance with the animal findings. For example, there is evidence for correlative effects of taste sensitivity and sweet avidity (Hirsch, 1997; Kampov-Polevoy et al., 1995, 1997, 1999 (review); Morabia et al., 1989; Pelchat and Danowski, 1992; Willenbring et al., 1989), novelty-seeking (Zilberman et al., 2003a,b), but not activity (Alessi et al., 2000), and drug abuse in several clinical studies. Thus, it is essential to extend this work in order to determine the mechanisms governing drug-associated vulnerable phenotypes. Novelty-seeking, locomotor activity, basal

cortisol levels and stress responsiveness will be examined as trait and state variables in this dissertation (Chapter II). Furthermore, we will examine which variables are predictive of vulnerability to S^R effects of cocaine. Such information will allow us to better characterize females at high risk for cocaine abuse.

Other temperament characteristics that are frequently studied in humans are high impulsivity, psychoticism, negative affect, and neurosis; these characteristics also correlate with substance abuse (Dalley et al., 2007, 2008; Kabbaj et al., 2004b; Kilbey et al., 1992; Miczek et al., 2004; Moeller et al., 2001b, 2002; Perry et al., 2005). Some of these characteristics are more difficult to study in animals generally due to an inability to obtain self-reported data which is widely used in clinical studies and lack of uniformly accepted definitions and measuring paradigms (Evenden, 1999a; Moeller et al., 2001a; Patton et al., 1995).

Impulsivity is a good example of a personality trait that has received considerable attention lately in psychiatry and addiction fields. Due to multidimensional nature of impulsivity, there are a number of various laboratory measurements used to study different components of impulsive behavior (Evenden, 1999b; Moeller et al., 2001a; Stoffel and Cunningham, 2008).

Dalley and colleagues (2007) used a five-choice serial reaction time (5-CSRT) paradigm that measured sustained visual attention. These investigators established that 5-CSRT performance was measuring a form of impulsivity that was strongly associated with individual variation in rates of cocaine self-administration. That is, highly impulsive rats acquired cocaine self-administraiton more rapidly than non-impulsive animals (Dalley et al., 2007). Furthermore, they revealed that impulsive rats had lower D2/D3

receptor availability in ventral striatum as measured by positron emission tomography (PET) using [^{18}F]fallypride. Since extracellular DA metabolite levels, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), did not differ in nucleus accumbens of impulsive and non-impulsive animals, it was concluded that D2/D3 receptor numbers but not DA levels were responsible for differences observed in impulsive behavior and cocaine self-administration. It is important to note that impulsivity measure on the 5-CSRT task inversely correlated with locomotor performance in the novel environment indicating that this "impulsive" phenotype is not the same as the HR-LR stimulant-sensitive phenotype.

In another study, Perry and colleagues (2005) used female rats in which locomotor activity was assessed prior to the rats being trained to respond on a different behavioral task thought to measure impulsivity, delayed discounting. Under these conditions, a smaller immediate reward was contingent on the rat pressing one lever and a larger delayed reward was contingent on pressing another lever. The rats were then categorized as more impulsive (HiI) or less impulsive (LoI) based on their mean adjusted delay scores (MAD), which was an average of all adjusting delays on the free-choice trials. Following assessment of locomotor activity levels, rats were trained in an operant chamber to lever press under an FR 1 schedule of cocaine (0.2 mg/kg) presentation. Locomotor activity did not differ between HiI and LoI rats, indicating that these behavioral endpoints describe different aspects of "impulsivity". As was seen in the Dalley et al. (2007) study, there was no relationship between locomotor activity and vulnerability. However, Perry et al. (2005) found a greater percentage of the HiI group acquired cocaine self-administration and at a significantly faster rate than the LoI rats.

This study highlights the importance of obtaining multiple measures of impulsivity and provides further evidence that different behavioral endpoints are assessing different types of impulsivity.

Contrary to Perry et al. (2005), Stoffel and Cunningham (2008) proposed that locomotor response to novelty in rodents is closely related to their behavioral disinhibition as measured by rats responding under a differential reinforcement of low-rate (DRL) schedule of food presentation. Reinforcement under a DRL contingency is dependent on the passage of a minimum amount of time between responses; animals that respond too soon will not receive a reinforcer. Thus, they need to inhibit responding for a minimum period after a response is made. Unlike a previous study (Bardo et al., 2006), they examined different DRL intervals and found that HR rats displayed more behavioral disinhibition relative to LR rats on DRL 20- and 35-sec schedules by making more responses and receiving less reinforcers. Therefore, in this case locomotor response to novelty appeared to be a useful assay which was related to measures of behavioral disinhibition in animals. Furthermore, in order to learn how this aspect of impulsivity relates to drug dependence, locomotor measures can be followed with self-administration studies.

The latter three studies displayed the multifactorial aspect of impulsivity and indicate that no single assay can adequately measure impulsivity. Furthermore, different measures of impulsive behavior do not correlate similarly with vulnerability to drug dependence or with other measures of impulsivity. Therefore, examining multiple behaviors believed to measure different aspects of impulsive behavior, prior to cocaine self-administration studies should provide a more comprehensive assessment of the

relationship between the phenotype described as "impulsivity" and drug abuse. In this dissertation, we extended novelty-induced locomotor activity measurement to also include novel object reactivity measurements to assess impulsive behavior in female monkeys and determine if the measures correlated and if they were predictive of future social rank.

B.1. DA and 5-HT system Impairment: Three biological variables that are closely associated with social rank and impulsive behavior and are associated with predisposition to drug abuse include frontal lobe deficits and impaired 5-HT and DA systems (Belin et al., 2008; Dalley et al., 2008; Evenden, 1999a; Fairbanks et al., 2001). Briefly, deficits in oribitofrontal (OF), prefrontal (PF), and anterior cingulate (ACC) cortices are associated with behavioral disinhibition, impaired impulse control and compulsive drug use in humans and laboratory animals (Brown et al., 2006; Dalley et al., 2008; Evenden, 1999a; Fletcher, 1993; Volkow et al., 2000). There is evidence that frontal lobe patients may show increases in specific kind of impulsivity defined as risk taking (Miller, 1992). In addition, selective lesioning of the medial PFC in rats impaired novelty- and stimulant-induced locomotor performance as well as responding in extinction of conditioned fear and emotional learning which are simple measures of behavioral inhibition (Dalley et al., 1999; Morgan et al., 1993; Quirk et al., 2006). While not assessed as part of this dissertation, MRIs of each monkey were obtained prior to the start of the experiment and may reveal volumetric differences related to future social rank and/or vulnerability to cocaine reinforcement.

Impairment of frontal-striatal DA system in attention-deficit hyperactivity disorder (ADHD) patients revealed one of the mechanisms responsible for poor impulse

control (Solanto, 2002). For instance, increased DAT availability and abnormal DOPA decarboxylase activity within the PFC was reported in adult ADHD patients (Dougherty et al., 1999; Ernst et al., 1998). Also, treatment with amphetamines is often prescribed to ameliorate the symptoms of ADHD, thereby implicating dysregulation of the DA and possibly noradrenergic (NA) systems in this disease. In preclinical studies with rodents, damage to nucleus accumbens which is densely innervated with DA neuronal projections increased impulsive choice as measured by a delay discounting task (Cardinal et al., 2001). In addition, administration of *d*-amphetamines decreased impulsive choice in healthy human volunteers as well as laboratory animals (de Wit et al., 2000, 2002; Isles et al., 2003; Winstanley et al., 2003). DA D2/D3 receptors were also found to be downregulated and a D2/D3 receptor antagonist but not a D1 receptor antagonist decreased the value of the delayed reward (i.e. increased impulsive behavior) in rodents (Dalley et al., 2007; Wade et al., 2000). Taken together these findings indicate that increased DA concentrations and low levels of D2/D3 receptors but not D1 receptors maybe more important in influencing reward size preference and in turn impulsive behavior. A similar relationship between D2/D3 availability and psychostimulant reinforcement has been observed in human subjects (Volkow et al., 1999).

Converging lines of evidence suggest that also lowering the brain 5-HT system function is associated with increased impulsivity, impulsive aggression and in turn increase risk for drug and alcohol abuse (Brown et al., 1979; Fairbanks et al., 2001; Fletcher, 1993; Higley et al., 1992, 1996a,b; Linnoila et al., 1983, 1994; Linnoila and Virkkunen, 1992). There is a wide variety of literature in ADHD field suggesting a strong involvement of the 5-HT system in impulse control and aggressive behavior in

humans. Low levels of 5-HIAA were observed in the CSF of children and adolescents with various behavioral disorders, including ADHD (Winstanley et al., 2006). Furthermore, trends towards correlation between low blood 5-HT and the severity of ADHD symptoms have been observed (Spivak et al., 1999). Although aggression is associated with serotonergic deficiency, the relationship is more readily detectable in aggressive individuals with impulsive component. For example, impulsive aggressive humans were found to have lower cerebrospinal fluid (CSF) 5-HT metabolite, 5-hydroxyindoleacetic acid (5-HIAA) than aggressive humans without impulsive component (Mehlman et al., 1994; Virkkunen and Linnoila, 1993). In this dissertation, CSF 5-HIAA concentrations were examined and correlated with measures of impulsivity and eventual social rank (Chapter II).

In rats, reducing 5-HT activity at the level of cell bodies, dorsal and median raphe nucleus, resulted in failure to demonstrate proper behavioral inhibition induced by reward removal when responding on an operant task (Fletcher, 1993). Moreover, depletion of forebrain 5-HT with 5,7-DHT induces impulsive responding in rats when assessed using DRL paradigm, as demonstrated by increases in responding and decreases in reinforcement frequency (Fletcher, 1995). In nonhuman primates, individuals with low CSF 5-HIAA concentrations exhibit impaired impulse control which is shown by excessive mortality, escalated aggression, more wounding, and greater risk taking as displayed by leaping longer distances between trees (Higley et al.,1992, 1996a-d; Higley and Linnoila, 1997). Furthermore, low CSF 5-HIAA levels were found to be trait-like and linked to increased alcohol consumption in NHPs (Higley et al., 1996a,b).

Another way to measure impulsive behavior in laboratory conditions is an Intruder Challenge which has been used as a standard test of behavioral inhibition in a social context (Fairbanks et al., 2001; Manuck et al., 2003). For example, an intruder paradigm was used to measure the relationship between CSF 5-HIAA levels and social impulsivity in socially housed male vervet monkeys (Fairbanks et al., 2001). A previously unfamiliar monkey was placed inside of a small cage which was positioned on the edge of the outdoor enclosure. The resident monkeys were then observed over the 30 minute sessions and latency to approach the intruder and aggressive and assertive behaviors were scored by the observers. These behaviors are believed to be indexes of impulsivity in this model. CSF 5-HIAA levels were found to be inversely correlated with impulsivity indexes of the resident monkeys in males and females. Furthermore, the latency to approach the intruder was determined to be the main contributor to the final score. In addition, when treated with SERT inhibitor fluoxetine (2 mg/kg, i.m.) for 9 weeks, the subjects showed lower impulsivity scores than the nontreated individuals (Fairbanks et al., 2001). These findings closely parallel the observations of Manuck and colleagues (2003) who used female cynomolgus monkeys and found a similar relationship between CNS serotonergic responsivity and impulsive behavior.

Kinnally and colleagues (2006) investigated the function of the peripheral 5-HT system and its contribution to impulsive behavior in pair-housed marmosets. A slightly modified intruder paradigm was used. Briefly, the day before the experiment an individual subject was removed from the pair housing condition and placed in a novel single cage overnight. The following day, an intruder was brought into the room and his cage was attached to the front of the experimental subject's cage. Various behaviors

constituting an impulsivity index (see Fairbanks et al., 2001) were recorded. Peripheral 5-HT and 5-HIAA concentrations were measured in each animal. Peripheral 5-HT and 5-HT metabolite levels were higher in animals that exhibited more inhibition in response to the stranger. Furthermore, when treated with oral fluoxetine or vehicle for 2 days, treated monkeys exhibited less inhibition towards the novel conspecific (i.e. intruder). This finding is surprising considering the previously described study discovered decreases in impulsivity following fluoxetine treatment. The reasons for the discrepant findings may be due to differences in treatment length, housing conditions as well as potential species differences.

The latter study demonstrated concordance between CSF and peripheral measures of 5-HT function and their relationship to social impulsivity. Peripheral 5-HT, like central 5-HT, appears to be stable in individuals suggesting that concentrations of this neurotransmitters are trait-like (Anderson et al., 2004; Higley and Linnoila, 1997); concentrations are also highly correlated between mothers and infants pointing to a potential genetic link (Anderson et al., 2004). Aggressive and impulsive children and adolescents exhibit lower rates of 5-HT uptake in platelets (Oades et al., 2002; Stadler et al., 2004). The association between behavior and peripheral 5-HT function is more likely due to the correlation between central and peripheral measures of 5-HT function rather than peripheral 5-HT effect on behavior (Kinnally et al., 2006). The high correlation between central and peripheral measures of the 5-HT system function provides a less invasive and risky procedure to indirectly measure CNS 5-HT function. In this dissertation, peripheral and central measures of 5-HT activity will be assessed in each monkey at various times throughout the experiments.

While DA system is believed to be involved in behavioral activation, the 5-HT system is linked with behavioral inhibition (Crean et al., 2002; Fairbanks et al., 2001; LeMarquand et al., 1998). However, there is considerable evidence indicating these neurotransmitter systems do not individually modulate impulsive behavior; it is the ability of the 5-HT system to modulate DA that affects impulsive behavior (Winstanley et al., 2006). Recent evidence suggests that stimulation of different 5-HT receptors can have radically different effects on impulsivity (Evenden and Ryan, 1999). Therefore, in this dissertation, 5-HT system function will be assessed by measuring CSF concentration of 5-HT metabolite 5-HIAA, SERT availability with PET, and peripherally using whole blood 5-HT levels. In addition, the relationship between 5-HT system function with several measures of impulsivity and social behavior in socially housed female monkeys will be assessed (Chapter II). Furthermore, it will be determined whether these markers are trait and/or state variables by examining them after stable social hierarchies have been established.

C.1. Environment: Environmental circumstances are very important determinants of drug-reinforced behavior in humans and animals. Certain conditions such as stress increase individual's vulnerability to abuse drugs (Kabbaj et al., 2004a; Morgan et al., 2002; Nader and Czoty, 2005). Chronic stress in the form of rearing conditions in rhesus monkeys resulted in higher alcohol consumption when they reached adulthood (Higley el al., 1993). In drug abusers, craving for cocaine and alcohol was increased when they were presented with stressful images (Sinha et al., 2000). In adult rats, cocaine and amphetamine self-administration was acquired more rapidly following physical (e.g. noncontingent electric foot shock; Goeders and Guerin, 1994; tail pinch; Piazza et al.,

1990b) and emotional (e.g. observing another rat being shocked; Ramsey and Van Ree, 1993) stressors. Acquisition of psychostimulant self-administration is also enhanced by aggressive attack by same-sex opponent or by a threat of attack by an aggressive opponent visually available to the previously defeated intruder in rats (Haney et al., 1995; Kabbaj et al., 2001; Miczek and Mutscheler, 1996; Tidey and Miczek, 1997).

Considerable evidence suggests that acute and chronic stress stimulates hypothalamic-pituitary-adrenal (HPA) axis which feeds back to stimulate mesocortical and mesolimbic DA systems and in turn facilitating acquisition of stimulant self administration (Miczek and Mutscheler, 1996; Piazza et al., 1989b, 1990b, 1991; Rouge-Pont et al., 1993; Tidey and Miczek, 1996). For example, Morgan and colleagues (2002) showed that chronic social stress and low social standing were associated with greater risk for drug abuse in an established model of chronic stress in male NHPs. Furthermore, differences in DA system function of dominant and subordinate monkeys were cited as possible cause (Morgan et al., 2002). They used positron emission tomography (PET) imaging technique to measure DA D2 receptor availability in male individually housed monkeys prior to them being randomly assigned to social groups. In humans, DA D2 receptors levels have been shown to be associated with risk factors for drug abuse (Volkow et al., 1999, 2006). Following social housing and establishment of stable social hierarchies (Kaplan et al., 1982), DA D2 receptor availability increased in the animals that became dominant but remained unchanged in the subordinate monkeys. Furthermore, when they self-administered cocaine under an FR schedule of reinforcement, cocaine did not function as a reinforcer in dominant monkeys (Morgan et al., 2002). The authors suggested that DA D2 receptor availability increased in dominant

animals after living in a more "enriching" environment which in turn resulted in increased protection from cocaine abuse. On the other hand, subordinates were subjected to constant social stress, spent more time alone, and less time being groomed by others, and had lower D2 DA receptors which resulted in higher responding for cocaine (see Nader and Czoty, 2005 for complete discussion). In addition to higher D2 receptor availability, subordinate animals possibly had higher extracellular DA levels than subordinates which was confirmed by rodent studies. Social stress, glucocorticoids (e.g. cortisol in primates and corticosterone in rodents), and mesoaccumbal DA activity appear to interact to facilitate DA release and sensitivity of postsynaptic DA receptors and in turn increase vulnerability to reinforcing effects of cocaine (reviewed by Piazza and Le Moal, 1996). In these dissertation studies, we extended this study to females to examine the relationship between social rank and cocaine reinforcement. In addition, we focused on the 5-HT system measures since little is known about its relationship with social rank and vulnerability to reinforcing effects of cocaine in female nonhuman primates.

The relationship between stress and substance abuse has been widely documented in both human and animal studies (de Wit et al., 2003; Goeders, 2002; Kabbaj et al., 2001; Kreek and Koob, 1998; Sinha et al., 1999, 2000, 2003); however, most studies are performed in male subjects. According to recent reports by Back and colleagues (2005), women may be more sensitive to subjective and physiological experiences of anxiety and social stress which could mean that in stressful situations women would be more vulnerable to use and abuse drugs. In addition, female rats were found to be more sensitive to chronic mild stress than males as depicted by disruptions in sucrose intake and alterations in estrous cycle (Dalla et al., 2005). Therefore, in this dissertation we will

extend work on stress and substance abuse to female monkeys and an animal model of chronic social stress (Chapter III).

V. Sensitivity to cocaine in females: As mentioned above, the focus of this dissertation is on cocaine abuse in females. Increased sensitivity to cocaine in women has been studied by several investigators in human and animal subjects. Most studies concentrated on comparing men and women when investigating behavioral and pharmacological effects of cocaine in order to uncover the underlying causes for the increased vulnerability to cocaine addiction in women (Becker, 1999; Lukas et al., 1996; Mello et al., 2002, 2007; Mendelson et al., 1999). There are several issues to consider when studying female subjects, including the importance of menstrual cycle. In this section, a brief summary of animal and human studies demonstrating differential cocaine sensitivity between males and females will be described followed by a more thorough analysis of the published literature implicating menstrual cycle phase and gonadal hormones in drug dependence in females.

Several animal studies, using different cocaine self-administration paradigms in laboratory animals, demonstrated that females acquire cocaine self-administration faster than males (Jackson et al., 2006; Lynch and Carroll, 1999). After self-administration has been established (maintenance phase), females had higher break points (BP) than males when responding on PR schedules of cocaine reinforcement (e.g. monkeys; Mello et al., 2007, e.g. rats; Roberts et al., 1989). Relapse and craving studies have been extensively studied in both sexes. Several findings suggested that women were more reactive to cues associated with cocaine taking and had higher scores of craving when presented with

cocaine cues than men (Kilts et al, 2004; Robbins et al., 1999); however, other groups reported the opposite (e.g. Fiorentine et al., 1997; Weiss et al., 1997). In animal studies, females earned significantly more saline injections during reinstatement of extinguished cocaine-reinforced responding following priming injection of cocaine (Lynch and Carroll, 2000). In addition, females had greater behavioral stimulation such as stereotypic and locomotor activity than male rodents (Glick and Hinds, 1984; Haney et al., 1995; Schindler and Carmona, 2002). In contrast, overall intake of cocaine has not been consistently shown to be higher in females (Hill and Powel, 1976; Lynch and Carroll, 1999; Morse et al., 1993 but not Haney et al., 1995; Roberts et al., 1989). Taken together, these findings suggest that reinforcing effects of cocaine may differ between males and females, although much work remains to better characterize these differences.

When measuring subjective and physiological responses to cocaine in women relative to men, lower, higher and no differences have been reported (Evans et al., 2002; Kosten et al., 1996; Lukas et al., 1996; Mello et al., 2002; Mendelson et al., 1999; Sofuoglu et al., 1999, 2000). In animals, drug discrimination paradigms have been widely used as a model of subjective effects in humans (Stolerman, 1993). The discriminative stimulus (S^D) effects of cocaine appeared to be equipotent in female and male rats across several cocaine doses (Anderson and van Haaren, 1999; Craft and Stratmann, 1996). Taken together, these animal and human data suggest that females may be more vulnerable to S^R effects while the subjective effects of cocaine appear to be less sensitive to sex differences. In this dissertation, cocaine-naive female monkeys were studied in models of drug self-administration in order to assess acquisition as well as maintenance (Chapter III).

IV. Menstrual cycle phase and gonadal hormones: As was mentioned above, menstrual cycle phase is of great importance as it relates to the behavioral effects of cocaine in female subjects. There is a growing body of animal and human literature examining the effect menstrual cycle phase has on reinforcing and subjective effects of cocaine. The effect of gonadal hormones on measures of subjective effects, reinforcing strength, and cocaine seeking behavior will be discussed in this section.

In humans, variation in the levels of PG and ES during the menstrual cycle appears to affect subjective responses to stimulant drugs. Across menstrual cycle, mood-altering effects of acute doses of these stimulants are greater during FP than the LP (Evans and Foltin, 2006; Justice and de Wit, 1999, 2000a,b; White et al., 2002). Although cocaine pharmacokinetics were not influenced by menstrual cycle phase, women in LP of the menstrual cycle showed attenuated response to the subjective effect of the drugs, compared with those who were in the FP (Evans et al., 2002; Evans and Foltin, 2006; Sofuoglu et al., 1999). Women in LP reported diminished ratings for a measure of "feel high" compared to women in the FP of the menstrual cycle and men (Sofuoglu et al., 1999). In addition, women in FP of the cycle had higher ratings of "good drug effect", "high", "stimulated", and "drug quality rating" than women in LP following smoked cocaine administration (Evans et al., 2002). Although one study reported differences in peak plasma cocaine levels as a function of menstrual cycle phase (Lukas et al., 1996), the differences in subjective effects observed are probably not due to cocaine's pharmacokinetic and pharmacodynamic effects but rather due to the changes in ES and PG during the menstrual cycle (Evans and Foltin, 2006; Mendelson et al., 1999).

In the Lukas et al. (1996) study, the effect of cocaine administration on plasma cocaine levels via the intranasal route did not produce consistent plasma cocaine levels (e.g., compare Lukas et al., 1995 and Lukas et al., 1996). Similar findings were reported when amphetamine (AMPH) was given to non-addicted women (Justice and de Wit, 2000a). Most of the physiological effects of AMPH did not differ as a function of menstrual cycle; however, ES treatment increased the magnitude of the effect of AMPH on "pleasant stimulation". AMPH produced smaller increases in ratings of "high", euphoria, energy, and intellectual efficiency when it was administered in the LP, compared to the FP (Justice and de Wit, 1999). In order to better understand the effect of ES alone on the subjective effects of AMPH, Justice and de Wit (2000a) examined AMPH effects on mood in early and late FP characterized by low and higher ES levels, respectively. They found no difference in the subjective effect rating; therefore, changes in ES alone were not enough to explain variability in response to stimulant drugs. Several lines of evidence suggest that PG administered during FP significantly decreased the positive effect of cocaine, providing strong support for the idea that higher PG during the LP dampens the subjective responses to cocaine and accounts for the cycle-related differences in psychostimulant drug effects (Evans and Folin, 2006; Sofuoglu et al., 2002). Taken together, these findings suggest that enhanced responses to stimulants may be due not to the presence of ES in the FP, but rather the absence of PG. However, it is important to note that PG concentrations administered were higher than physiological concentrations (Sofuoglu et al., 2002). In this dissertation, we will examine the effect of menstrual cycle phase on reinforcing effects of cocaine during the maintenance phase by determining cocaine dose-response curves in two phases of the menstrual cycle, follicular

and luteal (Chapter III). We hypothesize that cocaine would be a more potent reinforcer in the follicular compared to luteal phase of the menstrual cycle.

In animal studies using female subjects, different phases of drug dependence (i.e., acquisition, maintenance, and relapse) have been differentially affected by gonadal hormones. It has been described that female rats acquire cocaine self administration faster then male rats and a greater percentage of females do so than males when a low cocaine dose (0.2mg/kg/inj) was available in an auto-shaping procedure (e.g. Lynch and Carroll, 1999). In contrast, no sex differences were observed in days to acquire cocaine (1.5mg/kg/inj) self-administration under an FR 1 reinforcement schedule or in number of cocaine injections under a PR reinforcement schedule (Lynch and Taylor, 2004). While females acquire low-dose cocaine self-administration more rapidly than males, Caine and colleagues (2004) found that male rats acquired high-dose cocaine (1.5mg/kg/inj) self-administration under an FR 5 reinforcement schedule more rapidly than female rats. One consideration of these latter results is that using high cocaine doses in acquisition studies may have led to a ceiling effect (e.g., 100% of male and female rats acquire administration) – so the same maximal effect was observed in both sexes, but at different rates. In this dissertation, a low dose of cocaine (0.001mg/kg/inj), based on other published work, was first substituted for food in monkeys and increased by one-half log-units in order to determine the lowest dose necessary for acquisition. For these studies, acquisition was operationally defined as the lowest dose that functioned as a reinforcer (i.e., higher response rates compared to when saline was self-administered). The acquisition studies were also conducted with consideration of menstrual cycle phase so as to determine whether gonadal hormones influenced vulnerability (Chapter III).

In addition to studying acquisition, we examined how self-administration varied as a function of cocaine dose and menstrual cycle phase during maintenance phase of cocaine self-administration. There are studies suggesting that menstrual cycle phase and the respective gonadal hormone changes influence the reinforcing and subjective effects of stimulants in females. When female rat self administer cocaine on a PR schedule, they reached higher break points (BP) during the estrus phase of the cycle than during other phases (Roberts et al., 1989; Hecht et al., 1999) while responding maintained by a non-drug reinforcer remained unchanged (Hecht et al., 1999). Similarly, female rhesus monkeys reached higher cocaine BPs during the early FP of the menstrual cycle than any other phase; however, the differences were only found at the lowest dose of cocaine tested; this dose was not consistently reinforcing in most monkeys (Mello et al., 2007). On the contrary, reinforcing effects of cocaine were not different as a function of menstrual cycle phase or ovariectomy in rhesus monkeys when dose-response curves were determined on FR schedule of drug presentation; however, only two monkeys per group were evaluated (Mello et al., 2008). Similarly, Roberts and colleagues (1989) did not observe estrous cycle-dependent differences in cocaine self-administering rats on FR schedule of cocaine presentation; however, an FR 1 schedule was used, possibly obscuring the differences. Taken together, these studies suggest that in maintenance phase of drug dependence ovarian hormones may not have similar effects on cocaine taking and are dependent on schedule of reinforcement used.

In order to better assess the effect of ovarian hormones on different stages of cocaine abuse (i.e. acquisition, maintenance, and relapse), preclinical researches used exogenous hormone administration in ovariectomized (OVX) laboratory animals. For

example, Lynch and colleagues (2001) examined the effect of exogenous ES administration on acquisition of a low (0.2mg/kg/inj) cocaine dose in female rats. A greater percentage of intact, vehicle-treated and OVX rats treated with ES acquired cocaine self-administration compared to OVX female rats, or rats treated with tamoxifen, an ES antagonist. However, drug-naïve OVX rats did not acquire cocaine self-administration slower than intact rats when higher doses of cocaine where available (Caine et al., 2004; Grimm and See, 1997) or when OVX rats were treated with ES (Lynch and Taylor, 2005). However, cocaine dose-response curves (0.032-3.2mg/kg/inj) determined before and after ovariectomy in female rats did not differ from each other (Caine et al., 2004). Moreover, OVX rats and ES treated OVX rats did not produce any differences in dose-response curves as well. Cocaine-induced reinstatement studies demonstrate that ovariectomy reduced while ES treatments enhance responding when primed with cocaine (Larson et al., 2005). On the contrary, ES pretreatment did not alter cocaine discrimination dose-response curve in female rhesus monkeys (Mello et al., 2008) indicating that gonadal hormones may not affect subjective effects of cocaine in similar fashion as reinforcing effects.

In contrast to ES, PG has been shown to decrease stimulant-associated responding in laboratory animals. PG administration to OVX rats that were treated with ES replacement reduced acquisition of cocaine self-administration indicating that concurrent PG treatment counteracted the facilitory effect of ES on self-administration (Jackson et al., 2006). In intact rats, cocaine-induced reinstatement was attenuated by PG treatment (Anker et al., 2007). When PG was administered to intact female rhesus monkeys during

the maintenance phase, it dose-dependently reduced the reinforcing effects of cocaine (Mello et al., 2005).

It has been suggested that the mechanism responsible for ovarian hormone effects on the reinforcing effects of cocaine may be DA mediated (Bazzett and Becker, 1994; Becker and Cha, 1989; Becker, 1999). Evidence suggests that ES and PG affect the mesocorticolimbic DA system that is believed to be important for reinforcing effects of drugs (Koob, 1992). Specifically, estrogen is believed to enhance behavioral and neurochemical response to stimulants by changing neuronal excitability of GABAergic neurons which result in decreased $GABA_B$ receptor stimulation and in turn enhanced DA release (Becker, 1999). Furthermore, ES is believed to downregulate DA D2 autoreceptors, which may result in increases in DA release. In contrast, PG has been demonstrated to either inhibit or not alter DA release without prior ES priming (Becker, 1999). These findings are further supported by a recent PET imaging study that examined D2 receptor availability in female cynomolgus monkeys in the follicular and the luteal phases of their naturally occurring menstrual cycle (Czoty et al., 2008). It was found that D2 receptor availability was lower in the follicular phase when ES levels were increasing and PG concentrations were low compared to luteal phase of the menstrual cycle when ES levels were moderate and PG concentrations were high. Taken together, these findings suggest that ES enhanced and PG attenuated the abuse-related effects of cocaine by altering DA neurotransmission. However, the unit dose available for self-administration and the necessary presence of both hormones may also be an important determinant in influencing cocaine reinforcement.

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CHAPTER II

BEHAVIORAL AND NEUROBIOLOGICAL CHARACTERISTICS

INFLUENCING SOCIAL HIERARCHY FORMATION IN FEMALE

CYNOMOLGUS MONKEYS

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List of Abbreviations:

5-HIAA: 5-hydroxyindoleacetic acid; 5-HT: serotonin; ACTH: adrenocorticotropic hormone; ANOVA: one-way analysis of variance; CSF: cerebrospinal fluid; DA: dopamine;[^{11}C]DASB: [^{11}C]-3-amino-4-(2-dimethylaminomethylphenylthio)benzonitrile; DEX: dexamethasone; DVR: distribution volume ratio; HPA: hypothalamus-pituitary-adrenal; MRI: magnetic resonance imaging; NOR: novel object reactivity; PET: positron emission tomography; PRP: platelet-rich plasma; ROI: region of interest; SERT: serotonin transporter; WBS: whole blood serotonin

ABSTRACT

Socially housed monkeys have been used as a model to study human diseases. The present study examined behavioral, physiological and neurochemical measures as predictors of social rank in sixteen experimentally naïve, individually housed female cynomolgus monkeys (*Macaca fascicularis*). The two behavioral measures examined were novel object reactivity (NOR), as determined by latency to touch an opaque acrylic box placed in the home cage, and locomotor activity assessed in a novel open-field apparatus. Serum cortisol concentrations were evaluated three times per week for four consecutive weeks, and stress reactivity was assessed on one occasion by evaluating the cortisol response to adrenocorticotropic hormone (ACTH) following dexamethasone suppression. Measures of serotonin (5-HT) function included whole blood serotonin (WBS) concentrations, cerebrospinal fluid (CSF) concentrations of the serotonin metabolite 5-hydroxyindoleacetic acid (5-HIAA) and brain serotonin transporter (SERT) availability obtained using positron emission tomography (PET). After baseline measures were obtained, monkeys were assigned to four social groups of four monkeys per group. The two measures that correlated with eventual social rank were CSF 5-HIAA concentrations, which were significantly higher in the animals who eventually became subordinate and latency to touch the novel object, which was significantly lower in eventual subordinate monkeys. Measures of serotonin function did not change as a consequence of social rank. These data suggest that levels of central 5-HIAA and measures of novel object reactivity may be trait markers that influence eventual social rank in female macaques.

INTRODUCTION

Nonhuman primates living in social groups have been used to study psychiatric diseases, drug abuse, atherosclerosis and the neurobiology of behaviors such as aggression and impulsivity (Kaplan et al., 1982; Morgan et al., 2002; Shively et al., 1998). Social rank (an individual's relative position within a dominance hierarchy) has been shown to have significant effects on susceptibility and resistance to diseases, indicating a powerful influence of the environment (e.g., Abbott et al., 2003; Kaplan, 2004; Morgan et al., 2002; Sapolsky, 2005; Virgin and Sapolsky, 1997). The study of social rank and health in nonhuman primates has been frequently related to different levels of stress. When received chronically, stress can increase risks for numerous diseases (Sapolsky, 2005). In addition, dominance hierarchies produce marked inequalities in division of resources (e.g. food, space, access to sexual partners, etc.); thus, an animal's social rank can profoundly affect its quality of life and its health (Kaplan, 1987; Sapolsky, 2005), including increased risk for coronary artery atherosclerosis, poor ovarian function, and adrenal hypersecretion of cortisol (Kaplan et al., 19867; Shively et al., 1986, 1997). Studies assessing disease states as a function of social rank have typically examined the effects of "state" variables, that is, characteristics attributable to environmental circumstances. In order to more fully understand the role of the environment, including social rank, on disease states, other investigators have examined potential "trait" variables, that is, pre-existing characteristics that may underlie an individual's likelihood of occupying a high or low social rank. Such information is critical in understanding the interactions between the individual and the social context. The present study focused on several potential predictors of social rank in adult female

cynomolgus monkeys (*Macaca fascicularis*), including behavioral measures associated with reaction to novelty, hormone concentrations and measures of serotonin (5-HT) neurotransmission.

Macaques establish linear hierarchies based on outcomes of agonistic interactions (e.g., Kaplan et al., 1982). Variables such as body weight, age and kinship have been shown to affect eventual social rank (Cawthon-Lang, 2006; Gartland, 1968; Sapolsky, 2005). For example, in male cynomolgus monkeys, body weights prior to social housing were significantly correlated with eventual social rank, with heavier monkeys becoming the most dominant (Morgan et al., 2000). Such an outcome is consistent with observational studies indicating that physical appearance is an important variable in determining social rank in rhesus macaques (Bernstein and Mason, 1963; Bernstein et al., 1974; for review see Bernstein, 1991). The relationship between body weight and eventual social rank in female monkeys has not been explored. Other than body weight, the only other measure that predicted eventual social rank in our earlier study in male monkeys was locomotor activity in a novel environment (Morgan et al., 2000). In the present study, we examined this variable in female monkeys and extended the behavioral measures to include latency to touch a novel object. Other variables that have been examined as predictors of social rank in male monkeys include cortisol concentrations, which generally do not predict future social rank (e.g., Mendoza et al., 1979; Goo and Sassenrath, 1980; Morgan et al., 2000; but see Golub et al., 1979).

Finally, the present study extended our assessment of predictors of social rank to include measures of 5-HT function. The 5-HT system has been implicated in a variety of social behaviors such as grooming, approaching, feeding, as well as impulsive aggression

and impulsivity (Kaplan et al., 2002; Miczek et al., 2002, 2004a,b; Pattij and Vanderschuren, 2008). To assess 5-HT activity, the present study examined whole blood 5-HT (WBS) levels (Kinnally et al., 2006; Unis et al., 1997), cerebrospinal fluid (CSF) levels of the 5-HT metabolite 5-hydroxyindoleacetic acid (5-HIAA; Higley et al., 1991, 1992, 1996a,b; Higley and Linnoila, 1997; Kaplan et al., 2002; Westergaard et al., 1999; Yodyingyuad et al., 1985) and measures of 5-HT transporter (SERT) availability using positron emission tomography (PET; Frankle et al., 2005) in brain areas rich in SERT-containing terminals. Each 5-HT marker was examined in all monkeys allowing for comparisons of all dependent variables in a repeated-measures experimental design. We assessed whether these measures predicted eventual social rank (i.e., trait variables) and whether they changed after social group formation (i.e., state variables). Because recent work from our laboratory reported that measures of dopamine receptor availability were influenced by menstrual cycle phase (Czoty et al., 2008), PET imaging and endocrine experiments were conducted while monkeys were in the follicular phase.

METHODS

Subjects*:* Sixteen experimentally naïve adult female cynomolgus monkeys (*Macaca fascicularis*) 9-17 years old served as subjects. All the 16 monkeys were imported from Indonesia (Institute Pertanian Bogor, Bogor, Indonesia); eight animals were previously in a non-invasive study at the Wake Forest University Primate Center. While in quarantine, monkeys were fed *ad libitum*; their weights at the end of this period were used as an indication of free-feeding weights. After transfer to the laboratory, monkeys were weighed weekly and, to facilitate concurrent operant behavioral training involving food

reinforcement (data not shown), body weights were maintained at approximately 95% of free-feeding weights by limiting daily access to food (Purina Monkey Chow LabDiet #5038 and fresh fruit); animals had unlimited access to water. Monkeys were singly housed in cages in which they had visual, olfactory and limited manual contact with conspecifics. Quadrant cages (Model #PR242732I4, Allentown Caging Inc., Allentown, NJ) were equipped with removable metal partitions that separated the four animals into individual quadrants of the cage. Each quadrant measured 0.71 x 0.84 x 0.84 m. When partitions were removed, the total living space for the pen measured 0.71 x 1.73 x 1.73 m. Each animal was trained to sit calmly in a primate chair (Primate Products, Inc., Redwood, CA), as described previously (e.g., Morgan et al., 2000; Czoty et al., 2005).

The duration of the menstrual cycle was initially assessed by daily vaginal swabs over several months (see Czoty et al., 2008). The first day of bleeding was considered to be indicative of menses and was counted as day 1 of the cycle. Once a complete cycle of approximately 28 days was observed, experiments were scheduled to occur during days 2-10 (the follicular phase). Experiments scheduled to occur in luteal phase were conducted on days 19-26 of the menstrual cycle. To confirm that monkeys were in the follicular phase during PET experiments, on the day of a PET study, 3 ml of blood was drawn from the femoral vein and centrifuged (Beckman Coulter, GPR Centrifuge) at 4º C for 30 min at 3000 rpm. The serum was aspirated into an Eppendorf tube and stored at -30º C. Progesterone levels were assessed by the Biomarkers Core Laboratory of the Yerkes National Primate Research Center of Emory University in Atlanta, GA. Progesterone levels < 4 ng/ml were indicative of follicular phase. All animal procedures were carried out in accordance with the Declaration of Helsinki and/or with the Guide for

the Care and Use of Laboratory Animals as adopted and promulgated by the National Institutes of Health and were approved by the Animal Care and Use Committee of Wake Forest University. Furthermore, the number of animals used for the study was the minimal required to obtain significant statistical power and every effort was made to minimize their pain and suffering. Environmental enrichment was provided as outlined in the Animal Care and Use Committee of Wake Forest University Non-Human Primate Environmental Enrichment Plan. This included toys, mirrors and food enrichment, which was delivered via multiple types of foraging devices (Challenger balls, PVC cups, PVC tubes) and provided to each monkey a minimum of 2 times/week during their 2 hour feeding period while they are separated for feeding.

Social housing procedure and rank determination: After arriving in our laboratory, monkeys were individually housed for approximately 27 months while baseline behavioral (locomotor activity and novel object reactivity, NOR), neurochemical (5-HT function) and hormonal measures (menstrual cycle, cortisol concentrations and adrenal responsiveness) were obtained. Monkeys lived in social groups of four monkeys per pen. The initial placement of monkeys into groups was counterbalanced and based on body weight. Monkeys were ranked from 1-16 based on body weight and each of the 4 heaviest were randomly assigned to groups 1-4; the next 4 heaviest were then randomly assigned, etc. On the first day of social housing the steel partitions were removed and the animals were allowed to interact. Monkeys were monitored closely for the first 72 hr after placement into social groups. For the next 5 days, partitions were replaced at approximately 4:15PM and monkeys remained separated overnight. This precaution was

64

taken to prevent injury occurring when laboratory personnel were not present. After 1 week, monkeys were socially housed overnight and separated each morning, during which time monkeys were fed. Thus, time spent socially housed was approximately 22 hours per day. In rare cases of an injury that required veterinary care or other routine veterinary procedures, the entire pen was individually housed overnight (or until the injury healed).

For each group, social status was determined using the outcome of agonistic encounters (Kaplan et al., 1982). From week 2 through week 12 of social housing, two observers separately conducted a total of 34-36 observation sessions per pen (3 observations/week per pen). Each 15-min session began immediately after partitions were removed in the afternoon. Aggressive, submissive and affiliative behaviors were recorded according to an ethogram described previously (Kaplan et al. 1991; see Morgan et al. 2000, Table I) utilizing Noldus Observer software (Noldus Information Technology; Wageningen, The Netherlands). In these focal group sessions, both initiators and recipients of behaviors were recorded. The monkey in each pen aggressing towards all other monkeys and submitting to no one was ranked #1 (most dominant). The monkey aggressing at everyone except the #1-ranked monkey and submitting only to the #1-ranked monkey was ranked #2, etc. The monkey designated #4 displayed low levels of aggression and submitted to all other monkeys in the pen. Thus, a transitive, linear hierarchy was established in each pen.

Experiment 1. Behavioral measures.

Locomotor activity: Monkeys were placed in a 3 X 2 X 1.75 m open-field apparatus for 15 min to assess locomotor activity in a novel environment. The open-field apparatus was divided into 9 zones. Activity was recorded by 2 small cameras built into the top panels of the apparatus and a side-view camera set up on a tripod outside the apparatus. The primary dependant variable was number of crossings between compartments and was scored from the video.

Novel object reactivity: On the day of the test, the monkey in the quadrant adjacent to the test subject's home cage was removed, the test subject was moved to the neighboring cage and the partition was replaced. An opaque box (30.5 x 20.3 x 20.3 cm) made of black Plexiglas was placed in the subject's home cage. The partition was removed and activity was filmed for 15 min. Latency to touch the object was scored from the video. If the animal did not touch the box within 15 min a score of 900 sec was assigned.

Experiment 2. Measures of cortisol concentrations and adrenal responsiveness.

Serum cortisol: Blood samples (2 ml) were collected three times per week from each monkey, one day at 4:00 PM and two days at 7:30 AM, for 4 consecutive weeks. The samples were drawn by percutaneous stick from the femoral vein while the animal was awake and seated in a primate chair. The blood was centrifuged at 3000 rpm for 30 min and the serum was aspirated into an Eppendorf tube and stored at -20º C until analysis.

Adrenal responsiveness: Assessment of adrenal responsiveness was performed in the follicular phase of the menstrual cycle in all monkeys during individual housing and

repeated in dominant and subordinate monkeys after 3 months of social housing. While the monkey was seated in a primate chair, a blood sample was drawn from the femoral vein at 11:00 AM the day before the experiment to serve as a baseline. At 7:00 AM the following morning the animal was injected with 0.5 mg/kg dexamethasone (DEX) i.m. in her homecage. Four hours later, the monkey was seated in a primate chair and a baseline blood sample was obtained (time -15). Fifteen min later synthetic ACTH (Cortrosyn, 10 ng/kg) was injected into the saphenous vein followed by 3 ml sterile saline flush. Blood samples were drawn from the femoral vein 15- and 30-min following the Cortrosyn injection. Blood samples were centrifuged for 30 min at 3000 rpm. The serum was then aspirated into an Eppendorf tube and stored at -20º C until analysis. All blood samples were analyzed by the Biomarkers Core Laboratory of the Yerkes National Primate Research Center.

Experiment 3. Measures of serotonin function.

Whole blood 5-HT (WBS) measures: WBS was measured in each monkey in the follicular phase of the menstrual cycle while individually housed (n=16) and in dominant and subordinate monkeys following 3 months of social housing. Blood samples were obtained from the femoral vein while the animal was awake and seated in a primate chair. The blood samples were placed into EDTA-coated vacutainer tubes (Becton Dickinson, Franklin Lakes, NJ) and centrifuged at room temperature for 10 min at 1300 rpm to obtain platelet-rich plasma (PRP). The PRP-supernatant (200 µl) was then transferred into another tube with 800 µl of physiological saline and the total solution was centrifuged at 4° C for 10 min at 6300 rpm. The supernatant was then discarded and 200

μl of double-distilled water was added to the pellet. The final solution was stored at -80°C until analyzed by Dr. Richard R. Yeoman at the Oregon National Primate Research Center.

Cerebrospinal fluid (CSF) measures: While monkeys were individually housed, CSF was collected by a cervical puncture from 12 monkeys, once during the follicular phase and once during the luteal phase of a single menstrual cycle (i.e., two weeks apart) while the animals were anesthetized with ketamine (10 mg/kg, i.m.). These samples were used to assess whether 5-HIAA levels differed across menstrual cycle phases. In the remaining four monkeys, who were not cycling regularly at this time, two samples were taken two weeks apart. When it was determined subsequently that 5-HIAA levels did not differ across menstrual cycle phase (see Table I), data from the two samples were averaged for each monkey, including those that were not cycling, for use in determining whether CSF 5-HIAA levels predicted eventual social rank. Following social housing, CSF was collected from four dominant and four subordinate animals during the follicular phase. The back of the skull was shaved and cleaned with betadine and 95% alcohol. A 25-G, 1.5-inch needle was inserted into cisterna magna and approximately 2 ml of CSF was collected within 10 min of induction of anesthesia. CSF was then immediately transferred to chilled vacutainer tubes on ice. Samples were centrifuge at 4° C for 30 min at 3000 rpm. Aliquots were transferred to microcentrifuge tubes for storage at -30° C until analysis. The samples were assayed by Dr. John Mann at the New York State Psychiatric Institute.

PET imaging procedures: Prior to the first PET imaging study, magnetic resonance imaging (MRI) scans were acquired for each monkey under ketamine (20

mg/kg, i.m.) anesthesia (for details see Czoty et al., 2008). Images were used to anatomically define spherical regions of interest (ROI); data are shown for the caudate nucleus, putamen, anterior cingulate cortex (ACC), thalamus, hippocampus and cerebellum. PET scans to measure SERT availability were conducted using [^{11}C]-3-amino-4-(2-dimethylaminomethylphenylthio) benzonitrile ([^{11}C]DASB) during the follicular phase of the menstrual cycle, which was confirmed from blood samples taken prior to the PET study and analyzed for levels of progesterone. These regions were chosen because human postmortem studies suggest high to moderate densities of SERT (e.g., Mash et al., 2000) and significant occupancy by SERT compounds associated with improved anxiety scores (Kent et al., 2002). Serotonin has also been shown to influence basal ganglia function (e.g., Dray, 1981); a dopamine-rich region previously shown to be sensitive to changes in social rank (Morgan et al., 2002; Czoty et al., 2005). Scans were conducted in all monkeys prior to social group formation and again at least 3 months after formation of social groups in the four dominant and four subordinate monkeys.

Prior to the 90-min PET scan, the monkey was anesthetized with 10 mg/kg ketamine and transported to the PET Center, where she was intubated. Anesthesia was maintained by 1.5% isoflurane; this level of anesthesia dose not affect measures of [^{11}C]DASB availability (Milak et al., 2005). The monkey was placed in the scanner and a catheter was inserted by percutaneous stick into a vein. The venous catheter was used for [^{11}C]DASB administration and for administration of lactated ringers solution (5 ml/kg/hr) for fluid replacement throughout the study. To assure that the monkey did not move during the PET study, a paralytic (0.07 mg/kg vecuronium Br, i.v.) was administered 15 min before the start of the scan and respiration was maintained on a ventilator.

Supplemental doses of vecuronium Br (0.1 mg/hr) were administered throughout the study. During the scan, the temperature of the monkey was maintained through the use of a heating pad (temperature of the circulating water = 40º C). The following vital signs were monitored constantly throughout the scanning procedure: heart rate, blood pressure, respiration and body temperature. PET scans were performed using a primate microPET P4 scanner (Siemens/CTI Concorde) specifically designed for small-animal imaging (see Tai et al., 2001). Time-activity curves were generated for radiotracer concentrations in ROIs defined on each subject's co-registered MRI. Distribution volumes ratios (DVR) were calculated using the cerebellum as the reference region and the graphical method of Logan et al. (1996). For all regions, the right and left sides were then averaged and the DVR in each ROI was calculated.

Drugs and Chemicals: DEX (10 mg/ml) and Cortrosyn (250 µg/ml prepared on the day of the study) were purchased from the North Carolina Baptist Hospital Pharmacy. [^{11}C]DASB was prepared as previously described (Wilson et al., 2000) with slight modification: [^{11}C] methyl triflate was reacted with 0.5 mg of 3-amino-4-(2-methylaminomethylphenylsulfanyl)-benzonitrile (desmethyl-DASB) in 300 µL of acetonitrile. The reaction mixture was purified using reverse-phase HPLC. The radiochemical yields ranged between 20 – 30% with >95% radiochemical purity. The specific activity of [^{11}C]DASB was 4.73 ±0.13 mCi at the time of injection.

Statistical Analysis: Data are displayed for individual monkeys and as group means ± SEM. Data describing body weight and data from all pre-social housing experiments

except the DEX/cortrosyn study were analyzed using one-way analysis of variance (ANOVA) between the three groups (dominant, intermediate and subordinate); significant main effects or interactions were followed with a Newman-Keuls multiple comparisons post-hoc test. Because some monkeys did not touch the novel object and were thus assigned a score of 900, a (non parametric) Spearman's correlation was used to determine the relationship between latency to touch the novel object and CSF 5-HIAA levels. Data from the DEX/cortrosyn experiment were analyzed using a two-way ANOVA (social rank and time) as the main effects. Social housing data for WBS, CSF, and SERT availability were analyzed using unpaired t-test between dominant and subordinate groups. Correlations between measures were conducted by Pearson correlation. In all cases, significance was accepted at the 95% level of confidence ($p < 0.05$).

RESULTS

Experiment 1. Behavioral measures

Weight and age of the monkey. The body weight recorded immediately prior to social housing changed by less than 1% on average over the 3 months following social group formation, did not change significantly for the duration of the study (data not shown) and did not correlate significantly with social rank. There was no significant correlation between age or weight and any dependent variable examined in the present study, including social rank.

Locomotor activity and novel object reactivity: Locomotor activity ranged from 15 to 30 crossings over 15 min and did not differ as a function of eventual social rank

71

(Fig. 1, left panel). However, latency to touch the novel object (Fig. 1, right panel) differed significantly according to eventual social rank $(F_{(2,13)}=5.225; p<0.05)$. Post-hoc t-tests revealed that eventual dominant animals' latencies to touch the novel object was significantly longer $(p<0.05)$ than those of the eventual intermediate and subordinate animals. This relationship was observed in all pens. Latency to touch the novel object and locomotor activity scores were not significantly correlated.

Experiment 2. Measures of cortisol concentrations and adrenal responsiveness.

Serum cortisol: Mean cortisol concentrations were significantly higher in the AM compared to the PM samples (32.06 ± 2.23 vs. 20.79 ± 2.95 µg/dl, respectively; $t(3)=6.636$, $p<0.01$), but did not differ significantly as a function of eventual social rank (data not shown). During the first week of social housing, PM cortisol concentrations increased in all but three monkeys, but were not different in dominant and subordinate monkeys (see Fig. 2).

ACTH challenge: In assessments of adrenal responsiveness (Fig. 2, top panel), two-way ANOVA revealed a main effect of time $(F_{(3,39)}=42.75, p<0.0001)$, but no significant main effect of eventual rank and no significant interaction between eventual social rank and time. There were no significant differences in cortisol levels as a function of eventual social rank after DEX suppression or after an ACTH challenge as a function of eventual social rank. Moreover, following 3 months of social housing, the extent to which ACTH elevated cortisol levels did not differ between dominant and subordinate monkeys (Fig. 2, bottom panel).

Experiment 3. Measures of serotonin function.

Whole-blood 5-HT levels (WBS): While individually housed, WBS levels were highly variable between subjects, ranging from 101-1337 ng/ml (data not shown). There were no significant differences in WBS concentrations in monkeys as a function of eventual social rank, with mean values for eventual dominant, intermediate and subordinate groups of 554 ± 98.81, 611± 150.15 and 463 ± 184.54 ng/ml, respectively. Three months after social housing, these values had not significantly changed in dominant and subordinate monkeys, with means of 477.25 ± 101.89 and 329 ± 76.99 ng/ml, respectively.

CSF concentrations of 5-HIAA: In twelve normally cycling monkeys, CSF concentrations of 5-HIAA did not significantly differ as a function of menstrual cycle phase (Table I). Two samples were collected two weeks apart in four other monkeys who were not cycling normally. CSF 5-HIAA levels in those samples were similar to those in the normally cycling monkeys (subject #6805: 375.04 and 318.36, #6820: 453.54 and 404.6; #6818: 394.22 and 345.99; #6802: 346.79 and 338.06 pmol/ml). For determining the ability of social rank to predict future social rank, the two samples were averaged for each of the 16 monkeys. A one-way ANOVA indicated that CSF concentrations of 5-HIAA taken while individually housed differed significantly as a function of eventual social rank ($F_{(2,13)}=4.42$; $p=0.034$; Fig. 3, left). Post-hoc analyses indicated that 5-HIAA concentrations were significantly lower in monkeys that would become dominant compared to eventual subordinate and intermediate monkeys ($p<0.05$). This relationship was observed in three of the four pens. In the fourth, CSF 5-HIAA levels were similar. In addition, there was a significant negative correlation between baseline CSF 5-HIAA

concentrations and latency to touch the novel object (r= -0.57; p<0.05). Three months after establishing social groups, 5-HIAA levels did not significantly change from individual-housing baselines and remained significantly lower in dominant compared to subordinate monkeys (t(6)=2.86; p<0.05; Fig. 3, right).

SERT availability as measured by PET. There was a high level of uptake of [^{11}C]DASB and a linear rate of washout from all regions of interest. In the reference region, the cerebellum, there was low level of [^{11}C]DASB uptake and rapid rate of washout. For all regions of interest, there were no differences between left and right sides, so mean DVR data are shown (Fig. 4). SERT availability did not differ between eventually dominant and subordinate animals in caudate nucleus, putamen, hippocampus, thalamus, and anterior cingulate cortex (Fig. 4, top panel). Following 3 months of social housing, SERT availability did not significantly differ as a function of social rank (Fig. 4, bottom panel). There was not a significant correlation between CSF 5-HIAA levels and SERT availability, nor was SERT availability significantly correlated with any behavioral measure.

DISCUSSION

The primary goal of the present study was to examine several behavioral, physiological, and neurochemical measures as predictors of eventual social rank in female cynomolgus monkeys (i.e., trait variables) and to determine if these measures changed after social group formation (i.e., state variables). These measures included age, body weight, locomotor activity in a novel environment, latency to touch a novel object, HPA-axis function and 5-HT activity (peripheral and central). The only behavioral measure that was predictive of eventual social rank was a greater latency to touch a novel object among future dominant animals. We also found that CSF concentrations of the 5-HT metabolite 5-HIAA, but not other measures of 5-HT activity, predicted eventual social rank, and that no 5-HT measure changed after the establishment of stable social hierarchies.

The present study extends earlier research in male cynomolgus monkeys (Morgan et al., 2000) in which reliable predictors of social rank were the weights of the monkeys prior to social group formation and locomotor activity. Age, cortisol and testosterone measures were not significantly correlated. In the present study, neither weight nor locomotor activity in a novel environment was predictive of eventual social rank in female monkeys. The range of weights of the females prior to social housing was 2.4-3.7 kg, which was a narrower range than the males' weights (3.2-6.4 kg; Morgan et al., 2000), perhaps obscuring a significant correlation. Regarding locomotor activity, the apparatus was modified from the original study in males and is larger and allows for more vertical activity, which may preclude us from making direct comparisons between the two studies. In addition, it is possible that sex differences exist in locomotor activity

and are due to ovarian hormone fluctuations. In females, novelty and stimulant-induced locomotor behavior varied as a function of estrus cycle in rats (Davis et al., 2008; Sell et al., 2002), suggesting a source for sex differences in this dependent measure. To our knowledge, no studies have addressed this question in nonhuman primates. Overall, these findings suggest that physiological and behavioral variables that can be considered traits that predict social rank in male cynomolgus monkeys (body weight and locomotor activity) are not predictive in females.

In contrast to locomotor activity, NOR was predictive of eventual social rank in female monkeys. In a recent study examining locomotor activity and a behavioral measure of impulsivity (a 5-choice serial reaction test), Dalley et al. (2007) found that subjects determined to be more impulsive had lower locomotor activity. Taken together, both findings suggest that locomotor activity in a novel environment reflects a behavior that is different from impulsivity. In the present study, we hypothesized that latency to touch the novel object would reflect a trait measure related to temperament and that eventual subordinate monkeys would be more reactive (i.e., have lower latencies). Highly impulsive rhesus monkeys have been shown to occupy lower positions in the social dominance hierarchy and are more sensitive to the reinforcing effects of alcohol (Higley et al., 1992, 1996a,b). Similarly, high impulsivity is characteristic of human cocaine abusers (Moeller et al., 2001, 2002). We found that more reactive monkeys were more likely to become subordinate when socially housed, extending earlier work in well-established social groups to include this behavior as a trait marker. In addition, NOR latencies correlated with central 5-HT activity as indicated by low CSF 5-HIAA levels,

which is consistent with earlier work in nonhuman primates (Higley et al., 1996a,b; Raleigh et al., 1980, 1983; Westergaard et al., 1999).

CSF 5-HIAA concentrations have been shown to predict social rank in female rhesus and pigtail monkeys (Westergaard et al., 1999), but in a direction opposite to the present results. Westergaard et al. (1999) reported a direct relationship between CSF 5-HIAA concentrations and eventual social rank. In the present study, we found that individually housed female monkeys' CSF 5-HIAA levels were higher in eventual subordinate monkeys. The differences in results between the two studies may be that Westergaard et al. (1999) used adolescent female macaques that were approximately 2 years old, while monkeys in the present study were adults between 9-17 years old. This may suggest that developmental changes occur within 5-HT systems and that relationships apparent at a young age may be reversed as the subject ages. It is worthy of note, however, that we have observed a similar association between social rank and NOR in a group of male cynomolgus monkeys younger than the females used in the present study (n = 21, mean age =10.3 ± 3.2 years, unpublished data). Further studies using a within-subject design will be needed to more directly address this important issue.

As was observed in individually housed male monkeys (Morgan et al., 2000), cortisol levels were not predictive of eventual social rank. In the earlier study, plasma cortisol levels were only measured once; in the present study cortisol was measured three times per week for 4 weeks, but significant differences related to eventual social rank and cortisol levels were not observed at any time point, nor were differences observed after stable social group formation. Similarly, adrenal responsiveness, as assessed using DEX suppression and ACTH administration was not predictive of social rank, nor were there

differences following the establishment of stable social groups. A recent study from our group reported transient differences in cortisol levels in male monkeys early in social hierarchy formation (Czoty et al., in press). In that study, eventual subordinate monkeys had significantly higher evening cortisol levels compared to eventual dominant monkeys over the first three days of social housing, but by the fourth day no differences were observed (Czoty et al., in press). In addition, following social housing and DEX suppression, ACTH-induced cortisol release was significantly greater in subordinates (Czoty et al., in press). Taken together, these data suggest that social rank-related differences in basal cortisol concentrations are transient and that compensatory mechanisms are rapidly recruited that normalize circulating cortisol concentrations. In addition, it appears that there may be sex differences in adrenal responsiveness, with males being more sensitive than female macaques. It should be cautioned, however, that this conclusion is likely to be limited to the precise conditions employed in the present study.

Several measures of 5-HT function, which have been implicated in social behavior in nonhuman primates (Kaplan et al., 2002, Raleigh et al., 1983), were also examined in these female monkeys. WBS is purported to be an indirect measure of central 5-HT reuptake (Fekkes et al., 1997; Mann et al., 1992). WBS has been shown to be higher in adolescent boys with conduct disorder and increased aggression (Unis et al., 1997). In animal studies, WBS levels were higher in less impulsive marmosets as exhibited by more inhibition in response toward a stranger (Kinnally et al., 2006), as well as dominant male vervet monkeys (Raleigh et al., 1983). In the present study, WBS was not predictive of eventual social rank nor was it different between dominant and

subordinate monkeys during social housing. Moreover, WBS did not correlate with central measures of 5-HT activity, CSF 5-HIAA or SERT availability. It is not clear whether the lack of sensitivity of WBS in the present study compared to earlier work is related to sex differences or the duration of social housing (which was only 3 months in the present study). Nonetheless, this measure was insensitive to housing conditions in adult female monkeys.

PET imaging studies of dopamine D2 receptor availability have reported differences as a function of social rank in male and female monkeys (Grant et al., 1998; Morgan et al., 2002). Importantly, levels of D2 availability appear to be sensitive to changes in housing conditions, such that the transition from individual to social housing resulted in significant increases in D2 levels in dominant male monkeys but no change in subordinate monkeys (Morgan et al., 2002). The present experiment extended these studies to SERT availability. SERT availability was not predictive of eventual social rank and did not significantly change in any monkey following the establishment of stable social groups. These findings were surprising considering the observed rank-related difference in 5-HIAA levels. Based on earlier imaging work (see Laruelle, 2000), we hypothesized that SERT availability would vary with differences in extracellular 5-HT levels, which was suggested by the observed difference in 5-HIAA concentrations. One possible reason for the discrepancy is that it is presently unclear whether [^{11}C]DASB competes with endogenous 5-HT for the same SERT binding sites (Lundquist et al., 2005; Tabot et al., 2005; Yamamoto et al., 2007). Future microdialysis studies examining the neurochemical changes associated with social group formation would provide important information related to the role of this neurotransmitter system in social

behavior. The lack of a difference in SERT availability between dominant and subordinate monkeys was also unexpected in light of studies showing differences in SERT availability, SERT mRNA levels, and polymorphisms of the SERT gene in humans and laboratory animals that correlated with differences in impulsivity or social stress (Berton et al., 1999; Filipenko et al., 2002; Frankle et al., 2005; Paaver et al., 2007). This may also be due to the regions studied. However, other brain regions related to impulsivity, such as nucleus accumbens and prefrontal cortex, are difficult to study with PET because their target receptor regions are small with respect to the PET scanner's image resolution. Future studies examining the functional nature of the 5-HT system, using indirect and direct-acting agonists and antagonists, may uncover important differences based on social rank.

Acknowledgements

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Table I. Mean (± SEM) concentrations of 5-HIAA (pmol/ml) in CSF as a function of menstrual cycle phase in individual monkeys.

Monkey	Follicular Phase	Luteal Phase
	5-HIAA	**5-HIAA**
6804	307.54	362.13
6812	310.94	264.53
7371	346.67	352.82
7373	198.25	182.33
7376	349.56	376.13
7377	333.67	287.04
7370	330.29	293.90
7372	390.13	341.40
7374	459.16	388.37
6816	254.59	247.12
6817	314.63	272.58
7375	346.31	339.19
Mean	**328.48**	**308.96**
SEM	**19.36**	**18.50**

FIGURE CAPTIONS

Figure 1. *Left*: Mean (± S.E.M.) number of crossings in a novel environment over 15 min in monkeys that eventually became dominant (Dom, *n*=4), intermediate (Inter, *n*=8) and subordinate (Sub, *n*=4). *Right*: Latency to touch a novel opaque acrylic box as a function of eventual social rank. * $P<0.05$

Figure 2. Mean (± SEM) cortisol concentrations (μg/dl) at baseline (24 hrs), following DEX suppression and 15 and 30 min after administration of 10 ng/kg Cortrosyn, while individually housed (*top*) and following stable social hierarchies (*bottom*).

Figure 3. CSF 5-HIAA concentrations in female cynomolgus monkeys as a function of eventual social rank while individually housed (*left*) and following stable social group formation (*right*). Symbols represent individual subject data; horizontal lines represent group means for dominant (n=4), intermediate (n=8), and subordinate groups (n=4). All samples were obtained in the follicular phase. *p<0.05.

Figure 4. SERT availability as measured by [^{11}C]DASB in individually housed monkeys (*top*) and following stable social group formation (*bottom*) in the caudate nucleus (Cd), putamen (Pt), hippocampus (Hip), thalamus (Tha) and anterior cingulate cortex (ACC). Values represent mean ± SEM for dominant (n=4) and subordinate (n=4) ranked monkeys.
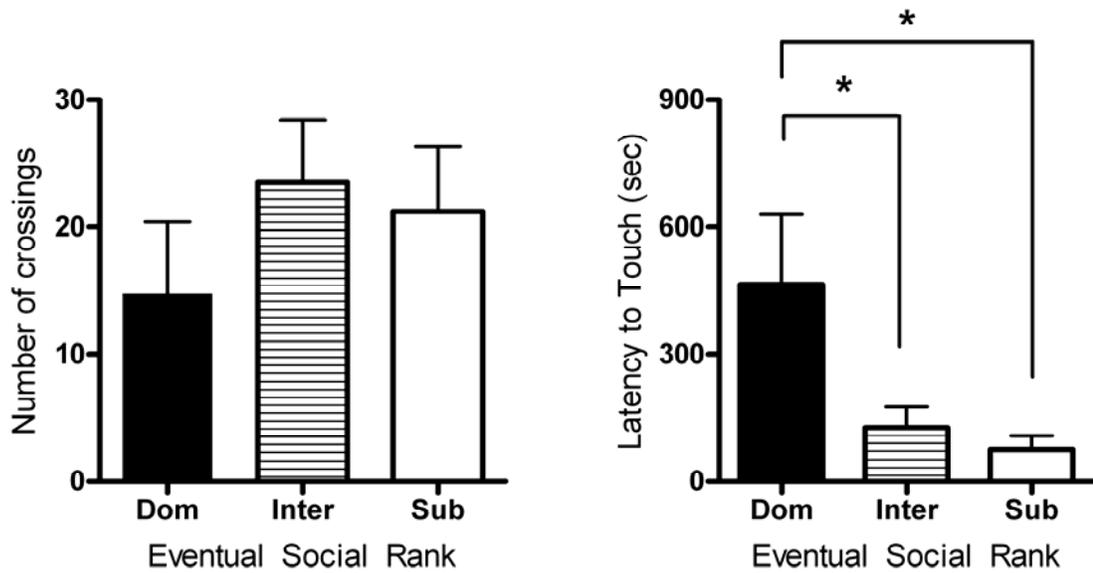
Figure 1.
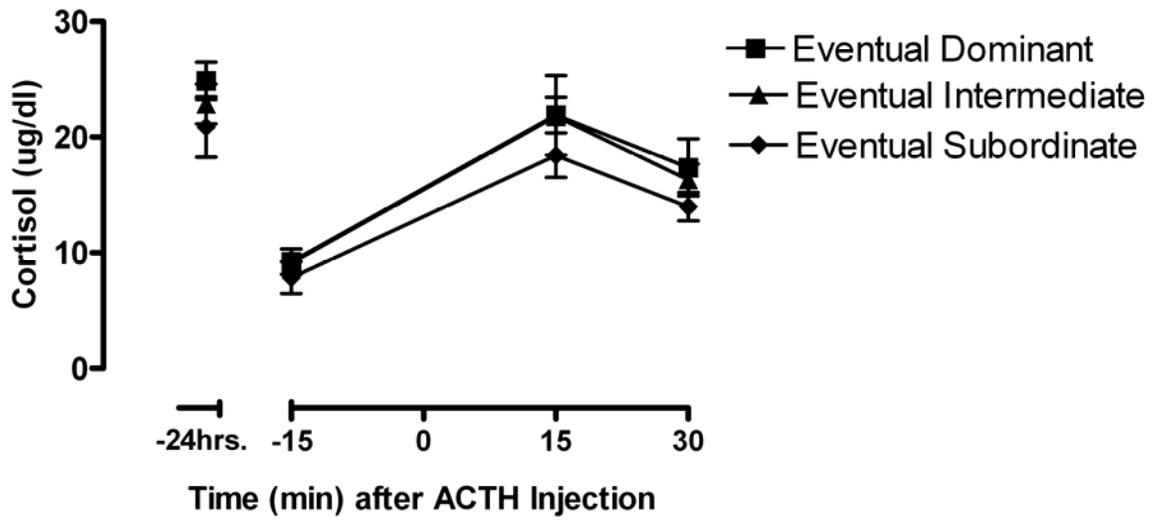
Figure 2.
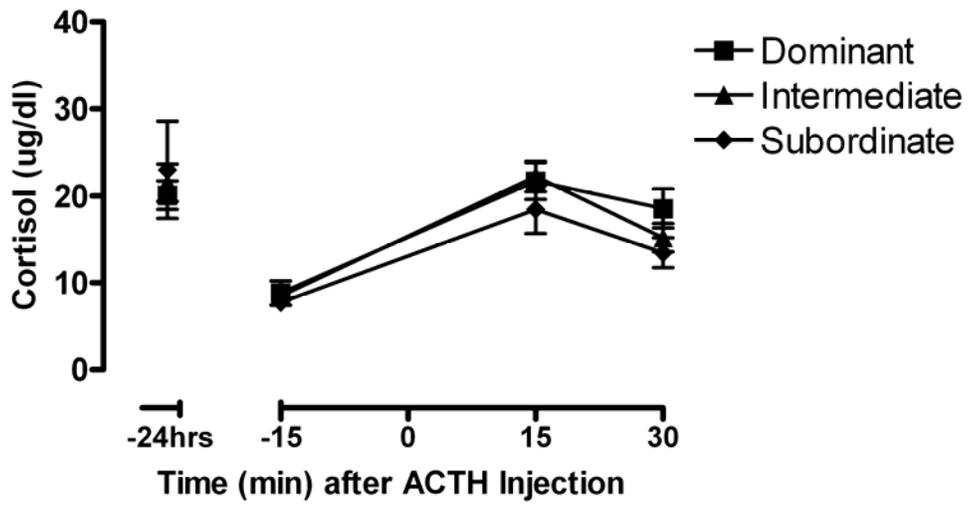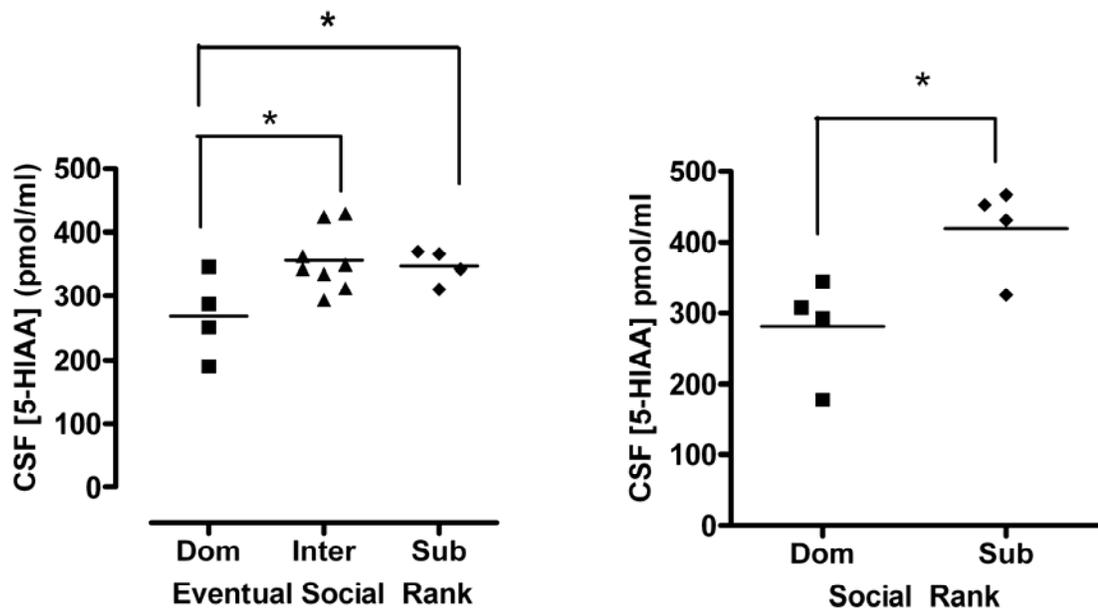
Figure 3.
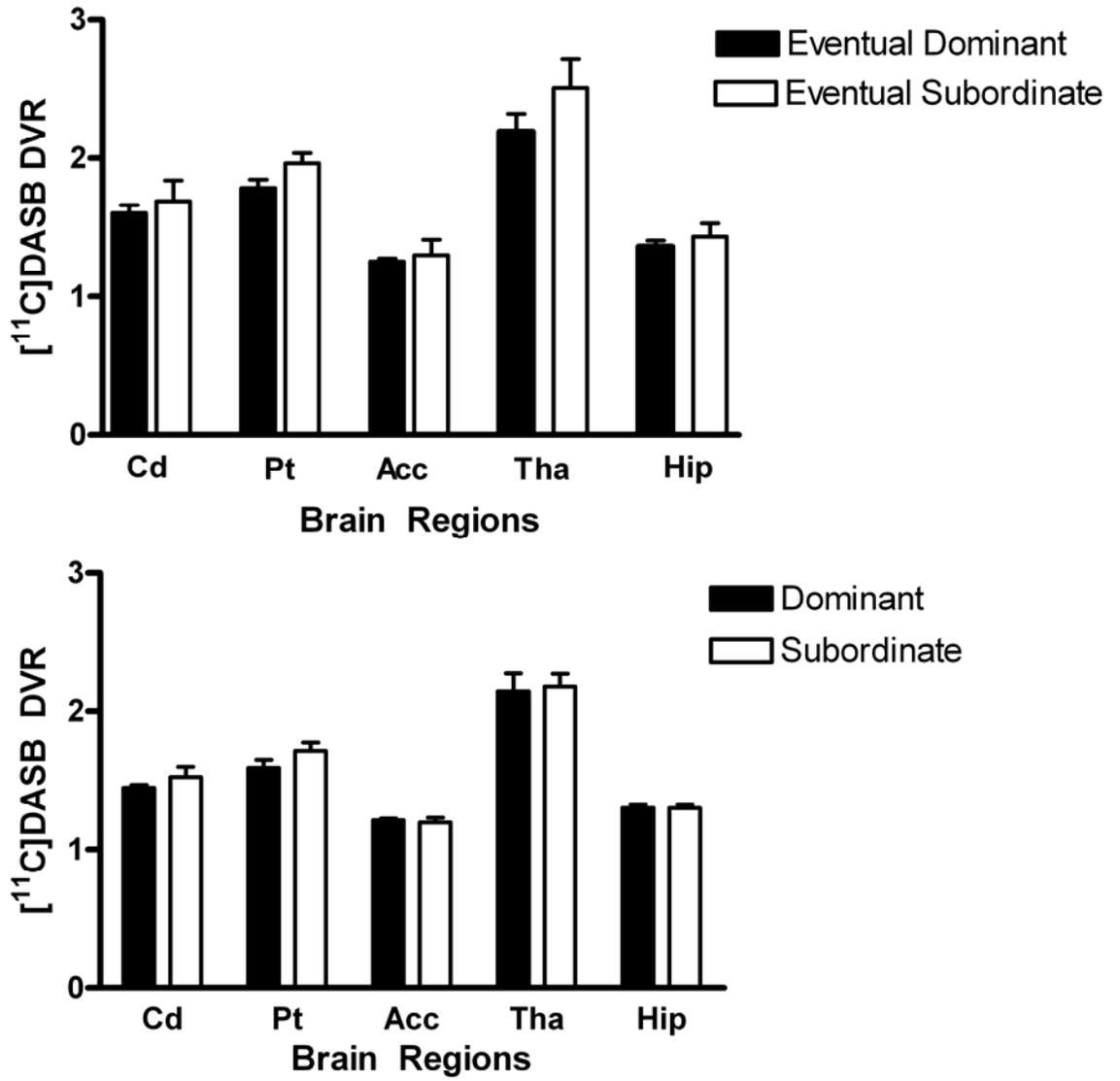
Figure 4.

CHAPTER III

SOCIAL RANK AND MENSTRUAL CYCLE PHASE INFLUENCE ON

COCAINE SENSITIVITY IN SOCIALLY HOUSED FEMALE MONKEYS

Natallia V. Riddick, Paul W. Czoty, Jay R. Kaplan, Michelle Icenhower, and Michael A.

Nader

ABSTRACT

Socially housed monkeys have been used to study stress influences on various disease states including drug abuse. Earlier work in male monkeys has shown that subordinate monkeys are more sensitive to cocaine reinforcement compared to dominant animals. The present study examined the influence of social rank and menstrual cycle phase on acquisition and early maintenance of cocaine self-administration in female cynomolgus monkeys (*Macaca fascicularis*) housed in groups of four monkeys per pen. The menstrual cycle was monitored by daily vaginal swabbing and was determined to be approximately 28 days in these monkeys. Each monkey was surgically prepared with an indwelling intravenous catheter and trained to respond under a fixed-ratio 30 schedule of food reinforcement. When stable, saline was substituted for food for at least 5 consecutive sessions. After a return to food-reinforced responding, increasing doses of cocaine (0.001-0.1 mg/kg) were substituted in ascending order until cocaine-maintained responding was significantly higher than saline self-administration. After acquisition, a complete cocaine dose-response curve was determined in both phases of the menstrual cycle. More dominant female monkeys (5 of 8) acquired cocaine self-administration at the lowest dose (0.001 mg/kg) than subordinate monkeys (2 of 7); this difference persisted at all doses studied in acquisition. After all doses were tested, the dose of cocaine that resulted in peak response rates for dominant monkeys was to the left of those for subordinate monkeys. There was no effect of menstrual cycle phase on cocaine self-administration in any monkey. These findings contrast earlier work in male monkeys and suggest that dominant female monkeys are more vulnerable to cocaine reinforcement

than subordinate monkeys and highlight the importance of sex differences in animal models of cocaine abuse.

INTRODUCTION

Drug abuse has become a major societal problem during the 20th century, costing billions of dollars in economic expenses which include health care, accidents, crime related to substance use and loss of productivity (WHO, 2004). In 2006 an estimated 20.4 million Americans used illicit drugs with 2.4 million specifically abusing cocaine (SAMHSA, 2006). Although there are more men than women illicit drug abusers in the US (10.5% vs. 6.2%, respectively), rate of stimulant use is similar and increasing in females (SAMHSA, 2006). A growing body of clinical and preclinical literature has revealed the sexually dimorphic nature of the reinforcing (S^R) and subjective (i.e. discriminative stimulus (S^D)) effects of cocaine prompting the importance of using female subjects in more studies (Lynch et al., 2002; Roth et al., 2004; White et al., 2002). Although cocaine use is less prevalent in females, they are more vulnerable to detrimental consequences of substance use (Greenfield et al., 2003). Women have increased sensitivity to drug cues and most importantly appear to be more vulnerable to abuse-related effects of cocaine (Kilts et al., 2004; Kosten et al., 1993).

Animal studies reveal a range of factors that can influence sensitivity to drugs in females. Ovarian hormones are the most frequently described risk factor for cocaine abuse (Campbell and Carroll, 2000; Lynch et al., 2001). In intravenous (i.v.) cocaine self-administration studies, estradiol (ES) was demonstrated to modulate cocaine S^R under some conditions (Hu and Becker, 2008; Jackson et al., 2006; Lynch et al., 2001, Lynch and Carroll, 1999; Lynch and Taylor, 2005; Roberts et al., 1989) but not others (Caine et al., 2004; Mello et al., 2008). For example in acquisition studies, female rats acquired cocaine self-administration faster than male rats (Lynch and Carroll, 1999). Intact and ES

treated ovariectomized (OVX) rats displayed enhanced acquisition compared to nontreated OVX and tamoxifen (i.e. ES antagonist) treated intact rats (Hu et al., 2004; Lynch et al., 2001).   The importance of studying multiple phases of cocaine reinforcement and sex differences can be observed in two rodent studies. Hu and colleagues (2004) found that ES facilitated acquisition of cocaine self-administration under an FR 1 schedule in females over males, but no sex differences were observed under maintenance conditions (Lynch et al., 2000). Similarly, in normally cycling rhesus monkeys responding under FR schedule of cocaine presentation, dose-response curves were not altered by acute ES pretreatments (Mello et al., 2008). Taken together, these findings suggest that ES may facilitate acquisition of cocaine self-administration but, once acquired, does not consistently affect maintenances of cocaine self-administration.

While ES is more commonly associated with enhancing reinforcing effects of cocaine, PG is thought to counteract the facility effect (Jackson et al., 2006). In intact rats, PG decreased cocaine-induced reinstatement (Anker et al., 2006), while, in monkeys, Mello and colleagues (2005) reported dose-dependent decreases in reinforcing effectiveness of cocaine following PG treatment.   In women, PG administration attenuated the positive subjective effects of cocaine (Evans and Foltin, 2006; Sofouglu et al., 1999, 2002). Similar finding were reported by Sofuoglu and colleagues (2004) on the subjective effects, however, no differences were found in subsequent self-administration studies. In rhesus monkeys, responding on PR schedule of cocaine presentation, break points were lower in luteal phase of the menstrual cycle when PG levels were high; however, this effect was only apparent for the lowest cocaine dose tested which was not consistently reinforcing in all the monkeys (Mello et al., 2007). Taken together, these

studies suggest that ovarian hormones can significantly impact cocaine reinforcement. The present study extends the examination of menstrual cycle phase and cocaine reinforcement in nonhuman primates to vulnerability to acquire cocaine self-administration and to changes in response rates under maintenance conditions in socially housed monkeys.

Although ovarian hormones are the more commonly described source of differential cocaine sensitivity in females, acute and chronic stress are commonly found risk factors for drug abuse in both human and animal studies (Back et al., 2005, 2008; de Wit et al., 2003; Miczek and Mutschler, 1996; Morgan et al., 2002). Physical stressors such as tail pinch and unpredictable foot shock potentiated acquisition of cocaine self-administration in rats (Goeders and Guerin, 1994; Piazza et al., 1990). Acute social stress, such as facing an aggressive opponent, led to increased cocaine self-administration (e.g. Miczek and Mutschler, 1996). In addition, control rats as well as rats with low propensity to self-administer psychostimulants (low responders in the novel open field) showed enhanced acquisition of cocaine self-administration following social defeat by an aggressive intruder (Kabbaj et al., 2001).

The study of social rank and health in nonhuman primates has been frequently related to different levels of stress. When received chronically, stress can increase risks for numerous diseases (Sapolsky, 2005). In addition, dominance hierarchies produce marked inequalities in division of resources (e.g. food, space, access to sexual partners, etc.); thus, an animal's social rank can profoundly affect its quality of life and its health (Kaplan, 1987; Sapolsky, 2005). Among female cynomolgus monkeys, social subordinates received more aggression and were groomed less often; these behavioral

outcomes have been associated with increased risk for coronary artery atherosclerosis, poor ovarian function, and adrenal hypersecretion of cortisol (Kaplan et al., 1987; Shively et al., 1986, 1997).   On the other hand, dominant animals are generally considered to be healthy due to their "enriched" environments. Dominance rank was found to be inversely correlated with alcohol intake during social housing but was not with control fluid intake in male and female squirrel monkeys (McKenzie-Quirk and Miczek, 2008). Furthermore, stress of extended social separation increased alcohol drinking in male monkeys but not females indicating sex differences in response to different type of social stressors. In socially housed male cynomolgus monkeys, cocaine was not a reinforcer in dominant male monkeys (Morgan et al., 2002), providing powerful evidence for the importance of social variables in cocaine reinforcement. The present study extended this work to female cynomolgus monkeys living in social groups of four monkeys per pen. Cocaine was made available under a fixed-ratio schedule similar to that used by Morgan et al. (2002). We hypothesized that subordinate females would be more sensitive to the reinforcing effects of cocaine compared to dominant monkeys.

METHODS

Subjects: Sixteen drug-naïve adult female cynomolgus monkeys (*Macaca fascicularis*) 10-20 years old served as subjects. While in quarantine, monkeys were fed *ad libitum*; their weights at the end of this period were used as an indication of free-feeding weights. Throughout the study, monkeys were weighed weekly and body weights were maintained at approximately 95% of free-feeding weights by food earned during experimental

sessions and by supplemental food (Purina Monkey Chow LabDiet #5038 and fresh fruit); animals had unlimited access to water in their home cage. Prior to the start of these experiments, one monkey became ill and died. Each monkey was socially housed (3 pens of 4 monkeys/pen and one pen of 3 monkeys) in quadrant cages (Model #PR242732I4, Allentown Caging Inc., Allentown, NJ), equipped with removable metal partitions (a wire mesh and a solid partition) separating the animals during feeding (see Riddick et al., 2008 for details). Each quadrant measured 0.71 x 0.84 x 0.84 m. When partitions were removed, the total living space for the pen measured 0.71 x 1.73 x 1.73 m. Prior to the start of this experiment, monkeys had lived in stable social groups for at least 5 months. Social rank was confirmed weekly.

Each animal was trained to sit calmly in a primate chair (Primate Products, Inc., Redwood, CA) and studied in operant chambers, as described previously (e.g., Morgan et al., 2000; Czoty et al., 2005). After the session, each monkey was placed into a separate quadrant and, after at least 30 min, given enough food to maintain body weight at approximately 95% of its free-feeding weight. Monkeys were given at least 2 hr to finish their food before the partitions were removed. Animal housing and all experimental procedures were performed in accordance with the 2003 National Research Council *Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research* and were approved by the Animal Care and Use Committee of Wake Forest University. Environmental enrichment was provided as outlined in the Animal Care and Use Committee of Wake Forest University Non-Human Primate Environmental Enrichment Plan.

Menstrual Cycle Phase Determination: The duration of the menstrual cycle was initially assessed by daily vaginal swabs over several months (see Czoty et al., 2008). The first day of bleeding was considered menses and was counted as day 1 of the cycle. Once a complete cycle of approximately 28 days was observed, experimental procedures scheduled during days 2-10 were designated as the follicular phase and procedures on days 19-27 were designated as the luteal phase of the menstrual cycle. To confirm that monkeys were in the appropriate phase during all the experiments, on the first day of each dose of cocaine availability 3 ml of blood was drawn from the femoral vein for hormone assessment. The blood was centrifuged (Beckman Coulter, GPR Centrifuge) at room temperature for 30 min at 3000 rpm, the serum was aspirated into an Eppendorf tube and stored at -30º C. Estradiol and progesterone levels were assessed by the Biomarkers Core Laboratory of the Yerkes National Primate Research Center of Emory University in Atlanta, GA. Progesterone levels < 2 ng/ml were indicative of follicular phase and higher were indicative of the luteal phase.

Apparatus: Experimental sessions were carried out in ventilated and sound-attenuated chambers (1.5 × 0.74 × 0.76 m; Med Associates, East Fairfield, VT) designed to accommodate a primate chair. An intelligence panel (48 × 69 cm) was located on the right side of the chamber, 0.72 m from the bottom. The panel contained two translucent Plexiglas response keys with three stimulus lights centrally located above (14 cm) each key. The keys were positioned to be within easy reach of the monkey sitting in the primate chair. Also located on the panel were a food receptacle and an attachment site for a tygon tube that delivered 1-g pellets from a feeder located on the top of the chamber. A

peristaltic infusion pump (Model 7531-10, Cole-Parmer Co., Chicago, IL) for delivering drug injections at a rate of approximately 1 ml/10 s was located on the top of the chamber. Different unit doses of cocaine were arranged by altering the concentration of the cocaine solution. Operation of the chambers and data acquisition was accomplished with a computer system and associated interfaces (Macintosh PowerPC; Med Associates, East Fairfield, VT).

Surgery: Each monkey was surgically prepared under sterile conditions with a vascular access port (Model GPV, Access Technologies, Skokie, Illinois), implanted under ketamine (15 mg/kg) and isoflurane (1.5%) anesthesia. An incision was made near the femoral vein, and the catheter was inserted into the vein for a distance calculated to terminate in the vena cava. The distal end of the catheter was threaded subcutaneously to an incision made slightly off the midline of the back. The vascular access port was placed within a pocket formed by blunt dissection near the incision. Antibiotic (25 mg/kg kefzol, BID; Cefazolin sodium, Marsam Pharmaceuticals, Cherry Hill, New Jersey) was administered prophylactically on the day of the surgery, given as a bolus approximately 1 hour before the surgery. A nonsteroidal anti-inflammatory drug, Ketoprofen (5 mg/kg), was administered i.m. for pain and inflammation for 3 days beginning on the day of the surgery.

Daily Self-Administration Sessions: Each morning (5−7 days/week), monkeys from each pen were individually housed by partitioning the cage into four quadrants. Next, each monkey was seated in a primate chair (Primate Products) and the chair was wheeled to the operant chamber (Med Associates, East Fairfield, Vermont). The back of the

animal was cleaned with chlorhexaderm and sterile water, and a 20 gauge Huber Point Needle (Access Technologies) was inserted into the port, connecting the infusion pump to the catheter. The pump was operated for approximately 3 s, filling the port and catheter with the dose of drug/vehicle available during the experimental session. At the end of each session, the port and catheter were filled with a solution containing heparin (100 Units/ml) in an effort to prevent clotting.

Experiment 1: Effect of social rank on acquisition of cocaine self-administration. In the presence of an illuminated white light over the right or left key, monkeys were trained to respond on the appropriate key to deliver a 1-g berry-flavored pellet (fixed-ratio 1; FR 1 schedule of reinforcement). Eight monkeys were trained to respond on the right key and seven were trained to respond on the left key. Following a response, the white stimulus light was extinguished, and a red light was illuminated above the depressed key and a pellet was delivered; the red stimulus remained illuminated for 10 s after which the white light was illuminated. Sessions ended after 15 food presentations or one hour. Over the next 2 weeks, the ratio requirement for pellet delivery was gradually increased until a final ratio of 30 was reached for each monkey (FR 30); sessions ended after 15 reinforcers were earned or 1 hour elapsed. When food-reinforced responding was deemed stable each monkey was implanted with a subcutaneous vascular port and intravenous catheter as described above. After at least 5 consecutive sessions and once stable food-reinforced responding was re-established (response rates were ±20% of the mean for the last 3 sessions), responding was extinguished by substituting saline for food. During saline substitution, the maximum number of stimulus presentations was increased to 30.

Saline was available for at least 5 consecutive sessions and until responding had decreased by at least 80% of food-maintained baseline. Following re-establishment of food-maintained responding, acquisition of cocaine self-administration was examined. At the onset of the acquisition studies, a low dose of cocaine (0.001 mg/kg) was substituted for food and doses were tested in ascending order until cocaine-maintained responding was higher than responding leading to saline injections. The same cocaine dose was tested in both (successive) phases of the menstrual cycle until acquisition has occurred; as with saline, the maximum number of injections was 30 per session and there was a return to food-reinforced baseline between doses. Cocaine doses were tested for at least 5 consecutive sessions and until responding was deemed stable. If responding was not stable by the end of a menstrual cycle phase, food was substituted for cocaine and that dose was retested when the monkey returned to that phase.

Experiment 2: Effect of social rank and menstrual cycle phase on cocaine self-administration. Following acquisition of the cocaine self-administration, a dose-response curve was determined for each animal in both phases of the menstrual cycle. Each dose was tested in random order within the dose range of 0.001-0.03 mg/kg for most animals. Each new dose substitution was separated by at least 3 sessions of food-reinforced responding. Unlike the acquisition phase, different doses of cocaine were tested in consecutive phases of the menstrual cycle.

Drugs and Chemicals: A stock solution of cocaine HCl (National Institute on Drug Abuse, Bethesda, MD, USA) was first prepared by dissolving it in sterile saline to a

concentration of 10-100 mg/ml. Doses (0.001-0.1 mg/kg) were prepared in 250 or 100 ml

sterile saline bags and delivered i.v. at a rate of approximately 1.5 ml/10 sec.

Statistical Analysis: For acquisition and maintenance of cocaine self-administration ($n =$

15), animals were considered either dominant (rank of 1 or 2, $n = 8$) or subordinate (rank

of 3 or 4, $n = 7$). The primary dependent variables were response rates and cocaine

intake. Paired t-tests were used to determine whether a particular cocaine dose was

reinforcing during the acquisition studies and to compare progesterone and estradiol

concentrations between the two phases of the menstrual cycle. A survival analysis and

Wilcoxon Rank Sum Test was used to compare the dose of acquisition and percentage of

dominant and subordinate monkeys acquiring cocaine self-administration. For menstrual

cycle phase and social rank effects on cocaine self administration (n=15), a repeated-

measures ANOVA with dose (4 levels excluding saline) and menstrual cycle phase (2

levels, follicular and luteal) as the within-subject variables and social rank (that is,

dominant and subordinate) as the between-subject variable was conducted. Fisher LSD

(Protected t-test) test was used for post-hoc analysis. A repeated-measures one-way

ANOVA was used to determine reinforcing doses in each group of monkeys in two

menstrual cycle phases. Dunnett Multiple Comparisons Test was used for post-hoc

analysis to compare each cocaine-maintained response rates to saline-maintained

response rates. Differences were considered statistically significant when p < 0.05.

RESULTS

When food-maintained responding was stable under the FR 30 schedule of reinforcement and prior to saline substitution, baseline response rates were not significantly different as a function of social rank, with mean rates for dominant and subordinate monkeys being 0.83 ± 0.23 and 0.91 ± 0.18 resp/sec, respectively (Table 1). When saline was substituted for food, response rates typically declined by at least 80% of baseline within 5 sessions in all monkeys. When saline self-administration was stable, mean response rates were not significantly different as a function of social rank (0.08 ± 0.02 resp/sec; Table 1).

Experiment 1: Effect of social rank on acquisition of cocaine self administration

Cocaine acquisition was operationally defined as the lowest dose that resulted in response rates that were significantly higher than response rates when saline was available. At the lowest dose tested, 0.001 mg/kg cocaine, approximately 62% (5 of 8) of the dominant monkeys acquired cocaine reinforcement, in contrast to approximately 28% (2 of 7) of the subordinate animals (Fig. 1). Differences between dominant and subordinate monkeys at this dose were not related to menstrual cycle phase. All but one dominant monkey acquired cocaine self-administration when 0.01 mg/kg cocaine was available (Fig. 1). For subordinate monkeys, all but one acquired cocaine reinforcement when 0.03 mg/kg cocaine was available. For the other two monkeys, a dose of 0.1 mg/kg cocaine was tested; the subordinate monkey acquired at this dose, the dominant animal did not. A Wilcoxon Rank Sum Test compared the distributions of the two curves (dominant vs. subordinate) with the outcome at each dose for each group being the

percent of monkeys that acquired cocaine self-administration (Fig. 1) and found that across doses, the curves were not significantly different [Z =-0.7402, p= 0.23].

Experiment 2: Effect of social rank and menstrual cycle phase on cocaine self-administration.

Following acquisition, the remaining cocaine doses were tested in random order in both follicular and luteal phases of the menstrual cycle (Fig. 2; Fig.3-6 for individual data). Within a social rank, both dominant and subordinate animals self-administered cocaine reliably across cocaine doses and during both menstrual cycle phases. There was no overall effect of menstrual cycle phase on rates of cocaine self-administration in dominant and subordinate animals (Fig. 2). Repeated-measures ANOVA demonstrated a significant effect of dose [$F(3, 39)=3.32$, $p=0.03$]. Irrespective of menstrual cycle phase, response rates were significantly higher at most cocaine doses in dominant compared to subordinate monkeys as demonstrated by Fisher post-hoc analysis. Repeated-measures one-way ANOVA and post-hoc analysis from mean data revealed that 0.003 and 0.01 mg/kg doses were self-administered above saline levels during the follicular and 0.003 mg/kg in the luteal phases by dominant monkeys (Fig. 2). Progesterone concentrations taken on the day when each new cocaine dose was tested confirmed that the monkeys were in the correct phase. Plasma progesterone concentrations differed significantly ($p<0.05$) between follicular and luteal phases in dominant (0.99 ± 0.07 and 9.45 ± 1.42 ng/ml, respectively) and subordinate (0.77 ± 0.09 and 7.32 ± 1.38 ng/ml, respectively) monkeys (Table 3); there were no differences between dominant and subordinate monkeys. There was no difference in estradiol concentrations as a function of menstrual

cycle phase in monkey of either rank. Despite differences in response rates as a function of cocaine dose, there was no significant difference in average cocaine intake per session as a function of rank or menstrual cycle phase (Table 2, Fig. 7).

DISCUSSION

The two goals of the present study were to evaluate the effect of social rank on acquisition of cocaine self-administration and to examine how social rank and menstrual cycle phase influence the reinforcing effects of cocaine in socially housed female monkeys. We demonstrated a trend for dominant female monkeys to be more vulnerable to reinforcing effects of cocaine by acquiring self-administration at lower doses than subordinate monkeys. Furthermore, across a wide range of doses, cocaine was a more potent reinforcer and resulted in higher response rates in dominant monkeys compared to subordinate animals. Finally, menstrual cycle phase did not influence the reinforcing effects of cocaine in any monkey. These findings support earlier work indicating the powerful effects of social variables in cocaine reinforcement and highlight the need for systematic studies of sex differences in animal models of cocaine abuse.

This is the first study to examine the effect of social rank on cocaine reinforcement in socially housed female monkeys. One goal was to replicate earlier work in male monkeys that reported greater sensitivity in subordinate monkeys when cocaine self-administered was studied (Morgan et al., 2002). While similar neuropharmacological effects were seen in relation to dopamine D2 receptor availability (unpublished observation), the present findings with cocaine self-administration were the opposite – dominant monkeys acquired cocaine at lower doses and maintained higher rates of

responding than subordinate female monkeys,. While the precise mechanisms accounting for these discrepant findings are currently being evaluated, these results suggest profound sex differences in cocaine self-administration in socially housed monkeys.

Examining the cocaine dose-response curves demonstrated that cocaine functioned as a more potent reinforcer in dominant females than subordinates. However, total cocaine intake per session did not differ as a function of rank which was likely due to the ceiling effect since maximum number of injection was preset at thirty. Each self-administration session ended when thirty injections were obtained or one hour expired. Because of the long session length, reinforcement frequency and, therefore, cocaine intake is not the best measure of sensitivity to cocaine reinforcement. While social rank differences in cocaine intake were noted in male monkeys (Morgan et al., 2002), these animals rarely earned all reinforcers at the high end of the cocaine dose-response curve.

Previous work by Mello and colleagues (2007, 2008) also demonstrated sex differences in cocaine self-administration in individually housed rhesus monkeys. They showed that regardless of ovulatory or anovulatory menstrual cycle, female rhesus monkeys had significantly higher cocaine break points under PR schedule of reinforcement than males, suggesting neurobiological substrates other than ovarian hormones to be responsible for sex differences observed (Mello et al., 2007). In the present study under an FR 30 reinforcement schedule, menstrual cycle phase did not have an overall effect on cocaine-maintained responding in dominant and subordinate monkeys or when examined independent of social rank. Similar to these findings, Mello and colleagues (2007, 2008) did not observe a consistent effect of menstrual cycle phase or estradiol pretreatment on reinforcing effects of cocaine in female monkeys responding

under FR schedule of cocaine presentation. However, under PR schedules of reinforcement, breakpoints tended to be higher in the early- and mid-follicular phase of the menstrual cycle compared to late-luteal when the cocaine dose tested was very low (Mello et al., 2007). Taken together, previous and present findings suggest that menstrual cycle phase has minimal impact on the reinforcing effects of cocaine in individually or socially housed female monkeys. Studies in humans have reported effects of menstrual cycle phase (Terner and de Wit, 2006), although these effects may be related more to subjective effects of cocaine rather than reinforcing effects. Future studies examining menstrual cycle phase and cocaine discrimination in monkeys is clearly needed.

There are several possible reasons for the observed sex differences in socially housed monkeys. First, the sex differences may be due to lower sensitivity to stress in female monkeys. Subordinate male monkeys had enhanced sensitivity to ACTH administration following dexamethasone (DEX) suppression compared to dominants, but no differences were found in female monkeys as a function of social rank (Czoty et al., in press; Riddick et al., in press). In addition, eventually subordinate male monkeys had higher cortisol concentration than dominants during the first three days of social housing, whereas the females only showed differences on the first day (Czoty et al., in press; unpublished data). Taken together, these findings suggest that females are likely less sensitive to stress-induced alterations in the adrenal gland function than males and HPA compensatory mechanisms may be recruited faster in females than males. Interestingly, in male rats, increased sensitivity to cocaine during early exposure (i.e. acquisition) was positively correlated with stress-induced increases in plasma corticosterone (for review see Goeders, 2002) Thus, unlike in males, adrenal gland responsiveness may not

contribute to rank-related differences in cocaine sensitivity suggesting sex differences in regulatory mechanisms contributing to increased cocaine sensitivity.

A second potential reason for sex differences is that ovarian hormones may affect the DA system in females by altering DA activity and/or DA receptor systems leading to differences in cocaine sensitivity. As mentioned earlier, subordinate male monkeys had significantly lower D2 receptor availability than dominant males which translated into enhanced vulnerability to reinforcing effects of cocaine in subordinate animals (Morgan et al., 2002). While a similar relationship between D2 receptors and social rank was reported in socially housed female monkeys (Grant et al., 1998) and in the monkeys used in the present study (unpublished observation), it was dominant female monkeys that were more sensitive to cocaine reinforcement. It has been suggested that in females DA neurotransmission can be directly increased by estrogen leading to increased sensitivity to stimulants regardless of the receptor availability (Febo et al., 2003). Future studies are needed to better understand the interactions of dopamine D2 receptor availability and estrogen levels in socially housed female monkeys.

It remains possible that ovarian hormones and specifically estrogen may be responsible for enhancing cocaine sensitivity in dominant females compared to subordinates. Several different groups demonstrated that subordinate monkeys have impaired reproductive cycles primarily due to suppressed levels of ovarian hormones. Specifically, estradiol and progesterone deficiencies were observed in subordinate female monkeys compared to dominants (Adams et al., 1985; Kaplan and Manuck, 1999; Shively et al., 1997; Williams et al. 1994). Subordinate monkeys typically had plasma estradiol concentrations of about 60 pg/ml whereas dominants averaged about 130 pg/ml

(Williams et al., 1994). It is important to note that stress did not cause amenorrhea, but caused deleterious effects on sex hormone concentrations similar to women with ovarian dysfunction that can be severe enough to cause infertility (Shively et al., 1997; Wu, 1990). ES is more commonly associated with enhancing reinforcing effects of cocaine and PG is thought to counteract the facilitory effect (Jackson et al., 2006). In the present study, dominant females were more sensitive to reinforcing effects of cocaine than subordinates in both the follicular and luteal phase of the menstrual cycle. Therefore, ovarian hormones and particularly overall higher estradiol concentrations may be responsible for enhanced reinforcing potency of cocaine in dominants over subordinates. While we did not find differences in estradiol or progesterone concentrations as a function of rank on the first day each cocaine dose was tested, these were always time points early in follicular and luteal phases when both hormone concentrations were low. Differences in peak concentrations of these ovarian hormones may have contributed to rank-related differences in cocaine self-administration. Future studies are warranted to address this important issue.

   While menstrual cycle phase did not affect cocaine self-administration, a recent study indicated that dopamine D2 receptor availability is significantly altered by menstrual cycle phase (Czoty et al., in press). Thus, it appears that in females, the influence of D2 receptor availability is not as profound as in male subjects. In fact, examination of many studies linking the DA system to cocaine reinforcement finds that the studies were conducted in males (e.g., Volkow et al., 1999; Morgan et al., 2002; Czoty et al., 2005; Dalley et al., 2007). It appears that female subjects respond differently to social and environmental variables as it relates to cocaine reinforcement. These

findings support the hypothesis that treatment options need to consider gender differences when evaluating novel strategies.

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TABLE 1:   Baseline Food and Saline Performance

Rank*	Subjects	Mean Food[a]	Mean Sal[a]	# Sessions[b]
1	C-6812	0.26(0.01)	0.01(0.00)	6
1	C-7373[c]	0.56(0.02)	0.12(0.03)	6
1	C-6805	1.43(0.07)	0.11(0.01)	7
1	C-6817	0.69(0.04)	0.16(0.00)	6
	MEAN (SEM)	**0.74(0.25)**	**0.10(0.03)**	**6.25(0.25)**
2	C-7370	0.21(0.02)	0.02(0.01)	5
2	C-7371	1.03(0.06)	0.06(0.02)	5
2	C-7375[c]	0.41(0.05)	0.01(0.01)	6
2	C-6816	2.05(0.09)	0.05(0.01)	8
	MEAN(SEM)	**0.93(0.41)**	**0.04(0.01)**	**6.00(0.71)**
3	C-7374	0.97(0.06)	0.16(0.01)	5
3	C-6804	1.53(0.08)	0.03(0.02)	6
3	C-6820	0.76(0.04)	0.11(0.02)	7
	MEAN(SEM)	**1.09(0.23)**	**0.10(0.04)**	**6.00(0.58)**
4	C-6818	1.01(0.09)	0.05(0.03)	5
4	C-7377[c]	0.38(0.02)	0.06(0.01)	5
4	C-7372	0.32(0.03)	0.06(0.01)	5
4	C-6802	1.41(0.09)	0.07(0.03)	6
	MEAN(SEM)	**0.78 ± 0.26**	**0.09 ± 0.03**	**5.25 ± 0.25**

[a]response rates expressed in responses/second [b]number of session to meet the extinction criteria *dominant are ranked #1&2; subordinates are ranked #3&4 [c]monkeys that lived in a group of three animals

TABLE 2: Average cocaine intake per session (3 day mean) for each individual function of dose.

		Follicular Phase			Luteal Phase		
	Subjects	Mean Intake (mg/kg/session) per cocaine dose					
Rank		**0.001**	**0.003**	**0.01**	**0.001**	**0.003**	**0.01**
1	C-6812	0.003	0.008	0.043	0.002	0.008	0.027
1	C-7373	0.030	0.090	0.300	0.029	0.090	0.257
1	C-6805	0.030	0.090	0.297	0.030	NA	0.300
1	C-6817	0.027	0.087	0.300	0.026	0.090	0.300
2	C-7370	0.003	0.012	0.010	0.001	0.022	0.140
2	C-7371	0.030	0.090	0.300	0.027	0.090	0.300
2	C-7375	0.000	0.006	0.017	0.002	0.005	0.147
2	C-6816	0.024	0.090	0.300	0.030	0.090	0.300
MEAN(SEM):		**0.02(0.00)**	**0.06(0.01)**	**0.20(0.05)**	**0.02(0.00)**	**0.06(0.02)**	**0.22(0.04)**
3	C-6804	0.001	0.008	0.077	0.002	0.011	0.047
3	C-6820	0.005	0.007	0.143	0.011	0.008	NA
3	C-7374	0.023	0.090	0.300	0.017	0.090	0.300
4	C-6818	0.023	0.090	0.293	0.030	0.059	0.300
4	C-7377	0.013	0.080	0.030	0.030	NA	0.290
4	C-7372	0.004	0.004	0.007	0.013	NA	0.007
4	C-6802	0.007	0.019	0.013	0.008	0.017	0.293
MEAN (SEM):		**0.01(0.00)**	**0.04(0.02)**	**0.16(0.05)**	**0.01(0.00)**	**0.04(0.02)**	**0.21(0.06)**

Table 3: Progesterone (ng/ml) and estradiol (pg/ml) concentrations on the first day each new dose was available for dominant and subordinate monkeys.

Rank	Menstrual Cycle Phase			
	Follicular Phase		Luteal Phase	
Dominants:	PG*	ES**	PG	ES
#1	0.96(0.09)	26.34(3.69)	6.39(1.56)	30.78(6.82)
#2	1.03(0.11)	31.35(2.76)	12.09(2.11)	31.86(6.56)
MEAN (SEM):	**0.99(0.07)**	**28.88(2.29)**	**9.45(1.42)**	**31.51(4.42)**
Subordinates:	PG	ES	PG	ES
#3	1.03(0.13)	44.72(7.33)	5.08(1.37)	30.77(8.84)
#4	0.56(0.10)	30.50(4.97)	8.77(2.03)	16.07(2.40)
MEAN (SEM):	**0.77(0.09)**	**36.75(4.41)**	**7.32(1.38)**	**20.15(3.51)**

*progesterone (ng/ml): average (±SEM); **estradiol (pg/ml): average (±SEM)

FIGURE CAPTIONS

Figure 1. Percent of dominant (n=8) and subordinate (n=7) monkeys that acquired self-administration as a function of cocaine dose. *Numbers above each point indicate the number of monkeys that first acquired at that dose (numerator) and the number of monkeys tested (denominator). Response rates at each dose were compared to saline levels for each individual subject by paired t-test.

Figure 2. Mean ± SEM response rates (responses/sec) as a function of cocaine dose or saline (S) for dominant (rank 1 and 2, filled squares; n=8) and subordinate (rank 3 and 4, filled circles; n=7) monkeys studied in follicular *(Left)* and luteal *(Right)* phase of the menstrual cycle. The 0.1 mg/kg cocaine dose was tested in one dominant and one subordinate monkey during acquisition. Data represent the mean of the last 3 days of availability for each animal. *statistically significant difference ($p < 0.05$) from subordinate monkeys at that dose; # statistically significant difference ($p<0.05$) from the appropriate saline point.

Figure 3. Individual monkey response rates (responses/sec) as a function of cocaine dose or saline (S) for #1-ranked monkeys in follicular (filled squares) and luteal (filled circles) phase of menstrual cycle. Data represent the mean ± SEM of the last 3 days of availability for each animal.

Figure 4. Individual monkey response rates (responses/sec) as a function of cocaine dose or saline (S) for #2-ranked monkeys in follicular (filled squares) and luteal (filled circles) phase of menstrual cycle. All other information is as described in Figure 3.

Figure 5. Individual monkey response rates (responses/sec) as a function of cocaine dose or saline (S) for #3-ranked monkeys in follicular (filled squares) and luteal (filled circles) phase of menstrual cycle. All other information is as described in Figure 3.

Figure 6. Individual monkey response rates (responses/sec) as a function of cocaine dose or saline (S) for #4-ranked monkeys in follicular (filled squares) and luteal (filled circles) phase of menstrual cycle. All other information is as described in Figure 3.

Figure 7. Mean ± SEM cocaine intake (mg/kg/session) as a function of cocaine dose or saline (S) for dominant (filled squares) and subordinate (filled circles) monkeys in follicular *(Left)* and luteal *(Right)* phase of the menstrual cycle. All other information is as described in Figure 2.
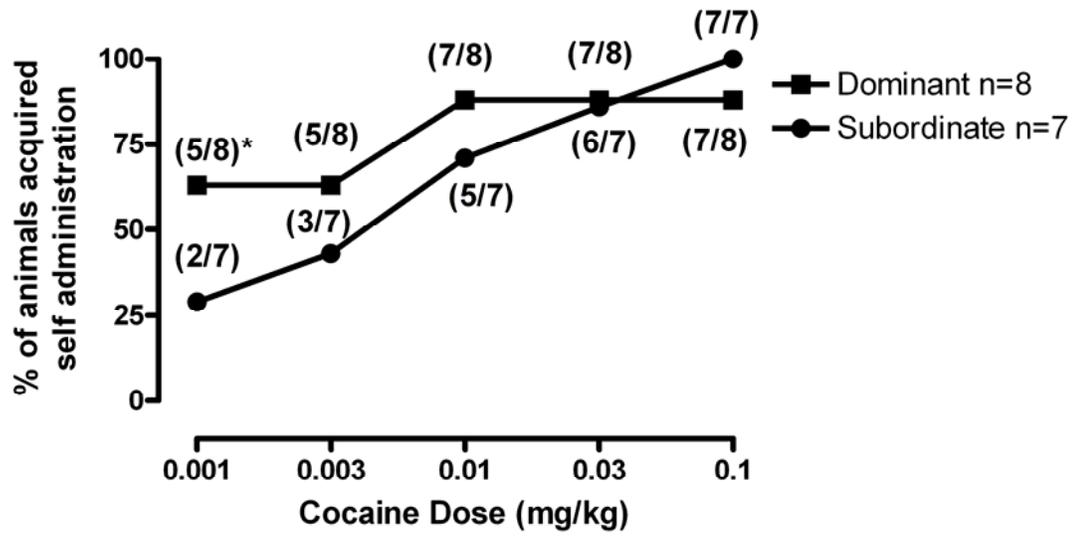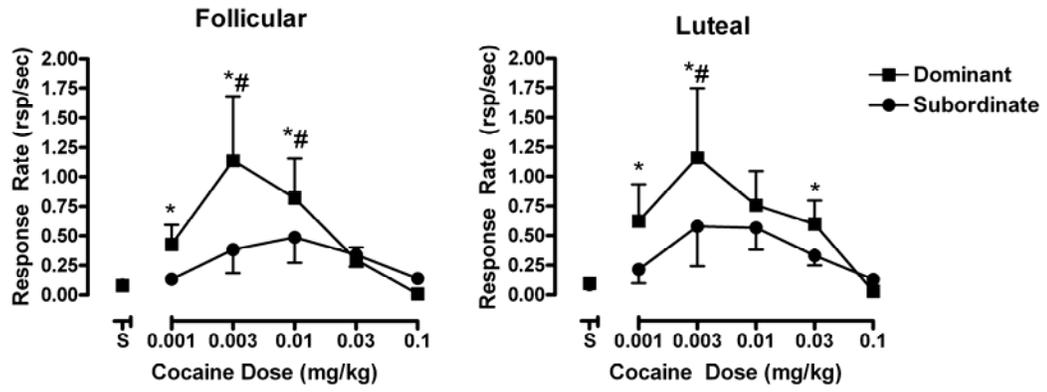
Figure 1.

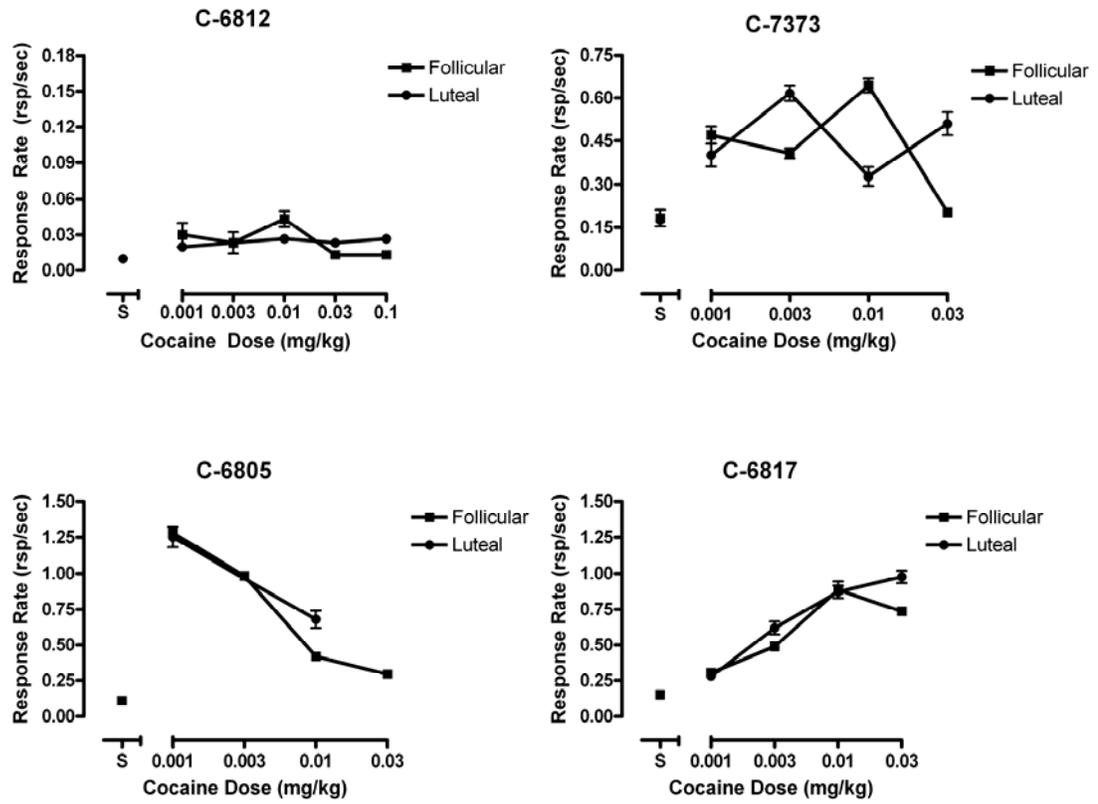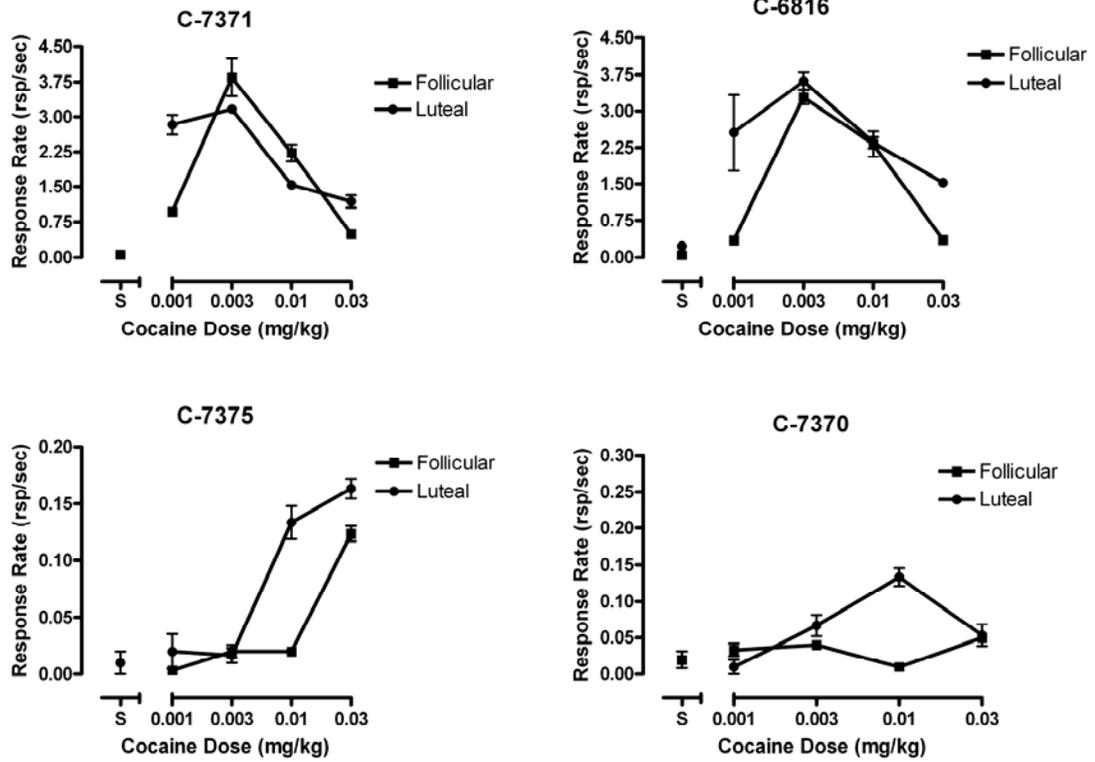Figure 2.

#1-Ranked Monkeys

Figure 3.

#2-Ranked Monkeys

Figure 4.

#3-Ranked Monkeys
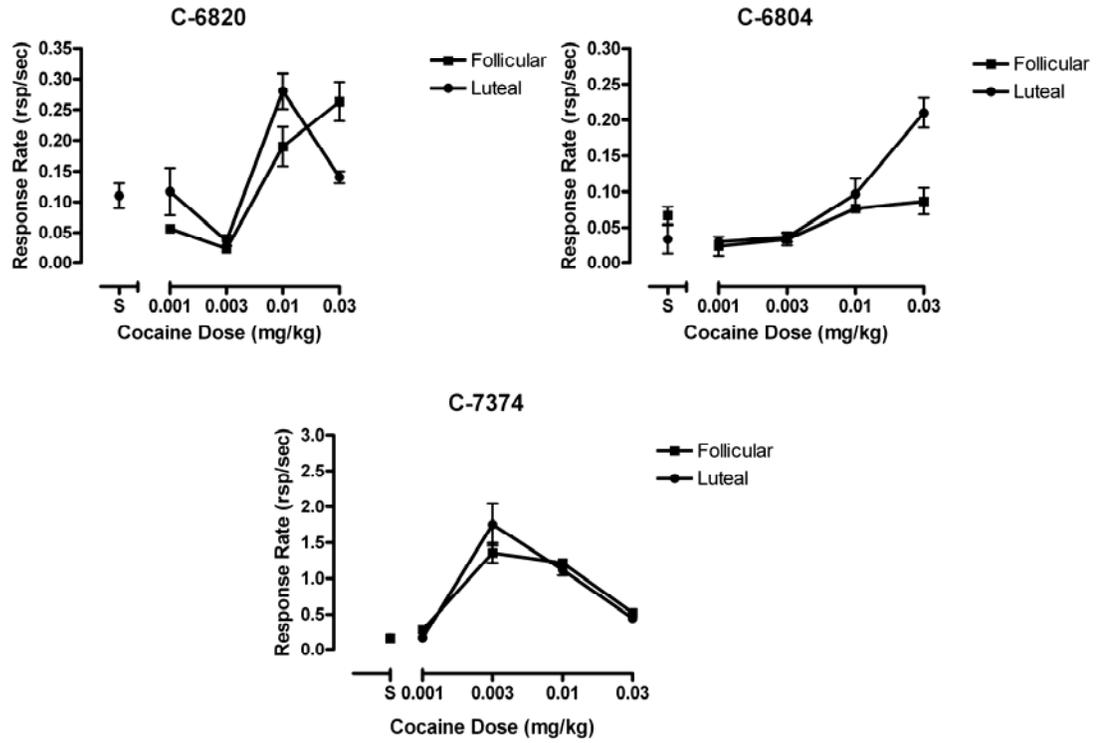
Figure 5.

#4-Ranked Monkeys

Figure 6.

Follicular Phase

Luteal Phase
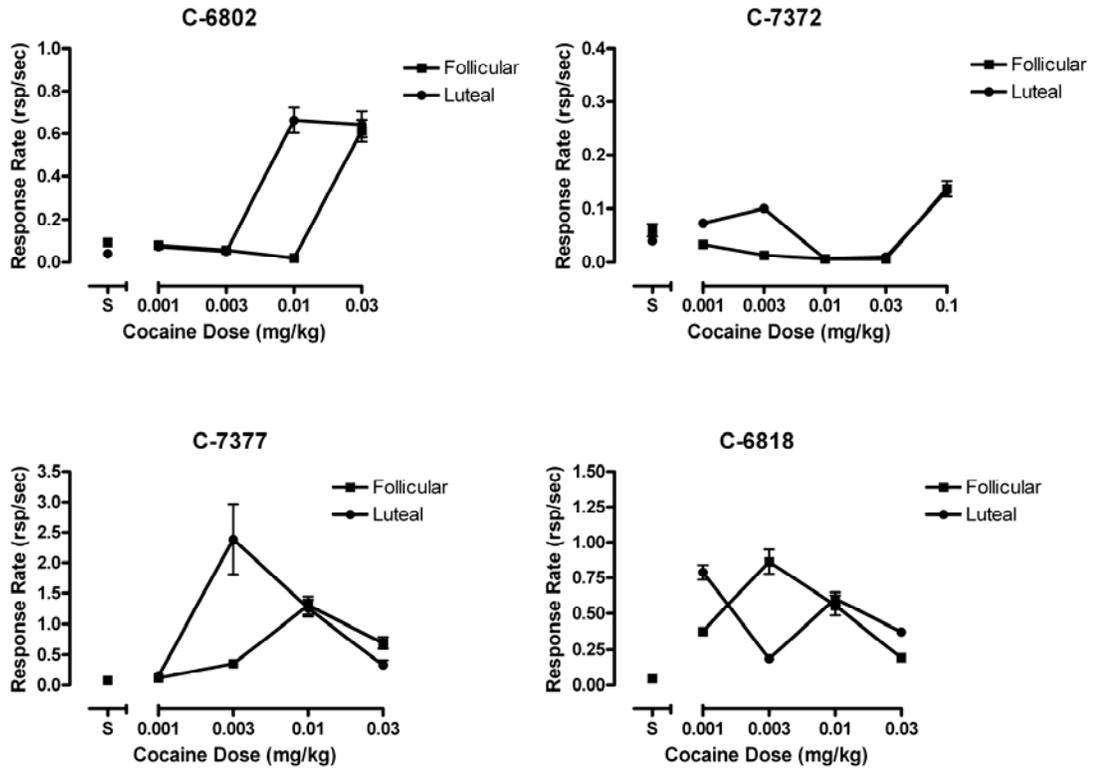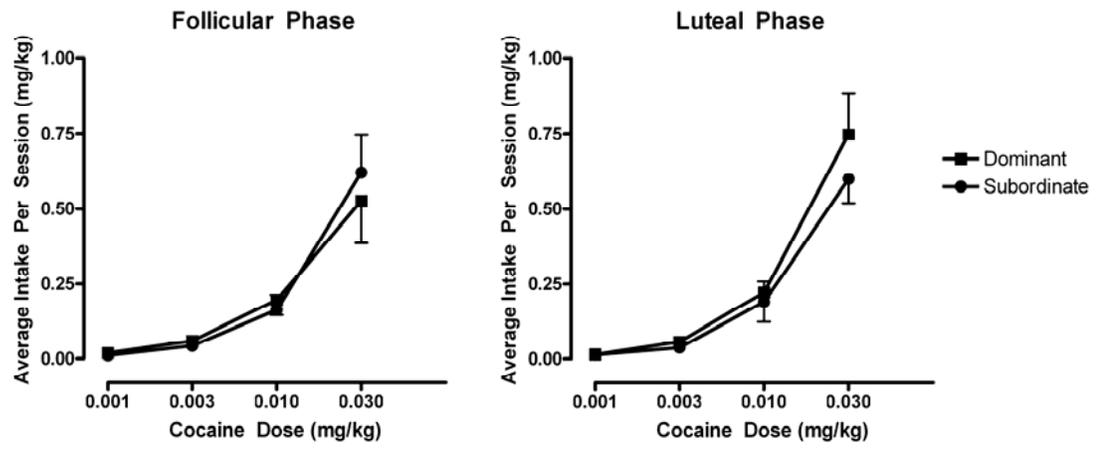
Figure 7.

CHAPTER IV

DISCUSSION

As James Mills once said: "Any disease - including drug addiction - depends for its spread on the three necessities: a susceptible individual, an infecting substance and an environment where the two can meet" (Mills, 1965). These "necessities" have been recently described in terms of the "agent", the "host" and the "context" (O'Brien, 2006). The research described in this dissertation was designed to investigate these factors, and their interactions, as they relate to vulnerability to drug abuse in a novel animal model in order to better understand why some individuals appear more susceptible to drug addiction than others. Accumulating evidence from both preclinical and clinical studies suggests that females may have an enhanced biological vulnerability to cocaine addiction compared to males (for review see Brady and Randall, 1999; Lynch et al., 2002). Vulnerability to reinforcing effects of cocaine can be influenced by behavioral, neurochemical, and physiological factors including temperament, impairments of DA and 5-HT systems, hormones, and psychological stress. The research described in this dissertation was designed to examine a number of these trait and state markers in socially housed female monkeys. In addition, the effects of menstrual cycle phase and social rank on cocaine sensitivity were examined in the same group of monkeys in both acquisition and maintenance phases of drug use. As will be described in this Chapter, perhaps the most significant findings relate to the relationships between these variables and drug abuse, which had been established in male subjects but appear to not have the same predictive validity with regards to vulnerability to drug abuse in females.

PHYSIOLOGICAL, BEHAVIORAL AND NEUROBIOLOGICAL

CHARACTERISTICS INFLUENCING SOCIAL HIERARCHY FORMATION

The studies in Chapter II were aimed at evaluating several behavioral, physiological, and neurochemical measures in individually housed female monkeys to determine if these measures would be predictive of eventual social rank (i.e., trait variables) and if they changed after social group formation (i.e., state variables). These measures included age, body weight, locomotor activity in a novel environment, latency to touch a novel object, HPA-axis function, and 5-HT activity (peripheral and central). The only behavioral measure that was predictive of eventual social rank was a greater latency to touch a novel object among future dominant animals. We also found that CSF concentrations of the 5-HT metabolite 5-HIAA, but not other measures of 5-HT activity, predicted eventual social rank, and that no 5-HT measure changed after the establishment of stable social hierarchies. Importantly for the generality of our findings, the dominant monkeys used in the present study were more aggressive than the subordinate animals (Table I) as has been reported by others (e.g., Kaplan et al., 1982).

To begin with, in contrast to previous studies in male monkeys (Morgan et al., 2000), body weight and locomotor activity were not predictive of eventual social rank in female monkeys. The body weight range in the female monkeys was a narrower 2.4-3.7 kg when compared to 3.2-6.4 kg in the males, which may have influenced a correlation. On the other hand, unlike in males, female monkeys' rank is established based on maternal social position; therefore, body weight may not be an important rank determinant in females. The fact that the heaviest monkeys do not necessarily become dominant suggests that there are other factors that mediate this outcome. Two behavioral

phenotypes, novel object reactivity (NOR) and novelty-induced locomotor activity, were measured in all individually housed monkeys before being placed into social groups (Chapter II). Only NOR was significantly different between eventual dominant and subordinate monkeys. Similar to what has been seen in rodent studies, novelty-induced locomotor activity in female cynomolgus monkeys differ from previous reports in male monkeys (Davis et al., 2008; Morgan et al., 2000).

Novelty-induced locomotor activity when measured in rodents is thought to be a behavioral phenotype related to impulsivity. For example, Stoffel and Cunningham (2008) proposed that locomotor response to novelty is closely related to behavioral disinhibition, which is believed to be one of the facets of impulsive behavior (Moeller et al., 2001). They measured behavioral disinhibition using a differential reinforcement of low rates (DRL) schedule of food presentation, and through evaluating a range of DRL intervals the rats were divided into high responders (HR) and low responders (LR) based on their locomotor performance in the novel environment. It was found that HR rats displayed more behavioral disinhibition relative to LR rats on DRL 20- and 35-sec schedules − in other words, the HR rats received fewer reinforcers due to excessive responding. On the contrary, two separate studies did not find novelty-induced locomotion to be related to impulsive behavior in rodents (Dalley et al., 2007; Perry et al., 2005). However, different assays were used to measure impulsivity: a five-choice serial reaction time (5-CSRT) paradigm and a delayed discounting procedure. Interestingly, Dalley and colleagues (2007) found that subjects determined to be more impulsive had lower locomotor activity while Perry and colleagues (2005) did not see any relationship between locomotor performance and delayed discounting mean delay

scores (MAD). Taken together, these studies suggest that novelty-induced locomotor activity may only correlate with the behavioral disinhibition component of impulsivity.

In contrast to the findings discussed in this dissertation, novelty-induced locomotor activity was found to be predictive of eventual social rank in male cynomolgus monkeys (Morgan et al., 2000). Of note, however, the open-field apparatus used in the present experiment is different from the one used in the male study. The apparatus was modified to allow for more vertical activity, thus, possibly preventing any predictive ability in female monkeys, although this seems unlikely to be the explanation. Alternatively, the sex differences observed may be due to ovarian hormones, since it has been documented in rodents that estrus cycle can alter overall locomotor activity (Davis et al., 2008). To our knowledge, no study has addressed this issue in nonhuman primates. Unfortunately, we did not monitor hormone levels or control for the menstrual cycle phase in this particular experiment. Therefore, it is possible that monthly hormonal variations may have contributed to the lack of correlation between the male and female studies.

The behavioral phenotype that was predictive of eventual social rank in female monkeys was NOR latencies. Eventual subordinate monkeys had lower latencies to approach the novel object than the eventual dominants (Fig. 1). Latency to touch the novel object may be interpreted as a measure of impulsivity (Stansfield and Kirstein, 1995). Due to multidimensional nature of "impulsivity", there is a number of laboratory measurements used to study different components of impulsive behavior (Moeller et al., 2001; Stoffel and Cunningham, 2008). The behavioral tasks are subdivided into three broad categories (Moeller et al., 2001): 1) punished responding or resistance to

extinction, where impulsivity is defined as perseverance of a response that is punished or unrewarded (Matthys et al., 1998); 2) reward size preference where impulsivity is defined as preference for a smaller immediate reward over larger delayed one (Perry et al., 2005); and 3) response disinhibition/lack of attention paradigms where impulsivity is defined as failure to withhold response or premature responses (Stoffel and Cunningham, 2008). Response to novelty as measured by locomotor activity in an open field has been suggested to be related to behavioral disinhibition (Evander, 1999; Stoffel and Cunningham, 2008) but was not predictive of eventual social rank or correlated with NOR latencies in our monkeys (Chapter II). Future studies examining other components of impulsivity (e.g., delay discounting, resistance to extinction) need to be compared with NOR latencies to better understand what component of impulsivity was associated with social rank.

In addition to the correlation with social rank, NOR latencies significantly correlated with cerebrospinal (CSF) concentrations of 5-HT metabolite, 5-hydroxyindoleacetic acid (5-HIAA) sampled from the cervical spinal region. It is still uncertain which serotonergic pathways and terminals the CSF metabolite originates, but the cortical prefrontal lobe has been suggested (Miczek et al., 2002). The present findings are not consistent with previous work in male and female nonhuman primates that showed an inverse relationship between behaviors related to impulsivity and CSF 5-HIAA levels (Fairbanks et al., 2001; Higley et al., 1996a,b; Westergaard et al., 1999). However, in one rodent study using the 5-CSRT task, higher tonic 5-HT concentrations in prefrontal cortex were correlated with loss of impulse control in rats (Dalley et al., 2002). When the above findings are considered as a whole, it seems likely that there are

many facets to 'impulsivity,' of which only some measures correlate with CSF 5-HIAA. Understanding these relationships may lead to a better understanding of risk taking behaviors that ultimately lead to addiction.

Although the behavioral and neurochemical data discussed above are not parallel to the results reported in Chapter II, Dalley and colleagues (2002) demonstrated a clear difference in 5-HT levels between highly impulsive and "normal" rats. Moreover, they showed that 5-HT concentrations did not change over time within subject. Similarly, in Chapter II studies we established that CSF 5-HIAA concentrations in female cynomolgus monkeys were a trait marker for social rank. Eventual dominant monkeys had significantly lower CSF 5-HIAA levels than the eventual subordinates. Moreover, following social housing the concentrations did not change and the relationship between rank and CSF 5-HIAA levels remained the same. What remains to be understood is why high CSF 5-HIAA concentrations in our monkeys was associated with lower NOR latencies.

Westergaard and colleagues (1999) demonstrated that CSF 5-HIAA levels predicted social rank in female rhesus monkeys and did not change following social rank formation. Of note, however, the direction of correlation was opposite from our findings. This difference in results may be due to the fact that Westergaard and colleagues used adolescent female monkeys that were approximately two years old. The present study utilized adult monkeys ranging from 9 and 17 years of age. This may suggest developmental changes taking place in the 5-HT system, and relationships apparent at a young age may be reversed as the subjects mature.

As it relates to our behavioral data on inter-hierarchy aggression, our finding that CSF 5-HIAA concentrations were lower in dominant and more aggressive monkeys than in subordinates is in concordance with several studies in nonhuman primates and humans that reported an inverse correlation between aggression and CSF 5-HIAA concentrations (Table I; Brown et al., 1979; Higley et al., 1992, 1996a,b,c; Kaplan et al., 2002). However, in contrast to our findings, some of the studies reported that aggressive male and female monkeys tended to be subordinate. For example, Higley and colleagues (1996c) measured CSF 5-HIAA concentrations in female rhesus monkeys that lived in indoor/outdoor enclosures with one or two adult males. Repeated CSF samples were obtained throughout the entire year and stable interindividual differences were observed during baseline and stressful conditions. They found that females with higher rates of spontaneous aggressive wounding had lower CSF 5-HIAA concentrations and tended to be subordinate.

Table I. Aggression and submission matrices for four groups of four monkeys

	Aggression						Submission			
	Recipient						Recipient			

Pen A:

Initiator	6812	7371	7374	7372	Total		6812	7371	7374	7372
6812	-	5	16	35	56		-	2	0	0
7371	5	-	7	29	41		15	-	0	0
7374	1	2	-	16	19		87	26	-	2
7372	3	0	1	-	4		214	72	316	-

Pen B:

Initiator	7373	7375	7376	7377			7373	7375	7376	7377
7373	-	50	29	47	126		-	0	0	0
7375	1	-	5	10	16		51	-	0	0
7376	1	0	-	21	22		218	25	-	0
7377	0	0	0	-	0		264	68	61	-

Pen C:

Initiator	6805	7370	6804	6802			6805	7370	6804	6802
6805	-	17	107	49	173		-	0	1	0
7370	3	-	35	20	58		19	-	0	0
6804	0	0	-	14	14		85	21	-	0
6802	0	0	1	-	1		160	22	11	-

Pen D:

Initiator	6816	6817	6820	6818			6816	6817	6820	6818
6816	-	97	48	37	182		-	46	0	0
6817	23	-	41	21	85		68	-	2	1
6820	0	2	-	3	5		129	150	-	0
6818	0	0	0	-	0		91	39	32	-

¶ Each block represents monkeys arranged by social rank (top-left is rank #1 and bottom-right is rank #4). Each row represents the number of acts initiated and each column represents the number of acts received. Aggression data are on the left- and submission data on the right-side of the table.

On the contrary and similar to the present findings, there is some evidence in captive female cynomolgus monkeys living in unisex groups that dominant females are more aggressive than subordinates (Kaplan et al., 2002; Kaplan and Manuck, 2004; Shively, 1995). Furthermore, lower central serotonergic responsivity to fenfluramine was observed in dominants compared to subordinates living in similar unisex social groups (Shively, 1995). Interestingly, although a previous study in free ranging female monkeys (Higley et al., 1996c) did not see similar correlations between aggression and social rank, they did find that aggression inversely correlated with serotonergic activity. Such disconnect between social rank findings are not surprising, however, given the evidence that social status reflects a relationship between social environment (i.e. living enclosures) and inherent characteristics of an individual (i.e. temperament, neurobiology) rather than just an unvarying trait of an individual (Shively and Kaplan, 1991; Kaplan et al., 2002).

In contrast to CSF 5-HIAA concentrations, other 5-HT measures, 5-HT transporter (SERT) availability and whole blood 5-HT (WBS), were neither predictive of eventual social rank nor changed with social rank formation. These findings are particularly surprising because of rank-related differences in CSF 5-HIAA levels. First, WBS is believed to be a peripheral measure of presynaptic 5-HT reuptake (Fekkes et al., 1997), however, under the present conditions WBS concentrations did not parallel CSF 5-HIAA concentrations. It is unclear whether the lack of differences were due to the monkeys being socially housed for only three months at the time of the blood draw or 5-HT in platelets not being as sensitive to environmental manipulations. It may also be related to statistical power, since WBS measures are generally used in human studies

with large number of subjects (Unis et al., 1997). In this dissertation, a total of 15 monkeys were used and these monkeys were further divided into social ranks, reducing the numbers even more. Nevertheless, further investigation is warranted in this matter since WBS is less invasive and requires less resources than measures of central 5-HT activity. Secondly, based on CSF 5-HIAA findings we hypothesized differences in SERT binding as a function of rank; however, no differences were observed. The main reason for the discrepancies could related to whether the radioligand used in this study, [11C]DASB, was affected by extracellular 5-HT (Lundquist et al., 2005; Tabot et al., 2005; Yamamoto et al., 2007). It remains possible that SERT densities were different between dominant and subordinate monkeys, but that differences in extracellular concentrations of 5-HT as a function of rank may have masked these differences. Future microdialysis and/or in vitro receptor autoradiography studies are needed to answer this important question.

As was observed in male monkeys (Morgan et al., 2000), cortisol levels were not predictive of eventual social rank in females. Similarly, adrenal responsiveness as assessed using DEX suppression and ACTH administrations were not predictive of social rank, nor were there differences following establishment of social hierarchies. Previous studies in male and female nonhuman primates have reported conflicting results. Hypercortisolemia has been observed in some subordinate monkeys living in stable social groups by some investigators (Sapolsky, 1989, 1992; Kaplan et al., 1986; Shively et al., 1997) but not others (Morgan et al., 2000; Shively et al. 1998). Furthermore, adrenal hypersensitivity to ACTH has been previously observed in subordinate female cynomolgus monkeys (Kaplan et al., 1986). However, we did not observe rank-related

differences in the present study. The reasons for these discrepancies could be the group composition, the temperament of the monkeys, or the social behavior in the group (e.g. amount of aggression versus affiliation). It may also be that the HPA axis responds to stressful situations rapidly and that "tolerance" developed within days of social group formation. Such adaptations seem necessary for the long-term health of the animals.

In summary, studies in Chapter II demonstrated that CSF 5-HIAA concentrations and NOR latencies were trait variables in female monkeys. Furthermore, we demonstrated that adrenal responsivity, other 5-HT measures, and novelty-induced locomotion were neither predictive of social rank in female monkeys, nor did they change with rank stabilization. Most studies examining disease states using socially housed nonhuman primates examine only state variables. However, it is clearly important to study pre-existing characteristics of the individual, in order to better understand the interaction between trait variables and the environment. Distinguishing between trait and state variables can aid development of better treatments for different diseases including drug abuse. If the variable is altered by the environment vs. was a stable trait of the individual provides important information as to whether the treatment must include changes in the environment (a much harder condition to maintain). These findings characterize the neurobiology, neurochemistry, and behavior of socially housed female monkeys and highlight the possible existence of sex differences in factors predicting social rank and stress sensitivity.

SOCIAL RANK EFFECT ON COCAINE SENSITIVITY

The first part of the studies in Chapter III was aimed at examining the effect of social rank on acquisition and maintenance of cocaine self-administration in female monkeys. We found a trend for more dominant monkeys acquiring cocaine self-administration at lower doses than subordinates. During the maintenance phase, cocaine functioned as a more potent reinforcer in dominant monkeys than subordinates when cocaine dose-response curves were established. These findings were the first describing intravenous cocaine self-administration in socially housed female monkeys and suggest (1) profound effects of social hierarchy on cocaine reinforcement and (2) an opposite relationship between social rank and measures of cocaine reinforcement in females compared to males (Morgan et al., 2002).

Previous research in rodents and monkeys demonstrated that acute and chronic stress may enhance the abuse-related effects of cocaine (for review see Campbell and Carroll, 2000; Goeders and Guerin, 1994; Haney et al., 1995; Morgan et al., 2002; Piazza et al., 1990; Ramsey and van Ree, 1993; Tidey and Miczek, 1996, 1997). Specifically, acquisition of psychostimulant self-administration is enhanced following an aggressive attack by a same-sex opponent and by a threat of an attack by an aggressive opponent in previously defeated intruders (Haney et al., 1995; Miczek and Mutscheler, 1996; Tidey and Miczek, 1997). Furthermore, cocaine has been shown to function as a more potent reinforcer in stressed rats compared to their nonstressed counterparts (Miczek and Mutschler, 1996). If the same relationships between acute aggression and cocaine self-administration were operating in our study, we would hypothesize that subordinate monkeys would be more vulnerable to cocaine self-administration than dominant animals

because the former received more aggression (see Table I). In contrast, we found that dominant monkeys were more sensitive to the reinforcing effects of cocaine than subordinates (Chapter III). The reason for the discrepancy is likely due to several procedural differences such as the model of social stress, acute vs. chronic stress, the paradigms utilized to measure acquisition of cocaine self-administration and cocaine dose-response curves, subjects' behavioral histories, and sex of the animals.

Nonhuman primate social behavior is a combination of social environment (i.e. behavior of all members of the group), individual temperament, and the underlying individual neurobiology that all contribute to the eventual social position occupied by a given animal (Kaplan et al., 2002). As discussed above, the results in Chapter II revealed that dominant animals had lower CSF 5-HIAA levels and were more aggressive (Table I) but less impulsive than subordinates. A low functioning 5-HT system, as indicated by the CSF levels of its major metabolite, as well as aggressive and impulsive behavioral phenotypes are both associated with higher risk for drug and alcohol abuse in human and nonhuman primates (Higley et al., 1996a,b; Cuomo et al., 2008; for review see Wrase et al., 2007). For example, increasing 5-HT by inhibiting its reuptake reduced cocaine self-administration in rodents (Carroll et al., 1990), while decreasing 5-HT was associated with an enhanced reinforcing effectiveness of cocaine (for review see Walsh and Cunningham, 1997). This behavioral outcome is believed to be due to the modulatory effect 5-HT has on the DA system (Di Matteo et al., 2000). Therefore, a lower functioning 5-HT system in dominant monkeys compared to subordinates may lead to disinhibition of the DA system, contributing to their increased vulnerability to the reinforcing effects of cocaine than subordinates. Future studies investigating the effect of

5-HT agonists and antagonists on cocaine self-administration in dominant and subordinate monkeys will better address this important issue.

In Chapter II we reported that lower NOR latencies predicted social subordination in individually housed monkeys and concluded that NOR was a trait variable that reflected a behavioral phenotype related to a component of impulsivity, temperament. Previous literature in humans and laboratory animals reported that impulsivity is a trait characteristic and is associated with increased risk for drug and alcohol abuse (Moeller et al., 2001; Higley et al., 1996c). In this dissertation, dominant females were tested when individually housed, and they appeared to be less impulsive than subordinates which led us to hypothesize that subordinate monkeys would be more vulnerable to the reinforcing effects of cocaine. However, the opposite results were obtained following self-administration studies. It remains possible that variables associated with the formation of social hierarchies were more relevant to cocaine self-administration than this trait variable, which we did not retest after stable social ranks were formed. It is important to note that other behaviors thought to measure some component of impulsivity, e.g., resistance to extinction, did not differ between dominant and subordinate monkeys. Due to the multidimensional nature of impulsivity, there are a number of various laboratory measurements used to study different components of impulsive behavior (Evenden, 1999; Moeller et al., 2001; Stoffel and Cunningham, 2008). Some impulsivity measures such as the 5-CSRT paradigm and delayed discounting were found to be useful in predicting vulnerability to stimulant abuse (Dalley et al., 2007; Perry et al., 2005). Future studies using other measures of impulsivity that can be assessed multiple times (when

individually housed and after social housing), such as the 5-CSRT or delay discounting, will better address the issue of impulsivity as a trait vs. a state variable.

In addition to the low functioning 5-HT system and an aggressive temperament, ovarian hormones may be involved in enhancing the reinforcing potency of cocaine in dominant monkeys. Several different groups have reported that stress-induced ovarian hypofunction in subordinate monkeys leads to suppressed levels of estrogen and progesterone (Adams et al., 1985; Kaplan and Manuck, 1999; Shively et al., 1997; Williams et al., 1994), the hormones thought to be involved in modulating the reinforcing effects of cocaine in females. Moreover, ovarian hormones are often described as one of the reasons for females' increased vulnerability to reinforcing effects of drugs compared to males (for reviews see Lynch et al., 2002; Roth et al., 2004). Estrogen is commonly associated with enhancing the reinforcing effects of cocaine while progesterone counteracts those effects (Jackson et al., 2006). In Chapter III, we reported rank-related differences in cocaine self-administration between dominant and subordinate monkeys when tested in the follicular and luteal phases of the menstrual cycle. We tested each cocaine dose in the follicular phase on days 1-10 of the menstrual cycle which is associated with low levels of estradiol and progesterone concentrations (Ferin et al., 1993). In the luteal phase, on days 19-27 estradiol concentrations were more likely moderate but opposed by high progesterone levels. These levels were confirmed in our monkeys (Chapter III). Therefore, based on these and previous findings, overall higher estradiol concentrations in dominants compared to subordinates may have been responsible for the enhanced reinforcing potency of cocaine in both phases. Taken together, it appears that there are several possible reasons for greater cocaine sensitivity

in dominant compared to subordinate monkeys, including ovarian hormones, temperament such as aggressiveness, and 5-HT system impairments.

MENSTRUAL CYCLE PHASE EFFECT ON COCAINE SENSITIVITY

The effect of menstrual cycle phase on cocaine self-administration in socially housed female monkeys was also examined in Chapter III. Menstrual cycle phase did not affect cocaine sensitivity in dominant and subordinate monkeys (Chapter III).

Reinforcing potency of cocaine was not different as a function of menstrual cycle phase nor was it altered by exogenous estradiol administration in normally cycling rhesus monkeys responding under an FR schedule of cocaine presentation (Mello et al., 2008). The reinforcing efficacy of cocaine, as measured under a PR schedule, was also not statistically different as a function of menstrual cycle phase; however, post-hoc analysis revealed differences at the lowest, not consistently reinforcing, dose of cocaine (Mello et al., 2007). Similarly, in this dissertation, we did not observe an effect of menstrual cycle phase on dominant and subordinate monkeys responding under an FR 30 schedule of cocaine presentation (Chapter III).

The rationale for hypothesizing differences in cocaine sensitivity as a function of menstrual cycle phase is based, in part, on rodent studies. Exogenous estradiol administrations and estrus phase of estrus cycle in rodents has been associated with enhanced cocaine self-administration (Hecht et al., 1999; Hu and Becker, 2008; Jackson et al., 2006; Lynch et al., 2000, 2001; Roberts et al., 1989). However, other studies demonstrated no or limited effects of menstrual cycle phase and exogenous estradiol

administration on cocaine reinforcement (Caine et al., 2004; Lynch and Taylor, 2005; Mello et al., 2007, 2008). Various procedural differences are possibly responsible for the discrepant findings in the literature, including using ovariectomized animals with exogenous hormone administration. Hu and Becker (2008) demonstrated that when administering estradiol exogenously, dose and length of treatment may affect cocaine reinforcement differently. For example, only physiologically relevant concentrations administered acutely enhanced the reinforcing effects of cocaine while chronic administration did not produce an effect. In addition, estradiol concentrations vary during the follicular phase of the menstrual cycle and initially are generally low and start to increase slowly after about day 7 peaking right before ovulation occurs (Ferin et al., 1993). Therefore, the discrepancies between the studies may also be due to cocaine being tested at different points in the follicular phase between studies.

Based on previous findings, lack of menstrual cycle phase-dependent differences in this dissertation (Chapter III) may be explained by low estradiol concentrations in the follicular phase of the cycle at the time of testing and high progesterone levels counteracting estradiol effects in the luteal phase (Ferin et al., 1993; Jackson et al., 2006). Unfortunately, in this study we did not measure ovarian hormone concentrations throughout the menstrual cycle; therefore, further studies are warranted to investigate the influence of ovarian hormones on cocaine reinforcement in dominant and subordinate monkeys. Taken together, we replicated previous studies that menstrual cycle phase does not alter the reinforcing potency of cocaine in normally cycling females, and we extended these findings to show that menstrual cycle does not differentially affect cocaine reinforcement in dominant or subordinate monkeys.

SEX DIFFERENCES IN SOCIAL RANK EFFECT ON COCAINE SENSITIVITY

Taken together, the findings in Chapter III and a previously published study from our laboratory in male monkeys suggest that sex differences may exist in cocaine reinforcement as a function of social rank. In that study, subordinate male cynomolgus monkeys housed in unisex groups self-administered significantly more cocaine than dominant monkeys (Morgan et al., 2002). Interestingly, cocaine did not even function as a reinforcer in the dominant monkeys. In contrast, Chapter III studies showed that dominant females were more sensitive to reinforcing effects of cocaine than subordinates.

Previous studies suggest possible sex differences between males and females with regard to chronic stress sensitivity. Although there was no baseline difference in cortisol concentrations in male cynomolgus monkeys as a function of social rank (Czoty et al., in press; Morgan et al., 2000), subordinates had enhanced adrenal responsivity to an ACTH challenge following dexamethasone (DEX) suppression (Czoty et al., in press). However, the rank-related differences were only significant at a 15-min. point following ACTH administration. Furthermore, transient differences in cortisol levels were also reported during social hierarchy formation in male monkeys. Specifically, eventual subordinate monkeys had higher cortisol concentrations only during the first three days of social housing (Czoty et al., in press). In contrast, there were no differences in adrenal responsiveness or baseline cortisol levels as a function of social rank in the female monkeys used in this dissertation research (Chapter II). Furthermore, cortisol concentrations were only higher in eventually subordinate monkeys during the first day of social housing (unpublished data). Taken together, these data suggest that female

cynomolgus monkeys may respond differently to stress than male monkeys, and HPA compensatory mechanisms appear to be recruited more rapidly in females than males.

The females appear to respond differently to stress than males, which was also demonstrated by differential sensitivity to the reinforcing effects of cocaine (Chapter III). This may be due to different types or intensities of stress experienced in all-female social groups compared to all-male groups. Different types of stressors as well as their predictability can differentially affect cocaine self-administration (for review see Piazza and Le Moal, 1998; Miczek et al., 2008). For example, the physical experience of stress is not necessary for increases in cocaine sensitivity – the mere psychological stress of witnessing another rat receiving foot shocks is sufficient to facilitate the acquisition of cocaine self-administration in rodents (Ramsey and van Ree, 1993). Given the difference in the way male and female monkeys maintain their hierarchies, the influence of the type of stressors experienced by male versus female subordinates may offer some insight. For example, male monkeys' relationships are both physical and aggressive, yet they are relatively transient. On the other hand, dominant females attack subordinates even in the absence of direct provocation. They constantly harass the subordinates causing a great deal of psychological stress and intimidation (Kaplan and Manuck, 2004). In addition, female cynomolgus monkeys generally inherit the rank of their mothers unlike the males who have to leave the troops they are born into (Kaplan et al., 2002). It is more natural for the males to have to join a group of unfamiliar monkeys than it is for females which can result in differences in stress levels to both dominants and subordinates when placed in unisex groups in captivity. Taken together, the type and intensity of stress varies

between male and female monkeys living in unisex groups which could lead to differences in the HPA axis activity but does not completely eliminate stress.

Ovarian hormones can be another reason for the sex differences suggested by the latter studies. For example, female rats have been shown to be more vulnerable to reinforcing effects of cocaine than males and ovarian hormones were the primarily described reason (e.g. Jackson et al., 2006; Lynch and Carroll, 1999; Roberts et al., 1989). Specifically, estradiol appeared to play a major role in modulating the behavioral effects of cocaine and may be a major factor underlying the increased sensitivity in females (Lynch et al., 2002; Festa and Quinones-Jenab, 2004). Estrogen can directly increase striatal DA release (Becker, 1990), enhance stimulant-induced DA release in nucleus accumbens (NAc) and striatum (Becker and Ramirez, 1981), and is implicated in regulating DAergic transmission in the brain (Becker, 1999). It has been suggested that estrogen's enhancing effects on stimulant-induced DA release could result via either D1 or D2 receptor signaling or both. Recent evidence suggests that D1 receptor blockade produced a more robust decrease in cocaine-induced locomotion in female rats compared to males (Festa et al., 2006; Schindler and Carmona, 2002). Although D2 receptor densities in the NAc or the prefrontal cortex do not appear to differ between males and females (Harrod et al., 2004), females have been reported to be less sensitive than males to the effects of D2 receptor stimulation (Arenas et al., 1999; Parra et al., 1999; Schindler and Carmona, 2002). Interestingly, estrogen does not appear to impact DA release in the NAc of males (Becker, 1990). Taken together these finding suggest that females are more sensitive to D1 but less sensitive to D2 receptor stimulation than males possibly due to the effects of estrogen.

Previous research demonstrated that subordinate females have lower D2 receptor availability than dominants (Grant et al., 1998) which is in concordance with the study in male monkeys (Morgan et al., 2002). D2 receptor stimulation is implicated in mediating the reinforcing effects of cocaine and can predict individual differences in vulnerability to the reinforcing effects of cocaine (Volkow et al., 1993; Nader and Czoty, 2005). Furthermore, evidence shows that baseline D2 receptor availability is inversely correlated with rates of cocaine self administration (Nader et al., 2006). However, in contrast to the expected effects related to D2 receptor availability, we found that dominant female monkeys were more sensitive to reinforcing effects of cocaine than subordinates (Chapter III). It is unclear at the moment if the difference is due to lower D2 or enhanced D1 receptor sensitivity in females or due to differential effect of estradiol on the DA release in dominant and subordinates. Estradiol has been demonstrated to curtail the D2 receptor autoregulatory mechanism in the ventral tegmental area (VTA) by decreasing G-protein coupling and, thus, increasing DA output in nucleus accumbens and striatum, brain regions thought to modulate the reinforcing effects of cocaine (Febo et al., 2003). Subordinate female monkeys may be protected from this effect due to estrogen deficiencies. Taken together, the evidence suggests that sex differences observed may be due to the interaction between stress sensitivity, ovarian hormones and DAergic neurotransmission differences.

In conclusion, the results of the experiments described in this dissertation have implications for the role of different factors on social rank and cocaine sensitivity as well as their interaction in female monkeys. In addition, this study suggests possible sex differences in how social dominance affects cocaine reinforcement and factors that

predict social rank formation. CSF 5-HIAA concentrations and novel object reactivity latencies predict eventual social rank in female monkeys while other 5-HT measures (WBS and SERT availability), HPA axis activity, and novelty-induced locomotor activity do not. Furthermore, no changes were observed in any of the measures after social rank formation. Dominant female monkeys were more sensitive to reinforcing effects of cocaine than subordinates possibly due to impairments in 5-HT neurotransmission, aggressive temperament, and ovarian hormones. Lastly, menstrual cycle phase did not have an effect on cocaine reinforcement in dominant and subordinate females.

The research presented in this dissertation further extends our understanding of neurobiological and behavioral underpinnings of social rank and cocaine reinforcement interactions in female monkeys and provides insight into a gender-specific "vulnerable" phenotype. Further studies of social rank-related differences in neurobiology and other individual traits are warranted to better understand vulnerability to drug abuse in females; such information will lead to identification of potential new targets for pharmacological and behavioral therapies of cocaine abuse.

In the end, this dissertation research demonstrates that social stress may have similar neurobiological but different behavioral consequences in women compared to men. For example, although similar rank-induced changes are observed in D2 receptor availability of females, behavioral consequences were found to be exactly opposite suggesting that neurobiology of vulnerability to reinforcing effects of cocaine is different between women and men. This profound sex difference demonstrates the importance of gender-specific pharmacotherapies and further investigation of the mechanism responsible for these differences. Future studies will further examine the mechanism for

stress sensitivity and DA and 5-HT system effect on cocaine self-administration in women.

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<div align="center">**SCHOLASTIC VITAE**</div>

<div align="center">NATALLIA VASHCHONAK RIDDICK</div>

EDUCATION:

2000-2004   Campbell University, Buies Creek, North Carolina
BS, Biochemistry; *Magna Cum Laude*

2004-2008   Wake Forest University School of Medicine
Winston Salem, North Carolina
PhD. in Physiology and Pharmacology
Dissertation: Factors that affect social hierarchies and the influence of social rank and menstrual cycle phase on cocaine self-administration in female cynomolgus monkeys
Advisor: Michael A. Nader, Ph.D.

POSITIONS AND HONORS:

2008   Graduate Student Travel Award,
American Society of Pharmacology and Experimental Therapeutics Annual Meeting in San Diego, CA

2006   Wake Forest University Alumni Travel Award to attend Society of Neuroscience Conference in Atlanta, GA

2004   Research Technician I
Wake Forest University
Winston Salem, North Carolina

2003-2004   General Chemistry II Laboratory Assistant
Campbell University
Buies Creek, North Carolina

2003-2003   Summer Intern,
CIIT, Centers for Health Research
Research Triangle Park, North Carolina

2002   PreMed Allied Honor Society
Campbell University
Buies Creek, North Carolina

POSITIONS AND HONORS (CONTINUED):

2002-2003	Laboratory Assistant
	Biology Department
	Campbell University
	Buies Creek, North Carolina
2002	Eta Sigma Honor Society
2001-2003	Pharmacy Technician
	CVS Pharmacy
	Clayton, North Carolina

PROFESSIONAL MEMBERSHIPS:

2005	Western Chapter of Society for Neuroscience
2006	Society for Neuroscience
2006	American Society for Pharmacology and Experimental Therapeutics

PUBLICATIONS:

Riddick NV, Czoty PW, Kaplan JR, Icenhower MI, and Nader MA. Social rank and menstrual cycle phase influence on cocaine sensitivity in female monkeys. (in preparation)

Nader MA, Czoty PW, Gould RW, **Riddick NV**. PET imaging studies of dopamine receptors in primate models of addiction. Philos Trans R Soc Lond B Biol Sci. 363: 3223-3232. 2008 Jul 18. Review.

Riddick NV, Czoty PW, Gage HD, Kaplan JR, Nader SH, Sandridge JM, Icenhower M, Bennett A, Pierre P, Garg PK, and Nader MA. "Behavioral and neurobiological characteristics influencing social hierarchy formation in female cynomolgus monkeys" Neuroscience (in press), doi: 10.1016/j.neuroscience.2008.11.016

Czoty, PW**, Riddick NV**, Gage HD, Sandridge, JM, Nader SH, Bounds M, Garg, S, Garg PK, and Nader MA. "Effects of Menstrual Cycle Phase on D2 Receptor Availability in Female Cynomolgus Monkeys" Neuropsychopharmacology 2008 Feb 6

PRESENTATIONS:

Riddick NV "Social rank and menstrual cycle phase influence on cocaine sensitivity in female monkeys" Slide presentation at Wake Forest -Emory University lab exchange event, Winston Salem, NC, September 19, 2008

Riddick NV, Czoty PW, Kaplan JR, Icenhower M, Gage HD, and Nader MA
"Trait and State Variables Associated with Social Rank and Cocaine Abuse in Female Cynomolgus Monkeys" Poster presentation for Addiction, Infectious Disease, and Public Health; 2nd Annual Conference for the Dissemination of Student Research, John Hopkins Bloomberg School of Public Health, Baltimore MD; April 21, 2008.

Riddick NV, Czoty PW, Kaplan JR, Icenhower M, Gage HD, and Nader MA
"Trait and State Variables Associated with Social Rank and Cocaine Abuse in Female Cynomolgus Monkeys" Poster presentation for Substance Abuse Research Day, Wake Forest University, Winston Salem NC; April 18, 2008 .

Riddick NV, Czoty PW, Kaplan JR, Icenhower M, Gage HD, and Nader MA
"Trait and State Variables Associated with Social Rank and Cocaine Abuse in Female Cynomolgus Monkeys" Poster Presentation at American Society for Pharmacology and Experimental Therapeutics Meeting, April 2008, San Diego, CA

Riddick NV, Czoty PW, Kaplan JR, Icenhower M, Gage HD, and Nader MA
"Trait and State Variables Associated with Social Rank and Cocaine Abuse in Female Cynomolgus Monkeys" Poster Presentation at Women's Health Research Day at Wake Forest University, April 2008, Winston Salem, NC

Riddick NV "Factors Predicting Social Status and Sensitivity to Cocaine in Female Monkeys" Annual departmental seminar, Physiology and Pharmacology, Wake Forest University, Winston Salem, NC, March 2008

Riddick NV "Identifying Risk Factors for Cocaine Abuse: Variables that Affect Social Hierarchy Formation in Female Monkeys" Slide presentation to the Department of Neuroscience, at Wake Forest-Emory University lab exchange, Yerkes National Primate Center, Atlanta, GA, September, 2007

Riddick NV "Central Serotonergic System Activity, Social Behavior and Vulnerability to Drug Addiction in Female Cynomolgus Monkeys" Annual departmental seminar, Physiology and Pharmacology, Wake Forest University, Winston Salem, NC, April 2007

Riddick NV, Czoty PW, Kaplan JR, Pierre P, Bennett A, Gage HD and Nader MA
"Behavioral and neurochemical measures as predictors of social rank in female monkeys" Poster Presentation at American Society for Pharmacology and Experimental Therapeutics Meeting, 2007, Washington, DC.

PRESENTATION (CONTINUED):

Riddick NV "Measures of impulsivity, serotonin neurochemistry, and D2 receptor availability as a function of menstrual cycle in female cynomolgus monkeys" Slide presentation at special session "Drug Abuse: Human and Animal Studies" Program No. 123.3. Society for Neuroscience, 2006, Atlanta, GA

Riddick NV "Understanding the role of serotonin in social behavior and cocaine sensitivity in female cynomolgus monkeys" Slide presentation for physiology and pharmacology department at Wake Forest-Emory University lab exchange, Winston Salem, NC, September, 2006

Riddick NV "Effects of menstrual cycle phase on "impulsivity", D_2 receptor availability, and CSF 5-HIAA levels in female cynomolgus monkeys" Annual Departmental Seminar, Physiology and Pharmacology, Wake Forest University, Winston Salem, NC, March 2006

Riddick NV, Czoty PW, Gage, HD, Sandridge, JM, Nader SH, Bounds M, Garg, S, Garg PK, and Nader MA. "Effects of menstrual cycle phase on D2 receptor availability in female monkeys" Poster Presentation, Graduate Student Research Day, Wake Forest University, Winston Salem, NC, March, 2006

Riddick NV "Choosing and surviving graduate school" Invited presentation to the Biology Department, Campbell University, Buies Creek, NC, November, 2005

Riddick NV "Effects of menstrual phase on "impulsivity" and D_2 receptor availability in female monkeys" Slide presentation to the Department of Neuroscience, at Wake Forest-Emory University lab exchange, Yerkes Primate Center, Atlanta, GA, September, 2005

Riddick NV, and Liu L. "Progesterone receptor transactivation with methoxychlor and genistein in HepG2 cells" End of summer internship accomplishment presentation to the scientific staff, CIIT, Centers for Health Research, RTP, NC, August, 2003

ABSTRACTS:

Riddick NV, Czoty PW, Kaplan JR, Icenhower M, Gage HD, and Nader MA
"Trait and State Variables Associated with Social Rank and Cocaine Abuse in Female Cynomolgus Monkeys" 2007 Experimental biology meeting abstracts [http://select.biosis.org/faseb]. *FASEB Journal*, **21: Program #719.9.**
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ABSTRACTS (CONTINUED):

Riddick NV, Czoty PW, Kaplan JR, Gage HD, Icenhower M and Nader MA. Trait and state variables associated with social rank and cocaine abuse in female cynomolgus monkeys. WFUSM Women's Health Research Day, April 3, 2008

Czoty PW, **Riddick NV,** Gage HD, Icenhower M, Bounds M, Kaplan JR, Bennett AJ, Pierre PJ, and Nader MA. Neurobiological and behavioral predictors of social rank in female monkeys. In *"Focus on Women and Sex/Gender Differences"* College on Problems of Drug Dependence, Quebec City, Canada, 2007, pp. 47-48.

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Nader MA, Czoty PW, Nader SH, Calhoun T, Sandridge JM, Gould R, Icenhower M, **Riddick NV**, Bounds MC, Gage HD. Brain imaging and monkey models of cocaine abuse. In: Di Chiara G, Carbon E, Valentini V, Aquas E, Bassareo V, Cadoni C, eds, *"Monitoring Molecules in Neuroscience: Proceeding of the 11th International Conference on In Vivo Methods"*. University of Cagliari, Cagliari, Italy, 2006, pp. 60-62.

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