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TABLE OF CONTENTS

LIST OF

ABBREVIATIONS........................................................................................................................................vi

LIST OF

TABLES..........................................................................................................................................................viii

LIST OF

FIGURES..........................................................................................................................................................x

ABSTRACT...........................................................................................................................................................xiv

CHAPTER

I  INTRODUCTION: PRECLINICAL MODELS AND CLINICAL STUDIES OF THE ROLE OF HORMONAL INFLUENCE ON COGNITIVE FUNCTION, SOCIAL STRESS AND COCAINE ABUSE..............................................................................................................................1

II  RELATIONSHIP BETWEEN ESTRADIOL AND PROGESTERONE CONCENTRATIONS AND COGNITIVE PERFORMANCE IN NORMALLY CYCLING FEMALE CYNOMOLGUS MONKEYS..................................................................................88

III  PREDICTORS OF SOCIAL RANK IN FEMALE CYNOMOLGUS MONKEYS.........................................................................................................................118

IV  EFFECTS OF RANK AND MENSTRUAL PHASE ON ACQUISITION OF COCAINE SELF-ADMINISTRATION, LOCOMOTOR ACTIVITY AND COGNITIVE PERFORMANCE IN FEMALE CYNOMOLGUS MONKEYS.............................................157

iv
V EFFECTS OF PRIOR COCAINE SELF-ADMINISTRATION ON COGNITIVE PERFORMANCE IN FEMALE CYNOMOLGUS MONKEYS..........................188

VI DISCUSSION..............................................................................................................222

SCHOLASTIC VITAE........................................................................................................268
## LIST OF ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-CSRTT</td>
<td>5-choice serial reaction time task</td>
</tr>
<tr>
<td>5-HT</td>
<td>serotonin</td>
</tr>
<tr>
<td>ACTH</td>
<td>adrenocorticotropic hormone</td>
</tr>
<tr>
<td>ADHD</td>
<td>attention-deficit/hyperactivity disorder</td>
</tr>
<tr>
<td>BNST</td>
<td>bed nucleus of stria terminalis</td>
</tr>
<tr>
<td>CANTAB</td>
<td>Cambridge Neurophysiological Test Automated Battery</td>
</tr>
<tr>
<td>CBT</td>
<td>cognitive behavioral therapy</td>
</tr>
<tr>
<td>CNS</td>
<td>central nervous system</td>
</tr>
<tr>
<td>CRF</td>
<td>corticotrophin releasing factor</td>
</tr>
<tr>
<td>D1</td>
<td>dopamine D1-like receptor superfamily</td>
</tr>
<tr>
<td>D2</td>
<td>dopamine D2-like receptor superfamily</td>
</tr>
<tr>
<td>DA</td>
<td>dopamine</td>
</tr>
<tr>
<td>DAT</td>
<td>dopamine transporter</td>
</tr>
<tr>
<td>DMS</td>
<td>delay match-to-sample</td>
</tr>
<tr>
<td>DNMS</td>
<td>delayed non-match-to-sample</td>
</tr>
<tr>
<td>DOPAC</td>
<td>3,4-dihydroxyphenylacetic acid</td>
</tr>
<tr>
<td>E2</td>
<td>estradiol</td>
</tr>
<tr>
<td>EPI</td>
<td>epinephrine</td>
</tr>
<tr>
<td>FR</td>
<td>fixed-ratio</td>
</tr>
<tr>
<td>GABA</td>
<td>gamma-Aminobutyric acid</td>
</tr>
<tr>
<td>HPA</td>
<td>hypothalamic-pituitary-adrenal</td>
</tr>
<tr>
<td>i.v.</td>
<td>intravenous</td>
</tr>
<tr>
<td>NAc</td>
<td>nucleus accumbens</td>
</tr>
<tr>
<td>NE</td>
<td>norepinephrine</td>
</tr>
<tr>
<td>NET</td>
<td>norepinephrine transporter</td>
</tr>
<tr>
<td>NHP</td>
<td>nonhuman primate</td>
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<tr>
<td>Abbreviation</td>
<td>Definition</td>
</tr>
<tr>
<td>--------------</td>
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<tr>
<td>P4</td>
<td>progesterone</td>
</tr>
<tr>
<td>PET</td>
<td>positron emission tomography</td>
</tr>
<tr>
<td>PFC</td>
<td>prefrontal cortex</td>
</tr>
<tr>
<td>PVN</td>
<td>paraventricular nucleus of the hypothalamus</td>
</tr>
<tr>
<td>S+</td>
<td>stimulus signalling reinforcement</td>
</tr>
<tr>
<td>S-</td>
<td>stimulus signalling trial termination, lack of reinforcement</td>
</tr>
<tr>
<td>SA</td>
<td>self-administration</td>
</tr>
<tr>
<td>SD</td>
<td>simple discrimination (cognitive tasks); standard deviation (statistics)</td>
</tr>
<tr>
<td>SDR</td>
<td>simple discrimination reversal</td>
</tr>
<tr>
<td>SEM</td>
<td>standard error of the mean</td>
</tr>
<tr>
<td>SERT</td>
<td>serotonin transporter</td>
</tr>
<tr>
<td>SNS</td>
<td>sympathetic nervous system</td>
</tr>
<tr>
<td>TSST</td>
<td>Trier Social Stress Test</td>
</tr>
<tr>
<td>VTA</td>
<td>ventral tegmental area</td>
</tr>
<tr>
<td>WGTA</td>
<td>Wisconsin General Testing Apparatus</td>
</tr>
</tbody>
</table>
LIST OF TABLES

CHAPTER II

TABLE I. Phase of cycle for each subject when acquisition occurred, number of total trials, errors and omissions to SD and SDR criterion……………………………………………………………………………………………………………………………………………………………….114

TABLE II. Phase of cycle for each subject when acquisition occurred, number of total trials, errors and omissions to SD and SDR criterion……………………………………………………115

CHAPTER III

CHAPTER IV

TABLE I. The effect of menstrual phase at each cocaine dose tested. Mean (±SEM) response rate and intake at the follicular phase and luteal phase of the menstrual cycle within subordinate and dominant monkeys. *p<0.05 between luteal and follicular at that particular dose………………………………………………………………………………………………………………………………………………………………………………184

TABLE II. Baseline response rates and reinforcers in socially housed female monkeys………………………………………………………………………………………………………………………………………………………………………………185

CHAPTER V

TABLE I. Subjects’ ages (years), weights (kg), lifetime cocaine intakes (mg/kg) and the individual delay times in the DMS task (seconds)……………………………………………………………217
TABLE II. Mean (± SEM) response latencies and pellet retrieval latencies (in sec) in cocaine-naïve (coc-naïve) and cocaine-experienced (coc-exp) monkeys………………218

CHAPTER VI
LIST OF FIGURES

CHAPTER II

FIGURE I. Mean (±SEM) estradiol (E2) and progesterone (P4) concentrations across the four selected phases of the menstrual cycle (n=12 monkeys per point).................. 115

FIGURE II. Relationship between concentrations of estradiol (E2; left panels) and progesterone (P4; right panels) and performance on a simple discrimination (SD; panels A-D) and SD reversal (SDR; panels E-H) task. Ordinate: Number of total trials (A, B, C, D) and errors (E, F, G, H) required to meet criteria performance. Abscissa: left: E2 concentrations (pg/ml); right: P4 concentrations (ng/ml).............................. 116

FIGURE III. Error distribution during acquisition of the reversal-learning task across the four phases of the menstrual cycle (n=14). Bars represent mean (±SD) number of error responses on each non-reinforced stimulus during the SDR stage. *, p < 0.05............ 117

FIGURE IV. Accuracy at short, medium, and long delays (n=11) during the four phases of the menstrual cycle. Points represent mean (±SD) ..................... 117

CHAPTER III

FIGURE I. The relationship between the weight of a monkey prior to social housing and the eventual rank of that animal following social housing........................... 151

FIGURE II. Average daytime locomotor activity prior to social housing (A) and during initial week of social housing (B). Points depict mean (n=4/group) ±SEM values...... 152

FIGURE III. Assessment of hormone concentrations as trait markers for eventual social rank. (A) Estrogen (E2), (B) progesterone (P4), (C) morning cortisol, (D) evening
cortisol, and (E) total testosterone concentrations prior to social housing as predictors of eventual rank. Points depict mean (n=4/group) ±SEM. * p< 0.05, **p<0.01 ……153

FIGURE IV. Assessment of hormone concentrations during the initial week of social rank formation. (A) Morning cortisol, (B) evening cortisol, and (C) total testosterone concentrations during initial week of social housing (Monday-Friday). Points depict mean (n=4/group) ±SD values. * p< 0.05, ** p<0.01, *** p<0.001………………154

FIGURE V. Assessment of hormone concentrations as state markers for social rank. (A) Estrogen (E2), (B) progesterone (P4), and (C) total testosterone concentrations following social hierarchy establishment (3 months after initial social housing). Points depict mean (n=4/group) ±SEM values. * p< 0.05, ** p<0.01………………………………………155

FIGURE VI. Performance on a delayed matching-to-sample task during initial week of social housing compared to prior baseline performance. Dependent measures include accuracy at the short delay length (A), medium delay length (B), long delay length (C) and number of omissions (D). Points depict mean (±SD) values…………………………………156

CHAPTER IV

FIGURE I. Dominant female monkeys and subordinate monkeys acquire cocaine reinforcement at similar doses. Percentage of dominant (open symbols) and subordinate (closed symbols) monkeys that reached criteria to acquire cocaine self-administration at various doses of cocaine available under a 30-response fixed-ratio schedule of reinforcement…………………………………………………………………………………………186

FIGURE II. Relationship between average circulating estradiol and dose at which cocaine first functioned as a reinforcer. ………………………………………………………………186
FIGURE III. Reinforcing effects of cocaine are greater in subordinate female monkeys compared with dominant animals. Mean (±SEM) rate of responding (responses/sec) when saline or various doses of cocaine were available per session for dominant (ranks #1 and ranks #2, open symbols, n=8) and subordinate (ranks #3 and #4, closed symbols, n=7) monkeys in the follicular (A) or luteal (B) phase of the menstrual cycle. Mean (±SEM) cocaine intake (mg/kg/session) during the follicular (C) or luteal (D) phase of the menstrual cycle. Each dose was available for at least five sessions until responding was stable; data represent to mean of the last 3 days of availability for each monkey. *p<0.05; **p<0.01 between dominant and subordinate monkeys at that particular dose…

CHAPTER V

FIGURE I. Performance during acquisition (total trials, errors and omissions to criterion) of the simple discrimination (SD) and reversal (SDR) phases in cocaine-naïve (coc-naïve, n=9) and cocaine experienced (coc-exp, n=5) monkeys. Data for errors was square-root transformed prior to analysis. Bars depict mean (± SD) values. *, p < 0.0125

FIGURE II. Error distribution during acquisition of the reversal-learning task in cocaine-naïve (n=9) and cocaine-experienced (n=5) monkeys. Bars represent mean (± SD) number of errors (square-root transformed) on each non-reinforced stimulus during the SD phase (left) and SDR phase (right). *, p < 0.0125

FIGURE III. Accuracy at (left), and absolute lengths of (right), the delays deemed short, medium and long in cocaine-naïve (n=8) and cocaine-experienced (n=5) monkeys. Points represent mean (± SEM)
FIGURE IV. Trials to criterion (top row) and errors to criterion (square-root transformed, bottom row) during the simple discrimination (SD, left column) and reversal (SDR, right column) phase for the three months following acquisition (A). Points depict mean (± SEM) values in cocaine-naïve (n=9) and cocaine-experienced (n=5) monkeys. Data from acquisition (A) are included for comparison.

CHAPTER VI
ABSTRACT

Kromrey, Sarah A

ENDOGENOUS HORMONE MILIEU OF FEMALE CYNOMOLGUS MONKEYS: EFFECTS ON SOCIAL RANK, COGNITIVE PERFORMANCE AND ACQUISITION OF COCAINE SELF-ADMINISTRATION

Dissertation under the direction of Michael A. Nader, Ph.D., Professor of Physiology & Pharmacology and Paul W. Czoty, Ph.D., Associate Professor of Physiology & Pharmacology

There are currently no FDA-approved drug treatments for cocaine abuse. The use of female subjects and a better understanding of the influence of hormones and cognitive function may provide valuable information to aid in pharmacotherapy development. The present studies utilized female monkeys housed in groups of four to examine the interactions of social rank and hormone concentrations on vulnerability to cocaine reinforcement and cognitive function. The overarching goal was to characterize how estradiol (E2) and progesterone (P4) concentrations influence cognitive performance, eventual social rank and vulnerability to cocaine self-administration (SA). We also examined how social rank altered these and additional hormonal measures.

Dopamine (DA) in the brain mediates cocaine abuse. E2 also influences DA concentrations and fluctuates across the menstrual cycle. The interaction between E2 and DA may impact cognitive function. Chapter II investigated the role of fluctuations of E2 and P4 on acquisition and subsequent performance of two cognitive tasks: a reversal learning task, measuring associative learning and behavioral flexibility, and a delay
match-to-sample task, measuring working memory. It was noted that phases with high E2 and low P4 were related to improved cognitive performance on the reversal learning task. Stress is also a factor influencing cocaine abuse and cognitive function. Social housing provides an ideal assessment of the factors that may contribute to the likelihood of occupying a particular rank. Chapter III investigated potential trait variables, and identified dysregulation of these factors as state variables. E2 concentrations in future #3-ranked monkeys were higher prior to social housing compared to all other ranks and rank-related effects on circulating cortisol and cognitive performance were observed.

Moreover, utilizing drug SA, Chapter IV identified that circulating E2 and occupying a subordinate rank increased vulnerability the reinforcing effects of cocaine. Cocaine users show cognitive deficits that persist into abstinence, although the extent and duration is not well established. In Chapter V, monkeys that previously SA cocaine performed worse, compared to cocaine-naive monkeys, during the reversal-learning task. Integrating cognitive and hormonal measures with social housing and cocaine SA extends our knowledge of hormonal influence on these variables and may contribute to sex-specific treatment strategies.
CHAPTER I

INTRODUCTION: PRECLINICAL MODELS AND CLINICAL STUDIES OF THE ROLE OF HORMONAL INFLUENCE ON COGNITIVE FUNCTION, SOCIAL STRESS AND COCAINE ABUSE IN FEMALES
1. FEMALES AND COCAINE ABUSE

Cocaine abuse and dependence remains a critical challenge for society. Cocaine addiction results in deleterious consequences on an individual’s health, work, and family as well as enormous societal economic burden and public health consequences (Karila, 2012). In the United States alone, an estimated 24.6 million people over the age of 12 were current illicit drug users, this includes 1.5 million cocaine users and almost 600,000 individuals used cocaine for the first time this past year (SAMHSA, 2014). These statistics advocate that not only is cocaine abuse an ongoing issue for our society, but is one that will continue to be a problem as future drug-dependent individuals are joining the ranks daily. Although males are consistently cited as having a higher prevalence of cocaine abuse over women, women may be more vulnerable to the reinforcing effects of cocaine (Greenfield et al, 2010). Furthermore, evidence on gender differences in cocaine dependence have been mixed with some studies providing evidence for women having an increased likelihood for experiencing cocaine dependence compared to men (O’Brien and Anthony, 2005; Wu et al., 2009).

It has been widely documented both in clinical cohorts and rodent preclinical models that females have a different trajectory to cocaine use and dependence. Specifically, females have been shown to initiate drug use at an earlier age (Chen and Kandel, 2002), progress from first use to dependence faster (Haas and Peters, 2000) and have more severe addiction symptoms entering treatment (Westermeyer and Boedicker, 2000; Lynch, 2006; Wagner and Anthony, 2007; Kasperski et al., 2011). It has also been suggested that women who seek treatment exhibit a more severe pattern of drug use-related social, medical and/or psychiatric problems compared to males (Denier et al.,
Abundant progress has been made to improve our understanding of drug addiction; however, pharmacological treatments for cocaine abuse have remained elusive. This may be in part due to the male-centric approach that researchers have historically taken when studying factors and/or treatments that influence drug abuse (Anker and Carroll, 2010). The lack of inclusion of female subjects in both preclinical and clinical studies has led to neglect of factors that may be underlying drug abuse in women, such as ovarian hormones. It should be pointed out that fewer novel drugs are being approved now than in the past, with most drugs failing in clinical trials (Hay et al., 2014). By considering sex differences earlier on in research, perhaps some of these disappointments could be circumvented. Specifically, ovarian steroid hormones (e.g., estradiol (E2) and progesterone (P4)) may influence the behavioral effects of drugs and contribute to susceptibility. An important direction for current research is to study the neurobiological basis for female vulnerability in substance abuse and its implications for treatment (Ashley et al., 2003; Marsh et al., 2004). A better understanding of variables that influence vulnerability to drug abuse in females is the goal of this dissertation research project.

Although pharmacotherapies have not been overtly successful, behavioral treatment strategies have demonstrated success in both men and women (DeVito et al., 2014). The success of behavioral strategies is largely dependent on cognitive
performance entering treatment (Aharonovich et al., 2006), therefore knowledge of
cognitive deficits produced by drugs of abuse may be extremely important in developing
treatment for cocaine abusers. However, whether sex affects cognitive performance in
cocaine abusers is not well understood because of the under-representation of women in
studies on drug abuse and cognitive performance (van der Plas et al., 2009). The studies
in this dissertation aim to elucidate the mechanisms by which hormones and environment
contribute to vulnerability to cognitive performance and cocaine abuse. Through such
research we may ultimately identify treatments that are more effective for females, which
is one step on the path to truly personalized medicine.

There is a longstanding sex bias in biomedical research, which leads researchers
to preferentially study diseases and test drugs in more male subjects than females in both
animal studies and human clinical trials. This practice not only puts women at unintended
risk, but also limits the scope of our scientific knowledge. This is especially concerning
as it is widely recognized that men and women manifest diseases differently, experience
illnesses differently, and treatments may be differently beneficial (i.e. Pilote et al., 2007;
Abel et al., 2010; Arnetz et al., 2014; Mostertz et al., 2010). These sex differences extend
to cocaine abusers as well. Epidemiological data demonstrate that while women report
lower rates of drug use compared to men, the number of current women users continues
to increase (SAMHSA, 2014). Additionally, women progress through the phases of drug
addiction differently in what is called a ‘telescoping effect’ where women transition from
casual drug use to dependence faster, experience higher levels of craving and relapse
during abstinence, take larger amounts of substance during bouts of relapse and are also
less likely to enter substance abuse treatment compared to men (Brady and Randall,
However, once women enter treatment, there are conflicting results as to whether gender itself is a predictor of treatment retention or outcome (Siqueland et al., 2002; Greenfield et al., 2007). Studies linking predictors of treatment outcome have linked certain characteristics with more favorable outcomes for men and women, such as fewer mental health problems and less severe drug problems (Greenfield et al., 2007; Green et al., 2002). Considering women are more likely to have comorbid mental health problems and more severe drug problems when entering treatment (Garg et al., 1999; Weiss et al., 2003), it makes sense that they may be at particular risk for treatment failure. In women-only samples, associations have been found between retention and characteristics including psychological functioning, higher levels of personal stability and social support, lower levels of anger, treatment belief, and referral source (Greenfield et al., 2007; Kelly et al., 2001; Loneck et al., 1997). To date, most substance abuse treatment models have been designed for men and based predominantly on male norms, however gender-specific interventions have resulted in mixed findings about whether these treatments are superior (Greenfield et al., 2007; Ashley et al., 2003; Kaskutas et al., 2005; Cummings et al., 2010). Over the past few decades, awareness of the importance of sex differences in addiction has grown, and dedication to characterizing sex differences in cocaine abuse continues (Evans, 2007).

The Introduction is divided into sections relevant to the design of the dissertation research. Because the studies utilized normally cycling females in models of cognition and cocaine abuse, the interactions of sex hormones and dopamine will be described first.
The second Chapter of this dissertation addressed how endogenous hormonal fluctuations influence cognitive performance, thus what is currently known about sex hormones and cognitive function and cognitive testing in nonhuman primates (NHPs) will be outlined next. A major strength of these studies is the use of socially housed NHPs. Therefore, the relationship between hormones and stress and how occupied social rank of an NHP represents differential stress exposure will be described. Additionally, the use of hormonal and cognitive measures to quantify these differences in stress exposure will be discussed. Finally, the last sections of this introduction will elaborate on cocaine abuse and what is known about how cocaine self-administration interacts with social rank and cognitive function.

2. DOPAMINE AND SEX HORMONES

The dopamine (DA) system plays a crucial role in motivated behaviors, goal-oriented movement, attention, reward and reinforcement (Carlsson, 1987; Robbins, 2003). The mesolimbic pathway originates in the ventral tegmental area (VTA) which project to the nucleus accumbens (NAc), bed nucleus of stria terminalis (BNST), and frontal cortex (Koob, 1992; Gardner, 2011). Neural activity in both the VTA and NAc is modulated via GABAergic, glutamatergic, serotonergic, and opioid peptidergic systems. Additionally, the VTA also receives noradrenergic input from the locus coeruleus (Hyman et al., 2006, Koob, 1992, Volkow et al., 2004; Wise, 2004). Drugs of abuse activate the mesolimbic DA pathway via stimulation of VTA neurons which result in DA
release into the NAc (Di Chiara and Imperato, 1988, Owesson-White et al., 2009; Pierce and Kumaresan 2006).

There are two superfamilies of DA receptors, the D1- and D2-like G-protein coupled receptors. The D1-like superfamily is composed of D₁ and D₅ subtypes, whereas the D2-like superfamily is made up of the D₂, D₃, and D₄ receptor subtypes. Importantly, both the D₁ and D₂ receptors are expressed in the areas previously mentioned with the DA pathways. The D2-like superfamily of receptors has been of particular interest to researchers interested in drugs of abuse. For example, positron emission tomography (PET) radiotracers have been developed to target the D2-like receptors and have been utilized extensively in both human and NHP studies (Volkow et al., 1993; Gould et al., 2014). In brief, NHP studies have found that a multitude of factors including stress and fluctuations in ovarian hormone concentrations affect measures of D2-like receptor availability (Morgan et al., 2002; Riddick et al., 2009). Additionally, D2-like receptor availability has been related to differences in sensitivity to the reinforcing effects of cocaine (Morgan et al., 2002; Nader et al., 2012).

Chronic enhanced activation of mesolimbic DA transmission can result in long-term functional changes within this pathway. Some of these functional outcomes will be discussed in this Introduction, specifically cognitive dysfunction. It is well established that chronic use of drugs “hijacks” normal motivated behaviors (Hyman et al., 2006) but endogenous substrates may also play an important role in mediating this system. Although most of what is known about DA, reinforcement, and motivation comes from studies conducted in male animals, over the past few decades, awareness of the importance of investigating the interaction of the female hormonal milieu and the DA
system has emerged. In fact, physiological and psychological responses to drugs of abuse are well documented and it is well established that the effects of E2 on DA systems are largely responsible for sex and menstrual cycle differences (Bobzean et al., 2014). Importantly, both human and animal studies have demonstrated that dysregulation of the DA pathway by drugs of abuse is influenced by sex. For example, rodent studies have shown sex differences in basal DAergic tone and activation (for review see Becker, 1999; Becker and Hu, 2008 and Becker et al., 2012), female rats have greater levels of basal DA concentration in the striatum than males (Castner et al., 1993; Volkow et al., 2004) and greater striatal concentrations of DA following cocaine administration (Walker et al., 2006). Human studies demonstrate similar findings; women have higher presynaptic DA synthesis capacity in the striatum (Laakso et al., 2002), and men have more basal DA release in the striatum (Munro et al., 2006). Moreover, evidence suggests that women may have lower striatal D2 receptor affinity than men which can be affected by both age and menstrual cycle (Pohjalainen et al., 1998).

The sexually dimorphic patterns of the DA system are likely large due to the influence of fluctuating levels of ovarian hormones associated with the primate menstrual and rodent estrous cycle. Preclinical microdialysis experiments support these sex differences showing that basal NAc levels of DA and its metabolite, DOPAC, are modulated by the estrous cycle in female rats (Shimizu and Bray, 1993). Additionally, ratios of extracellular striatal DOPAC/DA are highest during proestrus, suggesting a greater magnitude of DA turnover when circulating levels of E2 are high compared to other phases of the estrus cycle when E2 is low (Xiao and Becker, 1994). Evidence from preclinical studies supports E2 modulation of midbrain DA systems including pre-
postsynaptic components of DA transmission (Di Paolo et al., 1985; Becker and Beer, 1986; Becker, 1990; Bitar et al., 1991). Studies reveal that acute injections of E2 increase striatal DA release and turnover (Becker and Ramirez, 1981; Becker et al., 1984; Di Paolo et al., 1985; Becker and Beer, 1986) and increase the density of striatal DA uptake sites (Morissette et al., 1990). E2 also potentiated amphetamine-induced DA release, increased DA turnover in the NAc, and enhances cocaine-stimulated striatal DA release (Becker et al., 1984; Di Paolo et al., 1985; Peris et al., 1991; Thompson and Moss, 1994). Postsynaptically, the effects of E2 include increased number of striatal D1 DA receptors, decreases in high affinity agonist D2 DA binding, and increases in low affinity D2 DA agonist binding (Di Paolo et al., 1985, Levesque and Di Paolo, 1988, Levesque and Di Paolo, 1989, Morissette et al., 1990 and Shieh and Yang, 2008). Lastly, repeated cocaine administration to E2-treated ovariectomized (OVX) rats resulted in decreased striatal D2-like receptor binding (Febo et al., 2003). Overall, it appears that E2 demonstrates an augmented effect on striatal DA activity.

Although the majority of findings are characterized in the striatum, there are also sex and E2-mediated differences in DA within the VTA (Morissette et al., 2008; Gillies and McArthur, 2010; Johnson et al., 2010). Females have a significantly greater proportion of VTA DAergic neurons compared to males (Kritzer and Creutz, 2008). E2 removal reduced the number of cells of the rate-limiting enzyme for DA synthesis, while E2 replacement prevented this cell loss in female rodents (Johnson et al., 2010). Other studies exploring the effects of E2 on VTA DA transmission show that the basal firing rate, bursting rate, and spontaneous activity of VTA DA neurons is lowest in proestrus females but increases during estrus, when E2 is high (Zhang et al., 2008; Bobzean et al.,
From these studies, it appears that E2 plays a critical role in preserving the sensitivity of VTA DA neurons.

Facilitation of the reinforcing effects of drugs of abuse may also be affected by the interactions between DA receptors and E2 receptors. Numerous studies have demonstrated that D2-like receptor availability is related to differences in sensitivity to the reinforcing effects of cocaine (Volkow et al., 1993; Morgan et al., 2002; Nader et al., 2012). Because these midbrain DA systems contain high numbers of E2 alpha (ERα) and beta receptors (ERβ) (Creutz and Kritzer, 2002 and Creutz and Kritzer, 2004) and evidence suggests that E2 regulation of striatal D2 receptors occurs through the activation of the ERβ (Morissette et al., 2008), it is possible that E2 plays a more prominent role in the reinforcing effects of cocaine than what was previously thought. Support for this idea comes from a study by Febo and colleagues in 2003 demonstrating E2 treatment changes D2-like receptor function. Studies in male animals have demonstrated that the upregulation of these DA receptors is critical for reinstatement of drug-seeking behavior (Anderson and Pierce, 2005). Additionally, E2 produces indirect effects at D1 receptors and changes in levels of E2 regulate the firing rate of DA neurons; the downstream effect is changes in DA release and D1 receptor density in both the dorsal striatum and NAc (Becker, 1999; Becker and Rudick, 1999; Dazzi et al., 2007; Di Paolo, 1994; Laakso et al., 2002; Levesque and Di Paolo, 1989; McEwen and Alves, 1999; O'Dell and Torres, 2013; Zhou et al., 2002). Therefore, E2-induced activation of the ERβ in the NAc is involved in the upregulation of DA receptors. It is hypothesized that upregulation of these DA receptors is critical for changes in DA mediated behaviors. Taken together, these data provide support for the hypothesis that fluctuations in levels of ovarian hormones over
the estrous cycle of female rodents, and the menstrual cycle in both human and nonhuman primates, correspond with alterations in DA system activation. Since DA is implicated in cognition and drug abuse, these interactions will be a critical part of the studies described in Chapters II, III and IV.

2.1 SEX HORMONES AND COGNITIVE FUNCTION

Interest in how hormones may regulate cognitive function largely stems from evidence for sex differences in cognitive skills. Quantitative differences have been consistently found between males and females with females’ excelling on tasks of verbal skills and memory, on perceptual speed and accuracy, and on fine motor skills. Alternately, males tend to outperform women on tests of visual memory and on mathematical and spatial ability (Halpern, 1992; Shewin, 2013). These sex differences in cognitive performance are believed to be a result of differential exposure to hormones prenatally and referred to as “organizational effects”. A similar phenomenon is observed following puberty, when circulating hormones can amplify the neural “hard-wiring” laid down prenatally, which is referred to as the “activational effect” (Sherwin, 2012). Therefore, it is assumed that following puberty, circulating E2 and P4 concentrations may have profound effects on cognitive performance in females.

The ideal way to assess how fluctuations in circulating E2 and P4 may be associated with changes in cognitive function is to observe whether performance on a particular task changes in accordance with serum hormone levels during different phases of the menstrual cycle. Many investigations utilizing this approach failed to find any significant associations between cognitive function and menstrual cycle hormone
fluctuations in women (Pierson and Lockhart, 1963; Zimmerman and Parlee, 1973; Wuttke et al., 1975; Dor-Shav, 1976). Unfortunately, these studies suffered from methodological issues, including small sample size, failure to confirm menstrual cycle phase with hormone concentration analysis and use of inappropriate cognitive tasks to properly test the hypotheses (Broverman et al., 1981). Most subsequent studies that have identified these flaws and incorporated these factors into their experimental design have revealed changes in specific cognitive abilities across the menstrual cycle (Hampson, 1990a, 1990b; Maki et al., 2002). The majority of these findings suggested that estrogen facilitates verbal and fine motor abilities; however, these conclusions were drawn according to phases of the menstrual cycle during which P4 concentrations peak alongside E2. Therefore, the conclusions reached may be confounded by the omission of not including P4 concentration analysis. One study by Phillips and Sherwin in 1992 assessed how both E2 and P4 concentrations correlated with performance on a test of visual memory and found that scores were positively correlated with levels of P4 but not with E2. These findings demonstrate the importance of including both E2 and P4 assessments in studies aimed at assessing hormonal influence on cognition. Throughout all experiments as part of this dissertation, menstrual cycle was monitored and for certain studies, blood samples were taken to assess E2 and P4 concentrations in each monkey.

It has also been hypothesized that E2 and P4 may have differential effects depending on the task assessed. For example, performance on fine motor tasks are thought to be facilitated by E2, whereas visual and spatial tasks (where males typically excel) are better in women during phases of the menstrual cycle when E2 is low, suggesting that higher concentrations of E2 cause impairment (Maki et al., 2002;
Sanders et al., 2002). Support for this theory comes from preclinical research as well, where OVX animals that are not treated with exogenous hormones perform better on spatial tasks (Fugger et al., 1998; Lacourse et al., 2000). To further complicate things, there is a variable background of E2 secretion during all phases of the menstrual cycle which may also influence the effects the hormone have on aspects of cognitive performance. Therefore, although many studies investigate performance on a single task, the ideal study would assess multiple cognitive domains in an attempt to gain a larger understanding of the within-subject interaction between hormones and cognition.

*Therefore, the studies in Chapter II assessed how E2 and P4 concentrations at multiple time points across the menstrual cycle were associated with cognitive performance on tasks involving multiple cognitive domains. The goal of this study was to provide important information regarding how fluctuations in hormone levels within the physiological range may influence specific aspects of cognition in gonadally intact, normally cycling monkeys.*

2.2 COGNITIVE ASSESSMENT IN NHPs

NHPs are phylogenetically closely related to humans and macaques, including cynomolgus (*Macaca fascicularis*), are some of the most closely related NHPs approved for invasive biomedical research in the United States, making them the ideal model for translational research. Macaques have close homology to humans in terms of developmental and aging processes, neurotransmitter distribution, as well as complex social and cognitive behavioral repertoires (Weerts et al., 2007 for review). Specifically, humans and NHPs share greater than 95% overall gene homology and greater than 98% homology in monoaminergic transporters (Hacia et al., 1998; Miller et al., 2001).
Although many human studies reflect similar findings of those taken from rodent studies, it is important to develop an animal model that more closely reflects the human condition in order to provide insight into clinical responses prior to testing in humans. NHPs are the ideal animal subject for this research as they have provided the most generalizable findings to predict factors that contribute to human drug abuse (Weerts et al., 2007).

Additionally, there are differences in DA neuron innervation (Berger et al., 1991; Joel and Weiner, 2000) and affinity of receptors for DA between monkeys and rodents (Weed et al., 1998) which suggests other differences may exist between these species in drug biodistribution, pharmacokinetic or pharmacodynamic interactions within the DA system (Lyons et al., 1996; Roberts et al., 1999; Lile et al., 2003). Related to the current research, female macaques have similar duration (28-day) and hormonal fluctuations during their menstrual cycle as humans (Appt, 2004; Shimizu, 2005), making them the ideal species to use in studies investigating the influence of gonadal hormone fluctuations on cocaine reinforcement.

An additional advantage of using NHPs is the ability to have within-subject designs for long-term studies in a controlled laboratory setting. This is advantageous because baseline behavioral, hormonal, and cognitive measures can be collected and then correlated with changes following an experimental manipulation. In the current study, both housing condition and drug self-administration (SA) were manipulated while factors including nutrition and alternate environmental/pharmacological stressors were controlled over numerous years. Furthermore, NHPs can learn complex cognitive tasks similar to those administered to humans. Depending on the task being assessed, initial learning of the task and/or the effects of environmental or pharmacological manipulations
on a stable performance can be evaluated. These types of studies allow researchers to
gain a better understanding of the temporary and/or more permanent effects that stress or
drug exposure can have on specific cognitive domains. Importantly, because of the
multitude of cognitive tasks available and the comparable neurocircuitry between the
primate brain and the human brain (Roberts et al., 1996 for review), findings in monkeys
have high translational relevance to human disease states.

Importantly, the tasks included in these studies have been adapted from human
neuropsychological batteries and administered to monkeys using touch sensitive
computer screens under almost identical conditions. Tasks that are not as amenable to
NHP testing have been modified for use in monkeys to examine similar cognitive
domains as those tasks administered in humans. The Cambridge Neuropsychological Test
Automated Battery (CANTAB), the apparatus utilized in the current research, is
comprised of a series of visual and spatial tasks designed to probe regional brain function
by challenging extensively characterized, specific cognitive components (Weed et al.,
1999). Monkeys are an ideal model because not only can they be trained to perform these
tasks that assess performance in specific cognitive domains, but they can learn to execute
multiple tasks over successive days. This allows for a more complete understanding of
the cognitive processes that are sensitive to impairment and those that are resilient.
Furthermore, tasks can be tailored to probe cognitive functions known to be impaired in
specific diseases states and/or that are mediated by certain brain areas. Although the
CANTAB system was initially employed in humans, more recent applications have
extended to NHPs research investigating a multitude of variables including age, disease
progression, pharmacological manipulation, and CNS lesioning on task performance
(Cirillo et al., 1989; Dias et al., 1996a, 1996b, 1997; Voytko 1999; Weed et al., 1999, 2008; Porrino et al., 2005; Hampson et al., 2009; Gould et al., 2012; Gould et al., 2013).

The current research assessed working memory, a domain commonly associated with the hippocampus (Chapters II and III), and behavioral flexibility, a domain that involves multiple brain areas but mainly the prefrontal cortex (Chapters II and V).

Standard working memory tasks include both delayed match- and non-match-to-sample tasks (DMS and DNMS) which use either visual or spatial cues. Previous research indicates that structures within the medial temporal lobe, particularly the hippocampus, make up the crucial memory circuit responsible for successful performance on these tasks (Wilson et al., 1990; Monk et al., 2002). In either assessment, a stimulus is presented to the animal to which they must attend, and then that stimulus (sample) must be remembered across a variable delay interval. Following the variable delay, the animal must select from an assortment of stimuli one that is either the same as the sample stimulus (match) or different than the sample stimulus (non-match) which was presented prior to the delay. In these measures of working memory, increasing the delay length or the number of distracter images provided in the match/non-match stage of the task increases the cognitive demand which is visualized by a decrease in performance accuracy. For the research described in this dissertation, DMS was the task used to assess working memory.

The reversal learning task tests both the learning of simple stimulus discrimination, and the reversal of this previously acquired stimulus discrimination. In the former, associative learning is tested and in the latter, behavioral flexibility is measured which requires the monkey to inhibit a previously learned response while simultaneously
seeking out a new reinforced response. In the simplest form of this task, two stimuli are simultaneously presented, with one stimulus associated with a reward (S+) and the other stimulus with no reward (S-). The animal must learn to discriminate between the two stimuli and choose the stimulus associated with the reward. A ‘simple discrimination’ is said to have been acquired when performance meets an experimenter-determined criterion such as 8 correct responses in a row, or a serial acquisition criterion such as 18 correct responses out of 20 consecutive trials. To study behavioral flexibility, following this acquisition, the contingencies are reversed such that the previously reinforced stimuli is no longer associated with reinforcement, and the previously non-reinforced stimulus is now reinforced (i.e., the S+ now becomes the S- and vice versa). Importantly, this single task informs researchers of two different cognitive processes. Specifically, acquisition of a simple discrimination serves as a measure of associative learning, while reversal learning provides an indirect assessment of response inhibition, which is an aspect of behavioral flexibility. This reversal task relies largely on the prefrontal cortex (PFC) which operates as part of a network involved in reward-based learning and goal-directed behavior (e.g., Izquierdo et al., 2004). The studies in Chapter II will incorporate the SD/SDR, as well as the DMS to better characterize the role of menstrual cycle in various cognitive domains.

Importantly, the majority of previous cognitive studies in NHPs assessing either visual working memory or reversal learning have been conducted in individually housed male monkeys. In fact, the studies within this dissertation are the first of their kind to be conducted in socially housed female macaques. Cognitive performance is integrated into every chapter of this dissertation, offering imperative information regarding hormonal,
stress, and drug effects on women’s mental health. As mentioned previously, Chapter II demonstrates the role that monthly hormonal fluctuations play in cognitive performance. Chapter III utilizes cognitive performance as a behavioral outcome measure to compliment hormonal assessment during social hierarchy formation. In Chapter IV, monkeys previously trained to perform a cognitive task, began to SA cocaine in afternoon sessions and cognitive effects were assessed on subsequent mornings. Finally, Chapter V assesses how cognitive impairments following chronic cocaine SA may persist into abstinence.

3. HORMONES AND STRESS

Stress activates multiple neural and endocrine systems to allow an individual to respond to and survive in a changing environment. Typically, a stressful event triggers a stress response that elicits the activation of the sympathetic nervous system (SNS) and the hypothalamic–pituitary–adrenal (HPA) axis. Activation of the SNS stimulates norepinephrine (NE) and epinephrine (EPI) release from the adrenal medulla. Specifically, activated SNS afferents directly target peripheral organs and stimulate the adrenal medulla to increase circulating catecholamines, allowing an immediate “fight-or-flight” response to the stressor. The catecholamines NE and EPI mediate their effects on target immune cells via stimulation of the adrenergic receptors which activates downstream factors including pro-inflammatory cytokines (Tan et al., 2007). Through this pathway, the SNS plays an important role in stress-induced immune alterations and can profoundly impact behavioral responses (Wohleb et al., 2011).
The remainder of this dissertation will focus on the HPA axis and the cortisol response. Briefly, neurons in the paraventricular nucleus of the hypothalamus (PVN) release corticotrophin releasing factor (CRF) and vasopressin into the anterior pituitary gland to stimulate the synthesis and release of adrenocorticotropic hormone (ACTH). This, in turn, stimulates both synthesis and release of glucocorticoids (cortisol in primates and corticosterone in rodents) from the adrenal glands into general circulation to elicit an appropriate stress response. The presence of increased levels of glucocorticoids produces an inhibitory effect (negative feedback) on the stimulatory limb of HPA axis.

Numerous clinical and preclinical studies have demonstrated sex differences in HPA axis activation. In general, these studies show that females have higher levels of circulating cortisol, greater ACTH release in response to stress, faster onset of cortisol secretion after stress, and a faster rate in the rise of cortisol (Armario et al., 1995, Haleem et al., 1988, Handa et al., 1994, Heinsbroek et al., 1991, Jones et al., 1972, Kant et al., 1983, Kitay, 1961, Kitay, 1963, Le Mevel et al., 1979, Rivier, 1999, Young, 1996). Because of these sex differences, it is hypothesized that sex-hormones may be responsible. Although testosterone has been reported to suppress activation of the HPA axis in male rats (Handa et al., 1994), these sex differences in HPA axis regulation have been typically attributed to the activational effects of female ovarian hormones.

Additionally, it is well established that disturbances of homeostatic balance, for example through stress, can interfere with reproductive measures. Although reproductive suppression may be the result of numerous physiological pathways, it is accepted that behaviors and/or situations that chronically activate the HPA axis also disrupt the hypothalamic-pituitary-gonadal axis in women and cause ovarian dysfunction (Berga and
Loucks 2006). This dysregulation of the reproductive neuroendocrine axis may alter secretory patterns of gonadotropic hormones (for review see Tandon and Chintala, 2001; Berga and Naftolin et al., 2012). As stress increases circulating glucocorticoids, the glucocorticoids suppress the secretion of luteinizing hormone by the pituitary and of FSH, E2 and P4 by the ovaries (Laatikainen, 1991). The downstream effects of these changes, even a mild hormonal shift, result in menstrual cycle disturbance, amenorrhea and a number of other adverse health effects (Brucker-Davis et al., 2001). Although the knowledge of the deleterious systemic effects stress has on gonadal hormones exists, very few studies have been able to shed light on the progression of hormonal disruption that takes place between acute stressors and chronic exposure to stress. Therefore, the studies in Chapter III characterized the effects of social stress associated with establishment of a social hierarchy on alterations of hormonal profiles, both following acute stress and again after stable hierarchies were established to assess the effects of chronic stress.

3.1 SOCIAL RANK OF NHP AS A STRESSOR

The use of animal models of social behavior to better understand the behavioral, physiological and neurobiological alterations in human diseases is understudied. Although there are well characterized models of social behavior in rodent (i.e Duncan et al., 2006; Quadros and Miczek, 2009; Smith, 2012), the complex social and behavioral repertoire of NHPs allows for longitudinal, within-subject studies of disease states that are easily translatable to human beings (Weerts et al., 2007; Kaplan et al., 2010; Nader et al., 2013). It is known that within the hierarchies of group-housed monkeys, social rank can greatly influence the quality of life of the animals (for review see Sapolsky, 2005). It is speculated that the lower ranking animals can offer insight into the health of of low
socioeconomic status individuals who are shown to have increased risk of cardiovascular, respiratory, rheumatoid, and psychiatric diseases (Adler et al., 2000, Wilkinson, 2000, Kawachi and Kennedy, 2002; Siegrist and Marmot, 2004). The study of rank-health relations in such hierarchies has often been framed in the context of stress and the idea that animals of differing ranks are exposed to different patterns and levels of stress (Sapolsky, 2005).

Physical stressors are no doubt an external challenge to one’s homeostasis. However the psychosocial stress of anticipating that a physical stressor may occur can be equally damaging. As described previously, both types of stressors activate an assortment of endocrine and neural adaptations that differ when that status of the stressor is acute or chronic. Chronic activation, as observed in a social hierarchy, can increase the risk of numerous diseases or exacerbate preexisting diseases including, hypertension, atherosclerosis, immune suppression, reproductive impairments, and affective disorders (Sapolsky et al., 2000). The prevailing view is that lower-ranked animals carry the greatest risk of stress-related disease (Manogue et al., 1975; Creel et al., 1996; Cavigelli, 1999; Czoty et al.; 2009). It is believed that in captive NHP social groups, high-ranking individuals maintain dominance through psychological intimidation rather than physical aggression and this may be why subordinates show more frequent physiological stress compared to dominants (Sapolsky, 2005). Furthermore, when hierarchies are stable, subordinates are most socially stressed for the following reasons: 1.) high rates of physical and psychological harassment, 2.) their lack of social control and predictability and 3.) a lack of social outlets such as grooming or displacing aggression onto someone more subordinate (Sapolsky, 2005). Furthermore, NHPs in captivity may demonstrate an
exaggerated social stress response as they have fewer means to evade dominant individuals than they would in a natural setting (Creel, 2001).

Thus, a combination of the circumstances described above can drive the socially derived stress in subordinate NHPs even in established social groups. Furthermore, studies have shown that animals of specific social ranks tend to show characteristic stress-related physiological profiles. The most frequently studied physiological endpoint is blood concentration of glucocorticoids, adrenal steroid hormones that are secreted during stress, such as cortisol. Importantly, glucocorticoids typify the double-edged nature of the stress response, as they are helpful in mediating adaptation to acute stressors, yet are pathologic when secreted chronically (Sapolsky, 2005). An example related to the current research is the finding that group-housed, premenopausal female monkeys experience reproductive deficits induced by the psychosocial stress of social housing (Kaplan, 2008). Similarly, it is known that in male cynomolgus monkeys, obtaining a particular rank gives rise to a particular physiological profile, rather than vice versa, because the individual was studied before being placed in a social group (Morgan et al., 2000; Czoty et al., 2008). Additionally, Czoty et al. (2009) demonstrated that an acute stress response may be present in subordinate animals during initial social housing; while not observable after hierarchies stabilized. However, what is currently known in female monkeys regarding stress and social rank is largely drawn from studies of already established NHP groups (Shively et al., 1989; Kaplan and Manuck, 1999; Bauer et al., 2010) or recently stabilized hierarchies (Riddick et al., 2009). Chapter III utilized baseline measures of cortisol to investigate if circulating levels predicted eventual rank. Moreover, a novel approach was taken in assessing acute and chronic stress response, as
cortisol concentrations were analyzed during initial hierarchy establishment as well as once stable hierarchies were formed.

3.2 HORMONES AND SOCIAL RANK

As referenced above, glucocorticoid concentrations are not predisposing traits, but may differ as a result of occupied social rank. Animals of low social rank have been observed to have elevated basal glucocorticoid concentrations and a slowed on/off ‘switch’ of the stress response. This pathological condition has been observed in cynomolgus monkeys (Adams et al., 1985; Morgan et al., 2000), talapoin monkeys (Keverne et al., 1982), olive baboons (Sapolsky, 1990), and squirrel monkeys (Manogue et al., 1975). There are also studies that report exceptions to this hypersecretion of glucocorticoids in macaques (Gust et al., 1993; Bercovitch and Clarke, 1995), squirrel monkeys (Mendoza et al., 1978) and talapoin monkeys (Cavogelli, 1999). The mixed results of these studies may be due to the variety of circumstances that can influence the stress-related pathology of occupying a particular rank. The stability of the social group, the length of time that the social group has been together and any number of individual traits of the specific animals may influence hormonal outcome measures. Of note, a study by Czoty et al. (2009) in socially housed male cynomolgus macaques demonstrated transient rank differences in circulating cortisol between subordinate and dominant monkeys, with subordinate monkeys having increased circulating cortisol compared to dominant monkeys for only the first three days of social housing. The approach taken in Chapter III to analyze cortisol during the initial housing as well as following stable hierarchy formation will offer insight as to why some studies report increases of cortisol in subordinate monkeys, whereas others do not.
The role that reproductive hormones play in social stress is equally complex. It is known that fluctuating levels of ovarian hormones exert modulating effects on HPA axis functioning, including responsiveness and sensitivity to negative feedback (Young et al., 1995). For example, female rats have prolonged secretion of ACTH as well as increased corticosterone production during the proestrus phase of the estrous cycle, when circulating E2 and P4 levels are highest, compared to the diestrus phase of the cycle, when levels of these hormones are lower (Viau and Meaney, 1991). Female rats are also insensitive to the feedback effects of exogenously administered corticosterone on stress induced ACTH secretion; additionally, OVX increases the sensitivity of steroid feedback (Young, 1996). Similarly, women are less sensitive to dexamethasone feedback during the luteal phase of the menstrual cycle than during the early follicular phase (Altemus et al., 1997). These findings suggest that both E2 and P4 may influence the sensitivity to glucocorticoid feedback in females (Burgess and Handa, 1992, Ferrini et al., 1995, Patchev et al., 1995 and Rousseau et al., 1972). Hormone replacement studies have produced conflicting results with regards to the interactions of E2 and P4 with glucocorticoid concentrations. While some studies demonstrate that E2 enhanced onset and prolonged ACTH and corticosterone activity in female rats following stressful stimulation (Burgess and Handa, 1992, Carey et al., 1995 and Viau and Meaney, 1991), other studies have shown an inhibitory effect of E2 treatment (Russell et al., 2014; Young et al., 2001). The difference in these results is likely due to differences in hormone replacement dosing and treatment regimens.

In conditions involving social hierarchies, chronic stress inhibits reproduction in both sexes which is a classic example of stress suppressing a costly anabolic process until
more favorable times (Sapolsky, 2005). In female primates, this reproductive suppression can present itself as delayed puberty, decreased levels of E2 and P4, increased incidence of anovulatory cycles, impaired implantation, greater risk of miscarriage, prolonged interbirth intervals, and accelerated reproductive senescence (Sapolsky, 2005). As described previously, NHP studies show that the stress of occupying a subordinate rank is associated with decreased gonadal hormone levels, although these findings do not take into account the hormonal levels prior to social housing (Shively and Clarkson, 1994). In addition to cortisol measures, Chapter III assessed the influence basal E2 and P4 had on eventual social rank and tracked hormone concentrations rigorously throughout stable hierarchy establishment to pinpoint the alterations that occur in these reproductive hormonal measures over time.

4. STRESS AND COGNITIVE FUNCTION

Psychologically, acute stress is a subjectively negative experience, and can have positive as well as negative effects on cognition (Lupien et al., 2007; Starcke and Brand, 2012). The endocrine, immune and central nervous system are closely connected (Leonard and Song, 1999) and stress hormones can easily cross the blood-brain barrier to affect learning and memory (Buchanan and Lovallo, 2001; Takahashi et al., 2004; Lupien et al., 2007). The Trier Social Stress Test (TSST) is an acute stress protocol used to experimentally study the social stress response in human subjects and results in clear activation of key biomarkers of stress as well as its psychological effects (for review see Allen et al., 2014). Using the TSST, the relationship between acute social stress and
cognition is not simply linear; both high and low concentrations of circulating glucocorticoids can impair memory performance compared to moderate levels (Lupien et al., 2002). Furthermore, the cognitive effects of arousal states such as stress may depend on factors such as the difficulty of the task (Hanoch and Vitouch, 2004) and strategies employed during cognitive tasks in response to increasing stress.

When considering the interactions between stress and cognitive function, it is important to consider that the result of acute stress can be different from the changes observed following chronic activation of the stress cascade. On the molecular and cellular level, acute stress responses, grouped into the flight or freeze responses, are advantageous. However, when this stress response is sustained, the results can be detrimental. It is understood that animals occupying subordinate ranks are exposed to social stress for prolonged periods of time and undergo neurobiological changes. These changes involve inhibition of neurogenesis, dendritic atrophy, impairment of synaptic plasticity in the hippocampus (Magarinos et al., 1996; Kozorovitskiy and Gould, 2004) and altered patterns of apoptotic cell death with increases observed in the cortex while decreases are found in the hippocampus (Lucassen et al., 2001).

The secretion of hormones during acute stress responses has been shown to enhance cognition, augment immune responses, blunt pain perception and sharpen sensory thresholds (McEwen, 2004; Sapolsky, 2004). However, there is a glucocorticoid/neurotoxicity cascade hypothesis that predicts that cumulative glucocorticoid exposure reduces the resistance of neurons to insults, increasing damage and this augmenting cognitive function (Kudielka et al., 2009; Lupien et al., 2009). It is important to note that neurotoxic effects may be more prominent in certain brain areas,
such as the PFC or the hippocampus, and therefore chronically elevated cortisol may affect some specific cognitive abilities than others. One 35-year longitudinal twin study showed that increased stress resulted in increased cortisol levels and was associated with poor executive function, abstract reasoning, processing speed, and visual-spatial memory (Franz et al, 2011). This study is one of few human studies that have associated cortisol with executive function (PFC mediated tasks) as well as hippocampal function. Additionally, unlike many human studies in which glucocorticoids are exogenously administered to model a stress-like state (e.g. Newcomer et al., 1999), this study investigated the impact that living a chronically stressed life has on cognitive function; much like our socially housed NHP design aims to model.

Applying the knowledge of the stress system to cognitive processes is challenging due to the vast number of both stress assays and cognitive measures. This is particularly relevant because there are widespread differences in what is considered ‘stressful’ and in documented stress responses (Cohen and Manuck, 1995; Lazarus 1999). Furthermore, although a multitude of studies have attempted to address the question of how acute stress affects cognitive measures, not many preclinical models have endeavored to expand these investigations to chronic stress. This is surprising because social housing of nonhuman primates is a well-validated model of chronic psychosocial stress (Kaplan and Manuck, 1999; Czoty et al., 2009; Michopoulos et al., 2012). Chapter III is the first known investigation of how social housing affects cognitive function. Specifically, the studies described in Chapter III determine if future subordinate animals experienced impaired cognitive function initially and whether any noted impairments persisted after stable social hierarchies had been established.
5. COCAINE ABUSE

The reinforcing effects of cocaine depend on its ability to bind to the DA transporter (DAT) (Di Chiara and Imperato, 1988), although it binds similarly to the serotonin (5-HT), and NE transporters (SERT, NET, respectively; Ritz and Kuhar, 1989; Bennett et al., 1995). When cocaine binds, it blocks the removal of DA from the synapse, acutely elevating synaptic concentrations of all DA within the mesocorticolimbic system, this results in a buildup of DA in the synapse and increased stimulation of the receiving neuron (Di Chiara, and Imperato, 1988; Bradberry et al., 1993; Florin et al., 1994). Although the mechanism of action is fairly straight-forward, many factors may contribute the reinforcing effects of cocaine, including environmental and endogenous variables.

5.1 HORMONES AND COCAINE ABUSE

Studies show that E2 increases, whereas P4 potentially decreases the reinforcing effects of cocaine (for review see Bobzean et al., 2014). Preclinical studies in rodents have shown that phase of estrous cycle plays a role in sensitivity to cocaine’s reinforcing effects. Females are most vulnerable to cocaine’s reinforcing and locomotor effects (Roberts et al., 1989; Quinones-Jenab et al., 1999; Hecht et al., 1999; Feltenstein et al., 2009), and show resistance to extinguishing responding previously paired with cocaine (Kippin et al., 2005; Kerstetter et al., 2008) during estrus. These finding are further supported by research in OVX rodents which has confirmed a key role for E2 in acquisition rates of cocaine SA and cocaine-primed reinstatement of cocaine-seeking behavior (Becker, 1999; Lynch et al., 2001; Festa et al., 2004; Larson et al., 2005; Frye,
Replacement of E2 restores cocaine SA while administration of P4, and its metabolite allopregnanolone, attenuates cocaine seeking, cocaine-induced locomotor sensitization and blunts E2-augmented increases in cocaine sensitization (Becker, 1999; Lynch et al., 2001; Russo et al., 2003; Niyomchai et al., 2005; Jackson et al., 2006; Anker et al., 2007; Feltenstein et al., 2009).

Clinical studies have also been conducted to explore the extent to which endogenous hormone fluctuations across the menstrual cycle contributes to the subjective response to cocaine (Lukas et al., 1996; Mendelson et al., 1999; Sofuoglu et al., 1999; Evans and Foltin, 2006; Lynch, 2006; Sofuoglu et al., 2004; Collins et al., 2007). Some of these studies indicate E2 facilitates the subjective effects of cocaine. Women report increased feelings of high and increased heart rate during the follicular phase, when levels of E2 are high and P4 levels are at a minimum (Sofuoglu et al., 1999; Evans et al., 2002; Evans and Foltin, 2006). Furthermore, in the luteal phase, when P4 levels are at their peak, women report reduced positive subjective effects of cocaine (Sofuoglu et al., 1999; Evans et al., 2002; Evans and Foltin, 2006). It is important to note that E2 levels are also elevated at this time; for that reason the majority of studies involving humans and NHPs suggest that it is not the enhancing effects of E2 but the attenuating effects of P4 that strongly influence the rewarding effects of psychostimulants (Evans and Foltin, 2010).

In support of this, exogenous administration of P4 to women results in attenuated physiological and positive subjective effects of smoked cocaine (Sofuoglu et al., 2002; Evans and Foltin, 2006). However, when investigators administer intranasal cocaine to participants, findings are less systematic (Lukas et al., 1996; Collins et al., 2007). These
findings suggest that with different routes of cocaine, there may be differences in pharmacokinetics between cocaine hydrochloride, which is injected IV, and cocaine base, which is smoked. This possibility is supported by a large body of literature suggesting that route of administration may affect immediacy, duration and magnitude of cocaine’s effects (Hatsukami and Fischman, 1996). Additionally, although human studies have attempted to replicate the observed preclinical differences in cocaine SA as a function of estrous cycle phase in rodents, no orderly effects of menstrual cycle phase (Sofuoglu et al., 1999; Reed et al., 2011), or P4 administration (Sofuoglu et al., 2004; Reed et al., 2011) has been documented. Interestingly, studies investigating treatments for cocaine dependence have yielded results that differ depending on the sex of the individual. Preclinical studies examining baclofen, a GABAergic drug, reported reduced cocaine use particularly in women (Pettinati et al., 2008). In contrast, studies using bupropion found that this drug would not be as effective in women as men (Elkashef et al., 2008).

5.2 SOCIAL STRESS AND COCAINE ABUSE

The idea of examining the effects of environmental influences on SA behavior is not novel; in fact numerous preclinical studies have investigated this interaction by exposing animals to “enriched” or “stressful” environments. Enrichment is a broad term that encompasses greater access to resources within one’s environment such as food, novel stimuli, etc. This can be induced either through experimenter intervention, or through social housing resulting in attainment of a dominant position within the social hierarchy. As mentioned previously, stress is difficult to quantify, but is often introduced through an event that is unfamiliar, startling, and unpredictable or in a social setting where an individual is exposed to repeated aggression by others and resources are not as
easily gained, such is observed with occupying a subordinate position within the social hierarchy. Importantly, across several different species and models of environmental stress and enrichment, enrichment has been shown to result in neurobiological changes that reduce the vulnerability to self-administer psychostimulants, including cocaine (for reviews, see Nader et al., 2008; Stairs and Bardo, 2009; Solinas et al., 2010). Morgan et al. (2002) demonstrated that social rank of a monkey was associated with higher DA D2-like receptor availability, which has been shown to be a state variable that is associated with decreased vulnerability to drug SA (Morgan et al., 2002; Nader et al., 2006; Dalley et al., 2007). Interestingly, a human study complemented this preclinical data reporting that higher socioeconomic status was similarly related to higher DA D2-like receptor availability (Martinez et al., 2010). Furthermore, other studies suggest that strong social relationships, such as those between spouses, can facilitate recovery from addiction (Kosten et al., 1987) whereas weak social attachments may increase the vulnerability to substance abuse (Brennan and Shaver, 1995; Caspers et al., 2005).

Another influences on substance abuse is exposure to acute stressors. This results in increased cocaine seeking in rodents (Brown and Erb, 2007; Conrad et al., 2010) and increased relative reinforcing strength of cocaine in dominant monkeys following exposure to the stress of a rubber snake (Czoty and Nader, 2012). These data support the primary goal of behavioral treatment strategies because increasing exposure to positive, enriching environments (socialization with family, employment) and minimizing isolation and stress may reduce vulnerability to relapse and aid in sustaining abstinence.

Importantly, similar to environmental stressors, studies show that acute administration of most drugs of abuse activates the HPA brain stress pathway (Sinha,
Moreover, data from both human and animal studies demonstrate that regular and chronic drug use in addition to states of drug withdrawal and abstinence are associated with dysregulation of the HPA axis (for review, see Sinha, 2008). This is important because the same types of stressors that trigger activation of the HPA axis have also been shown to stimulate initiation of drug use, increase current drug use, and induce relapse to compulsive drug taking (Koob 2009; Sinha 2008). As previously discussed with acute vs chronic stress exposure; acute and long-term drug use active the HPA axis differentially as well. Specifically, both preclinical and human studies have also shown blunted HPA axis responses to stress challenge following long-term drug use (Adinoff et al., 2005 and Kreek, 1997). Not surprisingly, HPA axis dysfunction has been reported in substance use disorders (Gerra et al., 2008 and Walter et al., 2006).

Importantly, the majority of studies assessing the interaction between stress and cocaine abuse have been conducted in male animals and humans. The interaction between stress and drug abuse could be very different for females because ovarian hormones consistently show modulation of HPA axis stimulation and sensitivity and this, in turn, likely influences reward processing and addiction (Handa et al., 1994). Therefore, Chapter IV assessed how the rank (and therefore stress profile) of a female monkey influenced their susceptibility to the reinforcing effects of cocaine. Additionally, Chapter IV investigated the potential interaction of menstrual phase and this vulnerability.

5.3 PRECLINICAL MODELS OF COCAINE ABUSE

In preclinical models, the gold standard for measuring the reinforcing effects of drugs is intravenous drug SA (Bardo and Bevins, 2000). The classic SA studies involve operant
behavioral training where a stimulus (e.g. light, tone) signals the context in which a reinforcer (e.g. food, water, drug) will be delivered following a behavioral response (e.g. lever press, finger poke). To characterize whether a drug has reinforcing effects, the behavior maintained by the drug is compared to the level of the same behavior engendered by a neutral stimulus (e.g. the drug vehicle, typically saline). The drug is considered to have reinforcing effects if the drug presentation contingent on the rate of behavior occurs at levels greater than behavior leading to presentation of the neutral stimulus. Considering the complex nature of cocaine addiction, variations on this paradigm have improved the generalizability of SA across species, drug classes, and different stages of the addiction cycle being modeled.

The majority of animal models of drug abuse utilize simple schedules of reinforcement, specifically fixed-ratio (FR) schedules of reinforcement in which a constant number of responses results in delivery of a reinforcer. Behavior maintained under FR schedules does not allow researchers to directly compare the reinforcing “strength” of different stimuli. However, the current study used a specific procedure designed to measure sensitivity to the reinforcing effects of cocaine by making low doses of cocaine successively available until one dose is self-administered above the rate of saline. This was termed an acquisition paradigm as it allowed us to assess the lowest dose of cocaine that a monkey will ‘acquire’ SA. Furthermore, we compared rates of responding and drug intake to address additional questions regarding sensitivity to the reinforcing effects of cocaine at higher doses.

In addition to identifying what drugs are reinforcing, drug SA studies have been used for decades to also examine the effects of potential pharmacotherapies (Johanson
and Fischman, 1989; Woolverton and Nader, 1990; Mello and Negus, 1996). This is typically done by administering the putative pharmacotherapy acutely prior to the SA session and observing whether the reinforcer number or rate of responding is altered. Importantly, drugs that are abused by humans including stimulants, opiates, and barbiturates/benzodiazepines, are readily self-administered by both rodents and monkeys. Moreover, these drugs can be delivered via routes identical to those associated with humans drug use (e.g. oral, intravenous, inhaled), further contributing to the homology between these models. A major strength of the SA paradigm is that following minimal training, animals respond reliably and consistently; therefore, any observed deviations from this baseline response profile can be attributed to experimental manipulations. Additionally significant, is the fact that chronic cocaine SA in monkeys produce parallel neurobiological effects to those reported in human drug users including metabolic, structural and functional CNS alterations (Strickland et al., 1993; Volkow et al., 1993; Lyons et al., 1996; Beveridge et al., 2006). In this respect, drug SA studies have strong predictive and construct validity to human drug addiction. However, the majority of these preclinical studies use individually housed male rats and monkeys. Although this allows for a homogenous study population and ensures that results are easier to analyze and interpret, it may be limiting our knowledge into the profound contribution that variables such as hormones and social stress may have in human cocaine abuse.

Hormone-related differences in the motivation for and subjective effects of cocaine have been demonstrated in rodent studies. Specifically, female rats’ operant behavior is more robust than males’ during acquisition of cocaine SA, escalation of drug intake and reinstatement of previously extinguished drug seeking behavior (Lynch and
Furthermore, female rats acquire intravenous SA of cocaine more rapidly and at lower doses than males (Davis et al., 2008; Lynch and Carroll, 1999). Conditioned place preference (CPP) studies have also informed researchers on female vulnerability, showing reinstatement of cocaine CPP is greater for female animals (Bobzean et al., 2010).

Studying socially housed animals necessitates increased cost and experimental complexity to the experiment due to increases in number of animals. However, it can be argued that certain translational questions can only be answered using experimental designs incorporating animals living in a social environment (Nader et al., 2013). As described previously, the hierarchy continuum incorporates socially derived stress (in subordinate monkey), and environmental enrichment (in dominant monkeys) and has tremendous predictive, face, and construct validity (Nader and Czoty 2005). Most notably, social stress can influence the behavioral and reinforcing effects of drugs. Morgan et al. (2002) demonstrated that dominant males were less vulnerable to the reinforcing effects of cocaine than subordinate males. These findings were not replicated in female monkeys (Nader et al., 2012). Furthermore, exposures to environmental manipulations shifted the cocaine-choice dose-response curve differentially depending on social rank of the male monkeys (Czoty and Nader, 2005). These findings emphasize that a better understanding of the neurobiological mechanisms underlying the interactions of social rank with cocaine reinforcement may provide insight into improved treatment strategies (Miczek and de Almeida, 2012). Therefore, the goal of Chapter IV was to expand the studies of social rank on the reinforcing effects of cocaine to include female
monkeys and hormonal status. We examined the reinforcing effects of cocaine using an FR schedule of reinforcement and ascending low doses of cocaine.

6. COCAINE ABUSE AND COGNITIVE FUNCTION

Drug addiction is described as a chronic, relapsing, neurological illness characterized by a loss of control over drug seeking and intake. This loss of control is due to the fact that repeated use of drugs alters normal motivated behaviors via the dysregulation of brain reward circuitry (Hyman et al., 2006). By determining the cognitive alterations that are associated with cocaine use, we may better understand the mechanisms associated with the transition from first use to addiction. As mentioned above, the DA system underlies the reinforcing effects of cocaine. This system is comprised of four neuronal pathways originating in the midbrain with projections to various brain structures (for review, see Beaulieu and Gainetdinov 2011). The nigrostriatal pathway originates in the substantia nigra pars compacta, innervates the dorsal striatum (encompassing the caudate and putamen) and is involved in motor control. The mesolimbic pathway projects to the ventral striatum (nucleus accumbens), and other limbic structures including the amygdala, hippocampus, and cingulate gyrus. It is the key mediator of actions related to reward, reinforcement, emotion, and motivation. The mesocortical pathway innervates cortical regions and is implicated in learning and memory. Lastly, the tuberoinfundibular pathway projects to the hypothalamus and influences anterior pituitary gland function. Therefore, dysregulation of the DA system through neurodegeneration or pharmacological insult can have staggering functional
effects and contribute to a number of disease states including Parkinson’s Disease, depression, attention-deficit/hyperactivity disorder (ADHD), schizophrenia, and addiction (for review, see Vallone et al., 2000; Beaulieu and Gainetdinov, 2011). Therefore, the majority of drug development strategies for these conditions, including cocaine addiction, focus on direct and indirect mechanisms that influence the DA system.

Chronic cocaine use has a profound impact on brain function related to downregulation of dopamine D2 receptors in the striatum (Volkow et al., 1999), abnormalities in brain glucose metabolism (Baxter et al., 1988) and vascular hypoperfusion in subcortical, temporal and frontal regions (Strickland et al., 1993). Although it is understood that there is a difference between acute and long-term effects of drugs, there is not extensive insight into the cognitive effects during the temporal evolution of the development of drug addiction (Everitt and Robbins 2005). The loss of control over drug seeking and intake is not just a product of the positive reinforcing effects of drugs and drug-related stimuli; drug-seeking and taking behaviors are also promoted via negative reinforcement (Gardner, 2011 and Koob et al., 2014). This concept of a shift in motivation and neural systems is used to explain the characteristic persistent changes in motivation that are associated with drug dependence (Koob and Le Moal 2001; Koob and Le Moal 2008). Specifically, this reflects changes in activity of midbrain–forebrain systems that are shifting from impulsive to compulsive use, which represents a shift from prefrontal regulatory control of behaviors to limbic/striatal control (Everitt and Robbins, 2005; Piazza and Deroche-Gamonet, 2013).

The hallmark cognitive consequence of cocaine addiction is a disruption of executive function which includes all processes involved in learning, monitoring and
adapting to stimuli to produce complex, goal-oriented behaviors. Different tasks probe
the specific cognitive domains that are included in the umbrella term of ‘executive
function’. The specific cognitive domains that chronic cocaine users show impairments
compared to control groups include 1) updating, which includes monitoring and adapting
to cues relevant to a current goal and discarding/suppressing irrelevant information, 2)
shifting, which is the ability to redirect focus between multiple modalities or tasks, and 3)
inhibition, which is the ability to suppress or withhold a preplanned or impulsive
response (for review, see Miyake et al., 2000; Jovanovski et al., 2005; Beveridge et al.,
2008; Spronk et al., 2013). These impaired cognitive effects extend into abstinence,
although the degree of this extension is not fully known, but they can influence treatment
response, retention, and vulnerability to relapse.

Most paradigms investigating the effects of cocaine on memory assess either
immediate/delayed recall or working memory. These tasks are classically used to assess
the updating component of executive function and present a list of words or number that
must be recalled at a later point. Studies on the acute effect of cocaine on working
memory have not observed differences between users and non-users (Higgins et al., 1990;
Hopper at al., 2004; Haney et al., 2005). However, chronic cocaine users showed poorer
working memory performance on the n-back task, a test requiring an individual to recall
and identify a letter or number from a series that occurred n images prior to the current
one (Verdejo-Garcia et al., 2006; Tomasi et al 2007). Similarly, other tasks assessing
verbal or visual working memory (Hester and Garavan, 2004; Woicik et al., 2009;
Hanlon et al., 2011) revealed performance impairments in cocaine users and
demonstrated that increasing cognitive demand, either by the amount of information to
recall, the length of time, or the amount of distractions embedded between sample and recall phases, magnifies these cognitive deficits. *In Chapter V, the effects of cocaine and abstinence were assessed in monkeys with an extensive cocaine SA history and compared to performance in cocaine-naïve monkeys.*

Cognitive flexibility is a cognitive construct which involves switching between changing stimulus-reward associations. Cocaine users also show impairments in cognitive flexibility as measured via reversal learning and set shifting tasks (Kubler et al., 2005; Fillmore and Rush, 2006; Beveridge et al., 2008; Ersche et al., 2008; Hanlon et al., 2011). Reversal learning tasks probe specific aspects of the shifting component of cognition flexibility in the sense that the subjects must adapt their behavior according to changing contingencies. In the task, following a learned discrimination between two distinct stimuli the rules are reversed, unbeknownst to the participant, such that a previously non-reinforced stimulus is now reinforced and the previously reinforced stimulus now has no consequences. In this task, perseverative errors can be documented and studies have demonstrated that cocaine-dependent subjects adopt a more perseverative response style compared to controls (Ersche et al., 2008; Camchong et al., 2011; Fernandez-Serrano et al., 2012). Cocaine acutely may improve inhibitory control (Fillmore et al., 2002; Fillmore et al., 2005; Garavan et al., 2008), but following chronic use, studies clearly show impairments in response inhibition in cocaine users compared to non-users (Kaufman et al., 2003; Hester et al., 2007; Verdejo-Garcia et al., 2007; Liu et al., 2011; Fernandez-Serrano et al., 2012; Moeller et al., 2014).

Of importance for treatment development, these cognitive deficits are known to persist through weeks and months of abstinence (Verdejo-Garcia et al., 2006; Tomasi et
al., 2007). Nevertheless, most human studies compare currently or recently abstinent cocaine-dependent groups to cocaine-naïve control groups at only one timepoint which provides limited insight into whether acute abstinence from cocaine (e.g., several days) affects cognition differentially than longer durations of abstinence (e.g., weeks to months). This is extremely important seeing that Woicik and colleagues (2009) revealed that measures of attention and executive function were impaired to a greater extent in individuals abstinent from cocaine greater than 72 hours when compared to individuals that had used cocaine within 72 hours. This suggests that continuous or recent cocaine use may mask deficits that become magnified during abstinence and could increase vulnerability to relapse. Alternatively, another study showed greater impairments on tasks measuring working memory, planning and reaction time in current cocaine users compared to a group that maintained abstinence for 30 days (Hanlon et al., 2011). Interestingly, in that study, both of the cocaine history groups showed structural and cognitive deficits compared to cocaine-naïve controls. These studies highlight the need for additional research to elucidate the effects of cocaine, and abstinence from cocaine on cognitive performance. Utilizing NHPs we are able to control variables such as polydrug use, nutrition, and duration of cocaine use and/or abstinence. In Chapter V, the effects of cocaine and subsequent abstinence were assessed in monkeys with a SA history and compared to cocaine-naïve monkeys. These monkeys had been abstinent for 90 days, which provides an assessment of working memory and reversal learning performance during a critical window of abstinence during which treatment-seekers attempt to remain abstinent following treatment.
Interestingly, despite the numerous clinical studies demonstrating cognitive dysregulations in cocaine-experienced humans, there have not been very many studies examining the effects of cocaine on cognition in NHPs. The few studies that have addressed these effects in monkeys have been limited in the cognitive domains they have tested due to the extensive training required for most tasks. Overall, the most commonly assessed cognitive domains have been associative learning, measured using a simple discrimination task (SD); behavioral flexibility, measured using a reversal learning task (SDR); and working memory, assessed with a delayed matching-to-sample (DMS) task.

There have been only a few studies examining working memory in NHPs following a cocaine SA history. In a study by Liu et al. (2008), rhesus macaques (*Macaca mulatta*) with a cocaine history were trained on a delay alternation task to test working memory. The investigators found that monkeys with a cocaine history required a greater number of trials to acquire this task and that the cocaine-naïve monkeys improved at a significantly greater rate than the monkeys who continued to self-administer cocaine (Liu et al., 2008). An additional study by the same group found that, compared to a control group, monkeys who self-administered cocaine 4 days/week had impaired performance accuracy at the longest delay of a DMS task following 72 hours of abstinence during the first month, but tolerance developed to these disruptive effects (Porter et al., 2011). The effects of higher doses of cocaine and subsequent abstinence on working memory were assessed in a study by Gould et al. (2012). Initially, no differences in baseline working memory performance were observed between groups in a DMS task, but high-dose cocaine SA disrupted DMS performance, although tolerance developed to this. This study assessed abstinence from cocaine as secondary aim. Although acute abstinence did not
affect performance, by day 30 of abstinence performance accuracy increased significantly in cocaine-experienced monkeys, while performance of cocaine-naive monkeys remained unchanged. General distractibility during DMS was assessed during abstinence in a study by Porter and colleagues (2013). This study showed that although performance in the control group was not affected by either a novel or appetitive distractor, DMS performance was impaired in the cocaine group in the presence of the novel distractor. Taken together, these findings suggest that a history of cocaine exposure reliably alters working memory performance in NHPs.

Tests of behavioral flexibility following cocaine exposure are more common in the NHP cognition field. Jentsch et al. (2002) used the Wisconsin General Testing Apparatus (WGTA) to administer an object discrimination and reversal test to vervet monkeys (Chlorocebus pygerythrus) prior to and following two weeks of once daily, experimenter-administered injections of low (2 mg/kg) or high (4 mg/kg) doses of cocaine. Both groups of monkeys exhibited impaired abilities to learn the reversal portion of this task, but were not impaired in acquiring the initial discrimination (Jentsch et al., 2002). More recent studies have used touch-sensitive computer screens to administer a discrimination and reversal task prior to and following various cocaine SA paradigms (Liu et al., 2008; Porter et al., 2011; Gould et al., 2012). In a study by Liu et al. (2008), following chronic cocaine SA, monkeys were trained on discrimination contingencies between a high and low liquid reward. In this study design, a correct response depended on the monkey choosing the higher magnitude reward. It was determined that initial SD acquisition was impaired by chronic cocaine intake to such an extent that the SDR component could not be reliably conducted (Liu et al., 2008). Furthermore, Porter et al.
(2011) showed that monkeys who initiated cocaine SA did not show impaired percent accuracy in the first 15 trials, but did on sustained accuracy (defined as 27 of 30 consecutive trials correct). Additionally, when compared to controls, monkeys with a cocaine SA history had lower accuracy on the first 15 trials (Porter et al., 2011). When Gould and colleagues investigated behavioral flexibility in their cohort of monkeys, they found that cocaine-experienced monkeys required significantly more trials and committed more errors on reversal learning and multidimensional discriminations, compared with control monkeys (Gould et al., 2012). Effects of abstinence from cocaine on general distractibility during reversal learning was similarly investigated by Porter et al in 2013. In that study, stimulus discrimination was unaffected in cocaine-experience and control monkeys, whereas, reversal performance was disrupted by both the novel and appetitive distractors in only the cocaine-experience monkeys (Porter et al., 2013).

Overall, previous studies investigating how cocaine impacts behavioral flexibility have demonstrated impairments following exposure to the drug.

It has been proposed that cognitive deficits associated with cocaine use can perpetuate the cycle of drug use and increase propensity for relapse through both dysregulation of pathways controlling behavioral inhibition (i.e., increase impulsivity) and disruptions in executive function, perhaps perpetuating maladaptive behaviors (for review see Rogers and Robbins, 2001). This idea is supported by studies showing that neurobiological and behavioral measures obtained from cocaine-dependent treatment-seeking individuals prior to treatment initiation have been directly linked to rates of attrition and treatment success (Teichner et al., 2001; Turner et al., 2009; Martinez et al., 2011). Furthermore, the supposition that female cocaine users may be at an increased risk
for these cognitive dysregulations and relapse proclivity due to fluctuations in their hormonal milieu warrants additional research. *Therefore, in Chapter V, we complement these studies by examining the effects of abstinence from long-term cocaine SA on measures of working memory and behavioral flexibility in female monkeys.*

7. SUMMARY

Males and females differ in their magnitude of response to various properties of drugs of abuse. It is likely that the molecular neuroadaptations related to stress, hormones and chronic cocaine use, which develop over the course of addiction, contribute to females’ increased sensitivity to drug-associated cues, vulnerability and relapse to drug addiction. Furthermore, it is becoming increasingly apparent that females are more susceptible to the reinforcing effects of drugs of abuse compared to males. E2 has been consistently shown to facilitate drug SA by interacting with DA reward pathways and stress systems. The research in this dissertation extends these findings to socially housed, normally cycling female cynomolgus monkeys and intravenous cocaine SA.

The current studies begin to address the role of reproductive hormones in female substance abuse by investigating how hormonal fluctuations influence cognitive performance in drug-naïve, normally cycling females (*Chapter II*). The importance of hormones on environmental variables and vice-versa was assessed in *Chapter III* as females were socially housed into groups of four and hormonal measures were collected prior to and during the initial week of group housing and following establishment of social hierarchies. Additionally, alterations in cognitive performance were assessed
during this initial week of stress/enrichment for the monkeys (*Chapter III*). Subsequently, the effect of this environmental manipulation on cocaine SA was examined (*Chapter IV*). Potential involvement of circulating hormones (E2 and P4) on drug taking was also studied in this chapter. Finally, cognitive effects of chronic cocaine self-administration followed by abstinence were evaluated in *Chapter V* of this dissertation.

The studies included in this dissertation address an important sex bias that persists in neuroscience and biomedical research. For example, out of nearly 2,000 animal studies published in 2009, the use of male animals predominated in eight of 10 disciplines (Beery and Zucker, 2011). Neuroscientists used 5.5 males for every one female, pharmacologists used five, and physiologists used 3.7. Notably, despite the fact that women are twice as likely to suffer from major depression, fewer than 45 percent of animal studies related to these disorders used females. Ultimately, a careful consideration of the influence of sex on disease vulnerability, progression and treatment would work to everyone’s benefit. The studies conducted here support the premise that by understanding the mechanisms by which sex differences manifest in drug abuse, we may eventually identify sex-specific treatments.
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CHAPTER II

RELATIONSHIP BETWEEN ESTRADIOL AND PROGESTERONE CONCENTRATION AND COGNITIVE PERFORMANCE IN NORMALLY CYCLING FEMALE CYNOMOLGUS MONKEYS

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Abstract

Preclinical studies have demonstrated that cognitive function may be influenced by estradiol (E2) and progesterone (P4) concentrations, although few cognition studies involve normally cycling females. The present study examined cognitive performance in normally cycling female cynomolgus macaques (n=14), a species with similarities to humans in brain organization and a nearly identical menstrual cycle to women. Initial assessments compared cognitive measures to circulating concentrations of E2 and P4 (n=12). Once a relationship was characterized between hormones and cognitive performance, the menstrual cycle was divided into 4 distinct phases: early follicular (EF), late follicular (LF), early luteal (EL) and late luteal (LL), verified by the onset of menses and serum concentrations of E2 and P4. Concentrations of E2 were highest during the LF phase and P4 concentrations peaked during the EL phase. All monkeys were trained on two cognitive tasks: reversal learning, involving simple discrimination (SD) and reversal (SDR), which measured associative learning and behavioral flexibility, respectively (n=3-4 per phase) and a delayed match-to-sample (DMS) task which assessed working memory (n=11). P4 concentrations were positively correlated with number of trials and errors during SD performance, but not during acquisition of the SDR task or maintenance of the reversal-learning task. Across the menstrual cycle, significantly fewer errors were made in the SDR task during the LF phase, when E2 concentrations were high and P4 concentrations low. Working memory, assessed with the DMS task, was not consistently altered based on previously characterized menstrual cycle phases. These findings demonstrate a relationship between P4, E2 and cognitive performance in normally cycling cynomolgus monkeys that is task dependent. Knowledge of these interactions may lead to a better understanding of sex-specific cognitive performance.
**INTRODUCTION**

Executive function is largely responsible for the flexible adaptation to changes in the environment and encompasses a number of distinct tasks that involve the prefrontal cortex (PFC), frontostriatal networks and hippocampus. Executive function can be objectively measured by studying tasks that assess reinforcement learning in which behavior is shaped by stimulus-outcome associations (Eisenegger et al., 2014). Executive function includes 1) monitoring and adapting to cues relevant to a current goal and discarding/suppressing irrelevant information, 2) shifting, the ability to redirect focus between multiple modalities or tasks, and 3) inhibition, the ability to suppress or withhold a preplanned or impulsive response (see Miyake et al., 2000; Gould and Nader, 2015).

It has been known for some time that sex differences exist in cognitive performance, with women performing better on verbal tasks while men have better visuospatial skills (cf. Baros et al., 2015). When studying females, these sex differences may be attributed to fluctuations in estradiol (E2) and progesterone (P4). Hampson (1990) tested the hypothesis that at certain points in the menstrual cycle, hormone fluctuations in women would facilitate performance over males by studying performance on a series of cognitive tests in normally cycling women. In that study, women were tested twice on a battery of six cognitive and motor measures; testing occurred approximately 6 weeks apart, once to coincide with menses and the other during the preovulatory elevation of E2. Performance on spatial ability tasks was better during menses, when E2 and P4 concentrations are low, than during the preovulatory phase, while women performed better on motor tasks during the preovulatory phase compared to menses. In fact, they reported a curvilinear relationship between E2 concentrations and cognition. Although
they did not measure P4 concentrations, this study highlights the task-dependent nature of E2 (and perhaps P4) effects on cognition (see Lacreuse et al., 2015). In a recent review on the role of P4 in cognition, Baros et al. (2015) divide the review into studies that show detrimental effects and those that show positive effects. One of the limitations noted in that review was the lack of preclinical studies in normally cycling animals.

Female subjects are typically under-utilized in neuroscience research, partly due to changes in neurochemistry, neurohormones and behavior across the menstrual cycle. As mentioned above, there is evidence for differences in cognitive performance across the menstrual cycle (Drake et al., 2000; Lacreuse et al., 2001; Maki et al., 2002; Rosenberg and Park, 2002). In normally cycling women, the mechanism mediating interactions between menstrual cycle phase and cognition has been associated with E2 and P4 concentrations in specific brain regions (e.g., McEwen and Alves, 1999; Osterlund et al., 2000; Milad et al., 2010; Zhang et al., 2010; He et al., 2011; see Toffoletto et al., 2014 for review), stress pathways (e.g., Felmingham et al., 2012) and neurotransmitters. For example, clinical observations suggest that E2 fluctuations interact with dopamine (DA) to exert powerful effects on mood, mental state, behavior and memory (Fink et al., 1996; Carroll and Anker, 2010; Van Voorhees et al., 2012; Manovani and Fucic, 2014). Consistent with these findings, PET studies in female monkeys have shown significantly higher brain DA D2/D3 receptor availability in the luteal phase compared to the follicular phase of the menstrual cycle (Czoty et al., 2009). Less is known about the mechanism by which P4 may interact with the DA system or cognitive performance (van Wingen et al., 2008), but allopregnanolone, an active metabolite of progesterone, has been shown to influence GABA neurotransmission (see Baros et al., 2015).

The present study examined the effects of fluctuations in E2 and P4 concentration on cognitive performance in 14 normally cycling female cynomolgus monkeys. Old World
monkeys share many characteristics with humans in terms of endocrine physiology, cognition, neuroanatomy and a complex social hierarchy (Lacreuse and Herndon, 2002; Phillips et al., 2014; Lacreuse et al., 2015) and they have an approximate 28-day menstrual cycle with similar fluctuations of E2 and P4 as observed in women (Appt, 2004). After initial assessment of E2 and P4 concentrations over 3 months in each monkey, they were trained on two cognitive tasks and performance was evaluated in relation to hormonal concentrations. The first task assessed associative learning using a simple discrimination (SD) and behavioral flexibility (simple discrimination reversal; SDR), while the second task assessed working memory using a delayed match-to-sample (DMS) task. Based on findings suggesting improved cognition when E2 concentrations are high (Maki et al., 2002; Hatta and Nagaya, 2009), we hypothesized that a direct relationship would be revealed between learning and performance of the SD/SDR and DMS tasks and E2 concentrations, such that higher E2 concentrations (i.e., late follicular phase) would be associated with improved performance on both tasks. It is less clear how P4 concentrations would influence performance since some studies show high P4 is detrimental (Bimonte-Nelson et al., 2004) while others show high P4 (and high E2) lead to enhanced cognitive performance (Hatta and Nagaya, 2009). To examine whether phase-of-cycle influence on cognitive performance persisted following acquisition, performance was assessed for three consecutive months with re-exposure to the reversal-learning task using novel stimuli. We hypothesized that any observed differences in the SD/SDR task would dissipate in subsequent months based on previous studies that demonstrated rapid improvement on this task with repeated exposures (Kromrey et al., 2015).

MATERIALS AND METHODS
Subjects. Fourteen drug-naïve pair-housed adult female cynomolgus macaques (Macaca fascicularis) served as subjects (Table 1). Each monkey was fitted with an aluminum collar (Primate Products, Redwood City, California) and trained to sit in a standard primate chair (Primate Products). Monkeys were weighed weekly and fed enough fresh fruit and food (Nestle Purina PetCare Company, St. Louis, Missouri) to maintain healthy body weights as determined by physical appearance and veterinary exams; water was available ad libitum in the home cage which measured 0.71 x 1.68 x 0.84 m (Allentown Caging Inc., Allentown, New Jersey). All animals had a behavioral history of operant responding maintained by sucrose pellets but no drug history. A subset of these monkeys was included as a control group in a previous publication (Kromrey et al., 2015), but no approach was taken in that publication to address hormonal effects on cognition. Environmental enrichment was provided as outlined in the Institutional Animal Care and Use Committee’s Non-Human Primate Environmental Enrichment Plan. All experimental procedures were performed in accordance with the 2011 National Research Council Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research and were approved by the Wake Forest University Institutional Animal Care and Use Committee.

Verification of hormonal fluctuation across cycle. Blood sampling occurred in 12 of the 14 monkeys (see Table 1). Two of the monkeys included in the cognitive assessments were previously on a different study and were not trained for serum collection. Monkeys were trained to sit calmly in a primate chair in a quiet room, while ~3-mL blood sample was collected from the femoral vein. Blood draws occurred every other day across three consecutive menstrual cycles. E2 and P4 concentrations were performed using a Roche Diagnostics (Indianapolis, IN) Cobas-e411 assay instrument at the Endocrine Services Laboratory at the Oregon National Primate Research Center.
The assay sensitivity ranges were 5-4250 pg/ml for E2 and 0.035-59 ng/ml for P4. Intra- and inter-assay variation with the Roche Cobas-e411 is consistently less than 6% for E2 and P4. Four phases of the menstrual cycle were defined by counting backwards from menses and mean concentration of E2 and P4 during these phases were used to confirm menstrual cycle phase. These phases included early follicular (EF, menstrual cycle days 1-7), late follicular (LF, menstrual cycle days 8-14), early luteal (EL, menstrual cycle days 15-21) and late luteal (LL, menstrual cycle days 22- menstruation). Concentrations of P4 and E2 across the four-cycle phases were analyzed using separate one-way analyses of variance (ANOVA). Significant main effects were followed by post-hoc Tukey test.

**Cognitive assessments.** Cognitive testing was conducted 5 to 7 days per week between 9:00 am and 12:00 pm using the Cambridge Neuropsychological Test Automated Battery apparatus (CANTAB; Lafayette Instruments, Lafayette, Indiana) as described previously (Gould et al., 2012, 2013; Kromrey et al., 2015). Monkeys were first trained on the SD/SDR task and following completion of Experiment 1 (see below) trained on the DMS task with maintenance of performance on the SD/SDR assessed no more than once per week. Animals completed a maximum of 200 trials in the SD/SDR task and 80 trials in the DMS task. Only one task was assessed per behavioral session. For the SD/SDR task, total session length depended on task performance, as sessions terminated once reversal criteria were met or a maximum of 200 trials were completed (see below). In the DMS task, each animal completed 80 trials, divided up into three delay lengths (short, medium and long) which were performance based (see below), therefore the total session length varied between animals but was typically one hour.
Experiment 1. Influence of E2 and P4 concentrations on acquisition and maintenance of a reversal-learning task.

SD/SDR task: Acquisition. In the SD task, three geometric shapes (A, B, C) appeared in a horizontal row across the center of the screen while a light above the food pellet dispenser was illuminated. A response on one shape (A+) resulted in delivery of a 190-mg food pellet, illumination of a light inside the pellet dispenser and a 7-second inter-trial interval (ITI) during which the monkey retrieved and ate the food pellet. During the ITI, the light above the pellet dispenser was turned off. Responding on either of the other two shapes (B-, C-) resulted in a 10-second timeout (TO), followed by a 7-second ITI. During the TO the light above the pellet dispenser remained lit but no reinforcer was delivered. Shapes were pseudo-randomly distributed throughout the three possible positions on the screen with a maximum of 200 trials per day. Acquisition of the SD was defined as 18 correct responses out of the previous 20 completed trials. Once the acquisition criterion was met, the contingencies were altered in the SDR phase so that responding on the previously correct shape was incorrect (i.e., A-) while a response on one of the previous incorrect shapes was now the correct response (i.e., B+). The third shape, which was incorrect in the SD phase, remained incorrect in the SDR phase (i.e., C-). The same consequence for responding on the correct or incorrect stimuli and the same criterion for acquisition used in the SD phase was used in the SDR phase. Since initial acquisition of the task can only occur once, the 14 monkeys were randomly distributed across the four phases for the initial exposure to the task (EF, n=3; LF, n=4; EL, n=3; LL, n=4; see Table 2).

Data Analysis: The primary dependent variables were the total number of trials to criterion, the number of errors committed, and the number of omitted trials to acquisition during the SD and SDR tasks. Response and pellet-retrieval latencies were also recorded. Perseverative errors were calculated for the SDR task, defined as responses
on the stimulus that had been reinforced in the SD phase (A-). Incorrect responses on the stimulus that had not been reinforced in the SD phase (C-) were termed seeking errors. Multiple linear regressions were used to assess the relationship between E2 and P4 concentrations when acquisition occurred and the dependent variables of the reversal-learning task (i.e., total trials, errors, perseverative errors and omissions). Two-way ANOVAs were conducted using phase of cycle (EF, LF, EL, LL) and task phase (SD, SDR) as factors. Significant main effects were followed by post-hoc Fishers LSD tests. Multiple Pearson correlations were used to assess the relationship between mean E2 and P4 concentrations for the phase when acquisition occurred and the dependent variables of the reversal learning task (i.e., total trials, errors, perseverative errors and omissions). Because the data were not normally distributed, distributions were normalized with a square-root transformation to better comply with the assumptions of parametric analysis (Roberts et al., 1988; Wright et al., 2013). In all cases, differences were considered statistically significant at p < 0.05.

**SD/SDR task: Maintenance of performance.** Once all monkeys acquired the SD/SDR task, they were tested once each week for three months. On the other days of the week animals responded on the DMS task or no cognitive testing occurred. Shapes were presented in non-overlapping sets of three; the set of shapes used during each testing session was randomly selected from the “CAMCOG 0” list associated with the CANTAB system and was not re-used throughout this experiment. Task completion criteria were identical to those described above. Two monkeys stopped cycling regularly and two others were moved to another study, therefore the number of animals that completed testing for all three months decreased to 10 monkeys. For this experiment, each monkey was tested at each of the four phases of the cycle (EF, LF, EL, LL). Blood samples were not collected during this maintenance portion of the task. The within-subject variability in
sex hormone concentrations month-to-month in premenopausal subjects was not expected to be high considering previous investigations in women (Gann et al., 2001; Chatterton et al., 2005). Consistent with these reports, prior sampling of hormone concentrations in a subset of monkeys across 3 months did not significantly differ month-to-month (data not shown). Dependent variables included the total number of trials to criterion, the number of errors committed and the number of omitted trials to acquisition during the SD and SDR tasks. Three-way ANOVAs were conducted using cycle phase (EF, LF, EL, LL), stage of task (SD, SDR), and month as factors; significant main effects were followed by post-hoc Fishers LSD tests.

**Experiment 2. Effects of menstrual cycle phase on DMS performance.** In this task, a target image appeared on the screen and, following a response on the target, three images appeared after a 0 or 1 second delay. A response on the previously displayed image resulted in delivery of a 190-mg food pellet. A response on either of the other two images resulted in a 10-second time out and no pellet delivery. Once percent accuracy reached 80% for 3 consecutive days with this short delay, trials with gradually higher delays were included. Delay values were adjusted until task performance met predetermined criteria: short delay, >78% accuracy; middle delay, 55%-78% accuracy; long delay <55% accuracy (see Table 1 for short-, medium- and long-delay values for each monkey). Delays were randomly presented throughout each session so that there were ~27 trials per delay per session. DMS performance was deemed stable when accuracy at each delay length remained within these accuracy ranges for 5 consecutive days. Eleven of the 14 monkeys included in the analysis reached stability within 3 months of training initiation; three monkeys did not reach the stability criteria within 6 months and were therefore not included in statistical analysis (see Table 1). Once stability was reached, monkeys continued on the task at their individualized short-,
medium- and long-delay values for a minimum of 3 days per menstrual cycle phase (i.e., EF, LF, EL, LL).

Data Analysis: The primary dependent variables were percent accuracy at each delay length, response latencies for target and match phases and pellet retrieval latencies. To compare baseline delay-effect curves between phases of the menstrual cycle a two-way repeated measures ANOVA with phase of cycle (EF, LF, EL, LL) and delay (short, medium, long) as factors was conducted. Significant main effects were followed by post-hoc comparisons using Fisher’s LSD tests.

In both experiments, effect sizes for ANOVAs were estimated by calculating eta squared for each significant result. For pairwise comparisons, Cohen’s d was calculated using an online calculator at


RESULTS

Baseline E2 and P4 concentrations across the menstrual cycle.

Blood sampling occurred every other day across three consecutive menstrual cycles in 12 normally cycling female monkeys. Concentrations were measured and assigned a phase based on when menses was observed. A one-way repeated measures ANOVA comparing E2 concentrations across phases indicated that there was a significant main effect of menstrual cycle phase ($F_{3,36} = 31.83; p<0.001$; Fig. 1, open symbols; Eta squared = 0.59). Post-hoc comparisons revealed that E2 in the LF phase of the cycle was significantly higher than concentrations from all other phases (all p<0.001; Cohen’s d = 1.47 for all); none of the other three phases were significantly different from one another. There was also a significant main effect of menstrual cycle phase on P4 concentrations ($F_{3,36} = 64.86; p<0.001$; Fig. 1, closed symbols; Eta squared = 0.75). Post-hoc comparisons indicated that all phases were significantly different from one
another (all p<0.05; Cohen’s d: for EF vs EL, LF vs EL and EL vs LL = 1.47; for EF vs LL = 1.36; for LF vs LL = 0.97) except the LF vs. EF phases.

**Experiment 1. Influence of E2 and P4 concentrations on acquisition and maintenance of a reversal-learning task.**

**SD/SDR task acquisition: E2 and P4 correlates.** All the monkeys acquired the SD/SDR task within 7 days (mean 3 days ± 0.7 SEM) of initiation and therefore acquired within the same phase of the cycle as they were first exposed. Collapsed across all phases of the menstrual cycle, the mean number of total trials to meet criteria performance ($F_{1,10} = 10.86; p < 0.05; \text{Eta squared} = 0.15$), the number of errors ($F_{1,10} = 11.85; p < 0.05; \text{Eta squared} = 0.19$) and the number of omissions ($F_{1,10} = 14.49; p < 0.05; \text{Eta squared} = 0.11$) were significantly different in the SD compared to the SDR stage of the task (Table 2). Linear regression (n=12) revealed a significant positive correlation between P4 concentrations and the number of total trials ($r = 0.63, p<0.05; \text{Fig. 2B}$) and errors ($r = 0.66, p < 0.05; \text{Fig. 2D}$) during the SD stage. In contrast, there was not a significant correlation between P4 concentrations and total trials and errors during the SDR task (Fig. 2F, 2H). E2 concentrations, while not statistically significant, were negatively associated with SD and SDR performance (Fig. 2, left panels).

While there was not a significant correlation between E2 and P4 concentrations with SDR errors, using three stimuli in the SD/SDR task allowed for the determination of whether errors made in the SDR phase were perseverative. During the SD phase, monkeys made a similar number of errors on each incorrect stimulus, indicating that, prior to reversal, no stimulus bias existed (data not shown). During the SDR (Fig. 3), a main effect of error type was found ($F_{1,10} = 26.11; p < 0.001; \text{Eta squared} = 0.21$) and post-hoc tests revealed a significant difference between perseverative (i.e., A-) and seeking (i.e., C-) errors for the monkeys at all phases of the cycle except the LF phase.
(all p < 0.05; Cohen’s d for EF = 2.47, for EL = 1.83; for LL = 3.05). Moreover, fewer perseverative errors were made during the LF phase of cycle compared to both the EF (p < 0.05; Cohen’s d = 1.75) and LL (p < 0.05; Cohen’s d = 1.77) phases.

**SD/SDR task maintenance.** For the maintenance portion of these studies, after initial assessment of SD/SDR performance, monkeys were studied for 3 consecutive months on this task. A three-way ANOVA revealed a significant main effect of the task stage (SD vs. SDR) on the number of total trials (F_{1,231} = 7.96; p < 0.01; Eta squared =0.03), errors (F_{1,231} = 14.46; p < 0.01; Eta squared =0.05), and omissions (F_{1,231} = 3.87; p < 0.05; Eta squared =0.015) to criterion (data not shown). However, the positive relationship between P4 and SD performance did not extend to this period of maintenance (data not shown).

**Experiment 2. Effects of menstrual cycle phase on DMS performance.** A two-way ANOVA revealed a significant main effect of delay value on performance accuracy (F_{2,60} = 117.98; p < 0.001; Eta squared =0.71). There was no significant effect of menstrual cycle phase or an interaction between cycle phase and delay (Fig. 4). Response and pellet retrieval latencies were not significantly different across menstrual cycle phases (data not shown) nor were there significant interactions.

**DISCUSSION**

The goal of the present set of studies was to examine how fluctuations in estradiol and progesterone influence cognitive performance in drug-naïve, normally cycling female cynomolgus monkeys. Hormonal measures were assessed with serum concentrations of E2 and P4, which showed significant and orderly differences across the approximate 30-day menstrual cycle for three consecutive months. There was a
significant relationship between P4 concentrations and learning of a simple
discrimination (SD) such that monkeys with high P4 concentrations required more trials
and made more errors. There was no significant relationship between E2 concentrations
and SD performance. However, assessment of behavioral flexibility with the reversal
task (SDR) showed enhanced performance during the late follicular phase, which
coincides with high circulating E2 concentrations. These initial relationships between
hormonal concentrations and cognitive performance were not maintained when monkeys
were retested on the SD/SDR task over 3 months nor were differences noted in working
memory as assessed with DMS.

Although some previous studies in normally cycling women did not show variation in
cognitive performance across the menstrual cycle (Epting and Overman, 1998; Mordecai
et al., 2008; Mihalj et al., 2014), others have demonstrated differences in working
memory and verbal fluency when comparing luteal vs. follicular phases (Drake et al.,
2000; Maki et al., 2002; Rosenberg and Park, 2002). The majority of prior studies tested
women only twice, in the early follicular and mid-luteal phase, and observed
improvements in cognitive performance were typically attributed to elevated E2
concentrations (Maki et al., 2002; Mordecai et al., 2008; Hatta and Nagaya, 2009),
although measurements of E2 and P4 concentrations were not usually conducted.
Furthermore, the interaction between menstrual cycle phase and cognitive performance
appear to depend on the task assessed, the subject’s age and for studies in
ovariectomized animals, the duration of hormone deprivation (Gogos et al., 2014). For
example, researchers have shown that women perform better in tasks involving verbal
fluency, speed and fine motor skills during time points when E2 and P4 concentrations
are high (Hampson 1990; Drake et al., 2000; Maki et al., 2002; Rosenberg and Park,
2002; Yonker et al., 2003), whereas when concentrations of these hormones were low
they performed better in tasks involving spatial ability, target-directed motor tasks and
One advantage of an automated system such as CANTAB is that different memory tasks (e.g., behavioral flexibility and working memory) can be studied using the same motor components, thereby eliminating a potential confounding variable.

In order to better understand the relationships between normal fluctuations in E2 and P4 concentrations in normally cycling females and task sensitivity, two different cognitive tasks were used. The SD/SDR task measure executive function and behavioral flexibility (see Gould and Nader, 2015), which are believed to be mediated through the orbitofrontal cortex, medial striatum and ventrolateral prefrontal cortex (cf. Lacreuse et al., 2014). Lacreuse et al. (2014) reported a direct relationship between E2 concentrations and poor performance. That is, exogenously administered E2 to ovariectomized monkeys resulted in more errors during the reversal phase. In the present study, while E2 concentrations were not significantly related to SD performance, there was a relationship between E2 concentrations and errors during the reversal phase (SDR) of the task, with monkeys acquiring the reversal faster in the late follicular phase when E2 concentrations are highest. The differences in results may be due to the study of normally fluctuating E2 concentrations vs. the treatment regimen utilized when E2 is administered exogenous (see Lacreuse et al., 2014 for discussion).

An advantage of studying normally cycling female monkeys is that we can assess both E2 and P4 concentrations on cognitive performance. During the follicular phase, E2 concentrations were elevated while P4 concentrations were low, and under SD conditions, low P4 was associated with enhanced cognitive performance. Thus, it could be argued that elevations in E2 concentrations do not produce cognitive enhancement, but rather luteal increases of P4 concentrations may hinder cognitive performance. We found a direct relationship between P4 concentrations and the acquisition of a simple discrimination, such that monkeys with higher P4 concentrations performed worse than
those with lower concentrations. The present findings are consistent with human data where women administered a high dose of P4 performed more poorly on a delayed-recall and digit symbol substitution test (Freeman et al., 1993). Additionally, in ovariectomized rats, P4 administration resulted in compromised performance on a radial-arm maze task (Bimonte-Nelson et al., 2004). The role of P4 and memory has recently been reviewed (for review see Barros et al., 2015) and it is proposed that impairments are due to the relationship between P4 and GABAergic transmission. Specifically, certain metabolites of P4, such as allopregnanolone (3-alpha-hydroxy-5alpha-pregn-20-one), which have direct actions at the GABA_A receptor complex, alter the balance between excitatory and inhibitory functions of the central nervous system (Amin et al., 2006). These GABA_A receptors may also be related to the hormonal fluctuations across the menstrual or estrous cycle, since expression of these receptors is enhanced during phases when P4 peaks (for review, see Schumacher et al., 2014).

The present findings are consistent with other studies indicating that the influence of hormonal concentrations on cognitive performance is task specific (e.g., Islam et al., 2008). In fact, Barros et al. (2015) highlight the possibility that increases in P4 concentration that occur either before or after task performance can result in either enhancement or disruption of cognition. The study of normally cycling females on tasks that required several days to achieve stable performance, as done in the present study, controls for this mediating factor. With regard to E2 concentrations and task specificity, one possibility is that E2 influences cognitive function involving tasks reliant on the PFC, through the DA system (Shansky and Lipps, 2013). It is known that DA is critical for cognitive function (Arnsten, 2011) and E2 has been shown to increase the number of DA projections from the VTA to the PFC (Kritzer and Creutz, 2008) as well as enhance extracellular DA concentrations (Xiao and Becker, 1994). Our findings that high E2 concentrations during the LF phase of the menstrual cycle did not result in significantly
more perseverative responding offers indirect evidence for the involvement of DA in cognitive flexibility. The interaction of the DA system and perseverative responding is widely cited (Jentsch et al., 2002; Woicik et al., 2011; Vogel et al., 2013; Eagle et al., 2014), although to our knowledge, this is the first study to measure perseverative responding in normally cycling female monkeys.

Only one other study by Lacreuse and colleagues (2001) used direct hormonal measures to investigate whether cognitive performance fluctuated across the menstrual cycle in nonhuman primates. In that study, monkeys performed significantly better on a spatial delayed recognition span test during phases of the cycle when estrogen concentrations were low and no significant differences in a DMS task (Lacreuse et al., 2001). The lack of menstrual cycle effect on DMS performance in the present study is consistent with these results. The fact that phases when E2 concentrations are high were associated with fewer perseverative errors during the SDR task, but not working memory deficits during the DMS task may be due to different functional effects of E2 in different brain areas. For example, reversal learning is a PFC-dependent task and is highly influenced by DA signaling (Floresco and Magyar, 2006). Considering that E2 alters several aspects of the DA system (Becker, 1990; Jacobs and D'Esposito, 2011), this cognitive task may be particularly vulnerable to impairments and/or facilitation due to menstrual cycle phase. In contrast, the DMS task relies largely on the hippocampus. Although E2 receptors are found in the hippocampus (Quinlan et al., 2007), and E2 alters brain morphology and physiology in this region (Brinton et al., 2000; Woolley, 1999; Córdoba Montoya and Carrer, 1997), the functional significance of E2 in this region is yet to be fully understood. Our findings suggest that working memory as assessed with DMS may not be as susceptible to influence of natural hormonal fluctuation across the menstrual cycle as other tasks. That being stated, it should be noted that the extensive training required to establish a reliable delay curve in the DMS
task (on average 10 weeks) could account for the lack of menstrual cycle effect, since training occurs across all phases of the menstrual cycle. This possibility is supported by our findings that during maintenance of the reversal task, previously observed cycle effects are no longer evident.

Although we assessed cognitive performance across the menstrual cycle, an inherent limitation of these studies is that cognitive performance takes time to assess and monkeys may be studied in more than one phase before stable cognitive performance has been achieved. We were careful to design the tasks (e.g., SD/SDR) so that performance could be assessed while monkeys remained in one phase (e.g., LF), but even then, E2 and P4 concentrations can fluctuate. For the DMS task, training necessarily occurs over multiple cycles, making it impossible to directly assess the role of E2 and P4 on acquisition of this working memory task. In summary, our findings in normally cycling Old World monkeys indicate that P4 concentrations influence acquisition of a simple discrimination while E2 concentrations during the LF phase of the menstrual cycle appear to be associated with enhanced learning of a reversal-learning task. However, working memory, as measured by the DMS task, did not fluctuate with menstrual cycle phase. These studies add to the literature on how natural hormonal fluctuations across the menstrual cycle, particularly P4 and E2 concentrations, can have profound behavioral effects, particularly in regard to cognitive performance, that are task dependent.
ACKNOWLEDGEMENTS

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CONFLICT OF INTEREST: none

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may reveal neurocognitive processes implicit in the Wisconsin Card Sorting Test. 

Neuropsychologia 49, 1660-1669.


Table 1. Subject characteristics: weight (kg), age (years), average menstrual cycle length during serum collection (days) and the individual delay times in the DMS task (seconds).

<table>
<thead>
<tr>
<th>Subject</th>
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<th>Medium</th>
<th>Long</th>
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<td>5</td>
<td>29.67</td>
<td>3</td>
<td>15</td>
<td>35</td>
</tr>
<tr>
<td>C-7902</td>
<td>2.8</td>
<td>5</td>
<td>27.67</td>
<td>3</td>
<td>25</td>
<td>50</td>
</tr>
<tr>
<td>C-7664</td>
<td>2.9</td>
<td>6</td>
<td>30.33</td>
<td>2</td>
<td>25</td>
<td>40</td>
</tr>
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<td>C-7591</td>
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<td>33.33</td>
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<td>40</td>
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<td>32.67</td>
<td>1</td>
<td>15</td>
<td>35</td>
</tr>
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ND: Not determined; blood samples were not collected
Table 2. Phase of cycle for each subject when acquisition occurred, number of total trials, errors and omissions to SD and SDR criterion.

<table>
<thead>
<tr>
<th>Subject</th>
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<th>Errors</th>
<th>Omissions</th>
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<td>9</td>
</tr>
<tr>
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<td>LF</td>
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<td>520</td>
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</table>

Figure 1. Mean (±SEM) estrogen (E2) and progesterone (P4) concentrations across the four selected phases of the menstrual cycle (n=12 monkeys per point).
Figure 2. Relationship between concentrations of estradiol (E2; left panels) and progesterone (P4; right panels) and performance on a simple discrimination (SD; panels A-D) and SD reversal (SDR; panels E-H) task. Ordinate: Number of total trials (A, B, C, D) and errors (E, F, G, H) required to meet criteria performance. Abscissa: left: E2 concentrations (pg/ml); right: P4 concentrations (ng/ml).
Figure 3. Error distribution during acquisition of the reversal-learning task in the simple discrimination (SD; left) and discrimination reversal (SDR; right) stages of the reversal-learning task across the four phases of the menstrual cycle (n=14). Bars represent mean (±SD) number of error responses on each non-reinforced stimulus during the SD stage (left) and SDR stage (right). *, p < 0.05.

Figure 4. Accuracy at short, medium, and long delays (n=11) during the four phases of the menstrual cycle. Points represent mean (±SD).
CHAPTER III

PREDICTORS OF SOCIAL RANK IN FEMALE CYNOMOLGUS MONKEYS

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The following manuscript has been submitted to American Journal of Primatology in February, 2015. Stylistic variations are due to the requirements of the journal. Sarah A. Kromrey performed the experiments, analyzed the data and prepared the manuscript. Susan H. Nader and Thomas C. Register assisted in the hormonal analysis. Paul W. Czoty and Michael A. Nader acted in an advisory and editorial capacity.
ABSTRACT

Rationale: Physiological and behavioral differences between dominant and subordinate monkeys have been useful in investigating numerous disease states. It has been inferred that subordinate monkeys live in a context of chronic social stress and may be at risk for a variety of dysfunctions. Objective: To examine whether several phenotypic characteristics are trait markers for eventual social rank or are a consequence of the social context (i.e., state markers). Methods: Baseline measures of estrogen, progesterone, cortisol and testosterone concentration were obtained from 16 pair-housed female cynomolgus monkeys before and after the establishment of social hierarchies (n=4/group). Furthermore, effects of the initial week of social rank establishment on outcome measures of cognitive performance and home cage activity were examined. Results: Body weight and basal circulating estrogen were the only statistically significant predictors of eventual rank, with future subordinate monkeys being lighter and having higher estrogen concentrations. During initial hierarchy establishment, future subordinate monkeys had higher morning and afternoon cortisol concentrations, higher locomotor activity and impaired cognitive performance on a working memory task. Following stable hierarchy establishment (3 months), subordinate monkeys had blunted circulating estrogen and progesterone concentrations. Conclusion: These results, in female monkeys, indicate that estradiol may be a trait variable that predicts social rank, however basal cortisol and testosterone concentrations and HPA axis function are state variables that differentially reflect position in the dominance hierarchy. These data support the profound influence of social context on physiology and behavior.
**Key Words:** estrogen, progesterone, cortisol, testosterone, CANTAB, activity, female, working memory, social rank, cynomolgus monkey

**INTRODUCTION**

Nonhuman primate social hierarchies have been used to study a range of human diseases including drug abuse, psychiatric diseases, reproductive dysfunction and the neurobiology of behaviors such as aggression and impulsivity (Kaplan et al., 1982; Cameron, 1997; Shively, 1998; Morgan et al., 2002; Riddick et al., 2009). The relative position that a monkey holds within a dominance hierarchy has been shown to have profound effects on susceptibility and resistance to diseases (Riddick et al., 2009; Kaplan, 2008; Morgan et al., 2002; Sapolsky, 2005). For example, we have reported differences in vulnerability to the reinforcing effects of cocaine in dominant and subordinate monkeys that were attributed to their position in the dominance hierarchy (Morgan et al., 2002; Nader et al., 2012b). The majority of studies assessing disease states as a function of social rank have retrospectively examined the effects of “state” variables, which are characteristics attributable to environmental circumstances. However, individuals may have pre-existing characteristics that underlie their likelihood of occupying a high or low social rank, known as “trait” variables. Information regarding both state and trait variables is critical to understanding the influence of environmental and social context on behavior and disease states.

Monkeys establish linear hierarchies based on outcomes of agonistic interactions (Kaplan et al., 1982). By defeating other members of the social group in competitive interactions, high-ranking or “dominant” monkeys gain priority access to particular
resources (Morgan et al., 2000). While an animal’s initial position within the hierarchy is obtained through contact aggression, most often subordinate status is maintained by the threat of aggression from more dominant animals (Bernstein and Gordon, 1974; Bernstein, 1976; Shively and Kaplan, 1984). In captivity, variables such as age and body weight have been shown to affect eventual social rank (Morgan et al., 2000; Riddick et al., 2009). In a study involving 20 individually housed male cynomolgus monkeys, body weights and locomotor activity in a novel environment, assessed prior to social housing, predicted eventual social rank, with heavier monkeys becoming the most dominant and those with higher levels of locomotor activity more likely to become subordinate (Morgan et al., 2000). A similar study in female cynomolgus monkeys showed that both higher cerebrospinal fluid (CSF) concentrations of the serotonin (5-HT) metabolite 5-hydroxyindoleacetic acid (5-HIAA) and a lower latency to touch a novel object predicted the likelihood that a monkey would occupy a lower social rank (Shively et al., 1997; Shively, 1998; Riddick et al., 2009). Although previous investigations into locomotor activity as a rank predictor in females did not find a significant correlation (Riddick et al., 2009), the present study utilized a different approach to assessing locomotor activity, in which activity was measured in the monkey’s homecage rather than a novel environment; these measures were also taken during formation of social groups to also examine activity as a state variable. Additionally, a functional outcome of this stress response might be impaired executive and/or cognitive function as observed in numerous mental health disorders (Millan et al., 2012; Chrousos, 2009). Therefore, our study assessed cognitive performance of a working memory task, during social rank establishment.
It has been hypothesized that social subordination and dominance represent two ends of a continuum from chronically stressed to enriched, respectively (Nader et al., 2012a). This continuum has remarkable predictive, face and construct validity (Nader and Czoty, 2005), which is important considering that chronic stress is a significant causal and sustaining risk factor in a number of adverse health outcomes (Sapolsky, 2005; McEwen, 2008; Juster et al., 2010). The hypothesis that social status differentially affects the physiology of the hypothalamic-pituitary-adrenal (HPA) axis is most commonly validated through the measure of serum cortisol. There does not seem to be a direct relationship between pre-social housing cortisol concentrations and eventual social rank in macaque hierarchies (Goo and Sassenrath, 1980; Morgan et al., 2000; Czoty et al., 2009a). However, previous studies in male cynomolgus monkeys reported that while basal cortisol concentrations did not differ prior to social housing, future subordinate monkeys had significantly higher cortisol concentrations during initial social housing, although the effect dissipated within 3 days (Czoty et al., 2009a). The current study extended this characterization to newly formed social groups of female monkeys.

The present study also extended this characterization to sex hormones: testosterone, estrogen and progesterone. In male monkeys, there is not a significant relationship between eventual social rank and testosterone concentrations (Rose et al., 1975; Morgan et al., 2000; Czoty et al., 2009a). To the best of our knowledge, the current study is the first to utilize a within-subjects design to assess testosterone as a trait and state variable in female monkeys. Additionally, female cynomolgus monkeys have an approximate 28-day menstrual cycle with fluctuations in estrogen and progesterone concentrations across the cycle (Appt, 2004). However, prior to the present study, the
influence of basal estrogen and progesterone across the menstrual cycle on future social rank of female monkeys had not been investigated.

METHODS

Subjects. Sixteen adult female cynomolgus macaques (Macaca fascicularis) between 5 and 11 years old served as subjects (Table 1). All monkeys were fitted with an aluminum collar (Primate Products, Redwood City, CA) and trained to sit in a standard primate chair (Primate Products). Monkeys were weighed weekly and fed enough fresh fruit and food (Purina LabDiet 5045, Brentwood, MO) daily to maintain healthy body weights (2.6-3.2 kg) as determined by physical appearance and periodic veterinary exams; water was available ad libitum in the home cage. Home cages were divided into four equal quadrants by removable partitions. Each quadrant measured 0.71 x 0.84 x 0.84 m (Allentown Caging Inc., Allentown, New Jersey) and allowed visual, auditory and limited tactile interactions. When pair housed, the vertical partitions were removed; when housed in groups of four monkeys, the two vertical and one horizontal partition was removed and monkeys occupied the entire cage (0.71 x 1.73 x 1.73 m). Environmental enrichment was provided as outlined in the Institutional Animal Care and Use Committee’s Non-Human Primate Environmental Enrichment Plan. All experimental procedures were performed in accordance with the 2011 National Research Council Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research and were approved by the Wake Forest University Institutional Animal Care and Use Committee. Three of the 16 subjects had previously been surgically prepared under sterile surgical conditions with an indwelling venous catheter in a femoral vein, which
was connected to a subcutaneous vascular access port (VAP; Access Technologies, Skokie, IL) as described previously (Czoty et al., 2005).

Introduction of social housing conditions. All animals had previously been pair housed prior to social housing. Previously paired animals were not put into the same pen for the current study. The 16 monkeys were randomly assigned to four pens by weight, with the four heaviest monkeys designated to four separate pens, the next three heaviest randomly placed into those pens, and so on with the only additional exclusion variable being previous pair housing. The average weight of all subjects on the first day of social housing was 3.04 (range 2.6–3.7) kg and there were no significant differences in average body weight across pens. Initially, each monkey occupied one quadrant in their new pen and cage partitions remained in place, although monkeys were allowed visual, auditory and limited tactile contact with each future pen-mate. On the first day of social housing, three of the four partitions were removed, forming a “C” and allowing all four monkeys to be in direct contact with each other.

At approximately 09.00 h on the first day of social housing, partitions were removed and social interactions were video recorded for the first two hours. For the next 5 days, partitions were replaced at approximately 16.30 h and monkeys remained separated overnight. This precaution was taken to prevent injury occurring when laboratory personnel were not present. It is important to note that when monkeys were separated overnight all interactions other than physical (i.e., visual, olfactory and auditory) were maintained, as monkeys remained in close proximity. After the initial week, monkeys were socially housed overnight and separated each weekday morning, during which time monkeys were involved in cognitive behavioral sessions (see
below) and fed. They were socially housed each day beginning in the late afternoon (approximately 16.00 h). On weekends, pens were separated for 3 h for feeding. Thus, time spent socially housed was approximately 17 h per day on weekdays and 21 h per day on weekends. In rare cases of an injury that required veterinary care or other routine veterinary procedures, the injured monkey was individually housed until healed as determined by the veterinarian.

_Determination of ranks._ From weeks 2 to 12 of social housing, two observers separately conducted a total of 22 observation sessions per pen. Each 15-min session began immediately after partitions were removed in the afternoon. Aggressive, submissive and affiliative behaviors during these observation sessions and previously recorded initial 2 hours of social housing were recorded according to an ethogram described previously (Kaplan et al., 1991; Morgan et al. 2000) 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 none was ranked no. 1 (most dominant). The monkey aggressing at everyone except the no. 1-ranked monkey and submitting only to the no. 1-ranked monkey was ranked no. 2, and so on. The monkey designated no. 4 displayed a low frequency of aggressive behaviors and submitted to all other monkeys in the pen. Thus, a transitive, linear hierarchy was established in each pen (see Morgan et al., 2000).

_Hormonal measures._ Initial evaluations of basal cortisol, testosterone, estrogen and progesterone concentrations were performed when animals were pair-housed with no observable hierarchies in place between animals. These measures were repeated over the
course of 3 months of social housing. For assessing hormones across the menstrual cycle prior to social housing, blood draws occurred at approximately 09.00 h every other weekday across three menstrual cycles. The duration of the menstrual cycle was initially assessed by daily vaginal swabs over several months (see Kromrey et al., 2014). The first day of bleeding was accepted as menses and was counted as day 1 of the cycle. To obtain both pre- and post-social housing measures of estrogen, progesterone, cortisol and testosterone concentrations, monkeys were seated in a primate chair in a quiet room and trained to sit calmly while a ~3.0 mL blood sample was collected from the femoral vein and then centrifuged (Beckman Coulter, GPR Centrifuge) at 4 °C for 30 min at 3000 rpm. The serum was aspirated into an Eppendorf tube and stored at -70° C. Estrogen and progesterone concentrations were performed using a Roche Diagnostics (Indianapolis, IN) Cobas-e411 assay instrument at the Endocrine Services Laboratory at the Oregon National Primate Research Center. The assay sensitivity ranges were 5-4250 pg/ml for estrogen and 0.035-59 ng/ml for progesterone. Intra- and inter-assay variation with the Roche Cobas-e411 is consistently less than 6% for estrogen and progesterone. Cortisol and total testosterone concentrations were quantitated by RIA using commercially available kits (Coat-A-Count, Diagnostic Products Corp.). Assays were performed at the Clinical Chemistry and Endocrinology Laboratory at the Comparative Medicine Clinical Research Center, Wake Forest School of Medicine. Intra-assay coefficients of variation were less than 10% for these two assays.

**Experiment 1. Relationship between body weight, home cage activity and social rank.**

Each monkey was weighed approximately one week prior to social housing and a Spearman correlation was used to assess whether weight prior to social housing predicted
eventual rank. In order to quantify activity, each monkey was fitted with an Actical® (Phillips Respironics, Bend, OR) activity monitor, an omnidirectional accelerometer that measures the subject’s physical activity, secured to the collar. Activity was recorded in 30-s epoch lengths (i.e., 2880 samples/day) as the total number of activity counts per 30 seconds, 24 hours a day. For quantification of activity, data were downloaded and analyzed using Actical 3.0® (Phillips Respironics, Bend, OR). Only the period of activity during which the lights in the room were on (06.00 h-20.00 h) was analyzed. Monkeys had adapted to wearing the activity monitors for one month prior to data collection for this study. To assess locomotor activity as a trait variable, an average of four days of activity data while individually housed and prior to social housing was analyzed using a one-way ANOVA with eventual rank as a factor. As described previously, significant main effects were followed by post-hoc multiple comparisons testing using Fisher’s LSD tests.

The average activity per day for each monkey during the initial week of social housing (Monday-Friday) was analyzed using a two-way repeated-measures ANOVA with eventual rank and day of week as factors. The raw activity data did not pass the Shapiro-Wilk Normality Test, therefore a log transformation was conducted prior to analysis of these data. Furthermore, two of the monkeys removed their accelerometers during this week and were therefore not included in the analysis.

**Experiment 2.** Concentrations of progesterone, estrogen, testosterone and cortisol as trait and state variables related to social rank. Blood samples were obtained at three phases of the study: prior to social housing, during the initial week of social hierarchy formation and following stable social hierarchy formation (~ 3 months). For assessing
trait markers for estrogen and progesterone concentrations, average area under the curve (AUC) for each monkey was analyzed using a one-way analysis of variance (ANOVA). Significant main effects were followed by post-hoc multiple comparisons testing using Fisher’s LSD tests. To assess cortisol concentrations as a trait marker in this cohort, three of the blood draws across the last month of collection, prior to social housing, were timed specifically at 09.00 h and 16.30 h to account for the diurnal fluctuations of cortisol. An average of these morning and evening serum cortisol concentrations was used to assess whether social rank was predicted by morning or evening cortisol concentrations. Similarly, two total testosterone concentrations were averaged from the last month of blood collection to evaluate whether total testosterone predicted eventual social rank. Sigma Stat graphing software was used for all of the statistical analyses in these studies. For all analyses, p<0.05 was considered statistically significant.

During the initial week of social housing, blood draws occurred twice per day (09.00 h and 16.30 h). The concentration of cortisol at morning and evening each day of the initial week of social housing (Monday-Friday) was used to assess rank-related differences in cortisol concentrations during initial social housing. For morning cortisol, concentrations were only assessed Tuesday through Friday since social housing did not occur over the preceding weekend. A two-way repeated-measures ANOVA was conducted with eventual social rank and day of week as factors. Significant main effects were followed by planned post-hoc testing using Fisher’s LSD tests. Similarly, a two-way repeated-measures ANOVA was used with eventual rank and day of week as factors, followed also by a priori assigned post-hoc testing using Fisher’s LSD tests to determine whether there were rank-related changes in morning testosterone. Raw data of the
testosterone concentrations did not pass the Shapiro-Wilk Normality Test, therefore a log transformation was conducted prior to analysis of this data set.

To assess whether circulating estrogen or progesterone concentrations were modified following stable hierarchy establishment, blood draws occurred every other weekday for one month after animals had been socially housed for 3 months. A one-way ANOVA was conducted with AUC of both estrogen and progesterone as a factor of social rank. As previously described, selected blood samples taken during this month of stable social hierarchy were analyzed for total testosterone. These concentrations were also assessed using a one-way ANOVA with rank as a factor. Significant main effects were followed by post-hoc multiple comparisons testing using Fisher’s LSD tests.

**Experiment 3. Cognitive assessments of working memory as trait and state variables of social rank.** Prior to social group formation, cognitive testing was conducted 5 to 7 days per week between 9:00 am and 12:00 pm using the Cambridge Neuropsychological Test Automated Battery apparatus (CANTAB; Lafayette Instruments, Lafayette, Indiana) as described previously (Gould et al., 2012; Kromrey et al., 2014). In the delayed matching-to-sample (DMS) task, each animal completed 80 total trials with three delay lengths (short, medium and long, see below), therefore the total session length varied between animals but lasted one hour on average. For this working memory task, a target image appeared on the screen and a response was required to initiate a 1-sec delay. Following the delay, three images appeared on the screen and a response on the previously displayed image resulted in delivery of a 190-mg food pellet. A response on either of the two novel images resulted in a 10-second time out and no pellet was delivered. Once percent accuracy exceeded 80% for 3 consecutive days with a short (1-sec) delay, delays
were slowly increased. Baseline performance was established by increasing delay lengths so that similar individualized delay-dependent reductions in percent accuracy were reached. Specifically, there were three target levels of accuracy in each monkey: a delay that resulted in >78% accuracy was considered the “short” delay, a “medium” delay resulting in 55%-78% accuracy and a “long” delay resulting in <55% accuracy (Table 2). Delays were randomly distributed throughout each session so that there were ~27 trials per delay. Once accuracy remained within these ranges for 5 consecutive sessions, performance was deemed stable for each monkey and the animals completed the task only twice a week to maintain performance accuracy.

The monkeys completed DMS sessions daily for the week of initial social housing and percent accuracy at each delay was reported as a percent change from pre-social housing performance. A three-way ANOVA was conducted with day of week (Monday-Friday), delay (short, medium, long) and future rank (1,2,3 and 4) as factors. Significant main effects were followed by post-hoc multiple comparisons testing using Fisher’s LSD tests. Furthermore, number of omissions was assessed using a two-way repeated measures ANOVA with day of the week and eventual rank as factors. The raw omission data of did not pass the Shapiro-Wilk Normality Test, nor the Equal Variance Test, therefore a square root transformation was conducted prior to analysis of this data set. A square root transformation was used rather than a log transformation because there is the possibility of having zero omissions, of which a log cannot be calculated.
RESULTS

**Experiment 1.** *Relationship between body weight, home cage activity and social rank.* A Spearman correlation revealed a significant negative correlation between a monkey’s weight prior to social housing and their eventual rank in the hierarchy ($r = -0.54$, $p<0.05$; Fig. 1). That is, the lighter the monkey, the more likely she was to be subordinate.

Although future subordinate monkeys had higher home cage activity, a one-way ANOVA did not reveal a significant main effect of eventual rank and daytime activity (Fig. 2A). During the initial week of social hierarchy formation, there was a significant effect of the day of the week ($F_{4,36} = 17.45$, $p<0.001$; Fig 2B) and a significant interaction ($F_{12,36} = 2.85$, $p<0.01$; Fig. 2B) for daytime activity. Post-hoc comparisons showed that eventual rank 4 animals had significantly higher average activity compared to both eventual rank 1 ($p<0.05$) and rank 2 ($p<0.05$) monkeys (Fig. 2B).

**Experiment 2.** *Concentrations of progesterone, estrogen, testosterone and cortisol as trait and state variables related to social rank.* Across all phases of the menstrual cycle, there was a significant main effect between eventual social rank and average estrogen concentration ($F_{3,12} = 3.67$, $p<0.05$; Fig. 3A). Post-hoc testing demonstrated that this difference was driven by future rank 3 animals who had significantly higher circulating estrogen compared to all other ranks (rank 3 v. 1 $p<0.01$; rank 3 v 2 $p<0.05$; rank 3 v 4 $p<0.05$; Fig. 3A). In contrast, across all phases of the menstrual cycle, progesterone concentrations were not significantly different between eventual social ranks (Fig. 3B). Cortisol concentrations from morning (Fig. 3C) and evening (Fig. 3D) and testosterone concentrations (Fig. 3E) were not predictive of eventual social rank. When correlations between hormone concentration and social rank were conducted to assess potential
relationships, no significant correlations were found for any of the hormones (i.e. estrogen, progesterone, total testosterone, or morning/afternoon cortisol (data not shown).

During the first week of social housing, a two-way repeated measures ANOVA of morning cortisol revealed a significant main effect of eventual rank (F3,35 =4.92, p<0.05) and of day of week (F3,35 =3.774, p<0.05; Fig. 4A), but no significant interaction. Post-hoc analysis showed that future rank 1 monkeys had significantly lower cortisol in the morning throughout the initial week of social housing compared to future rank 3 (p<0.05) and future rank 4 monkeys (p<0.01; Fig. 4A). Furthermore, post-hoc comparisons of the days of the week demonstrated that morning cortisol concentrations on Wednesday (Day 3) were significantly higher compared to both Thursday (Day 4) (p<0.01) and Friday (Day 5) (p<0.05; Fig. 4A). Importantly, significant differences between future rank 1 and future rank 4 animals remained through Friday morning (p=0.01; Fig. 4A).

Afternoon cortisol concentrations revealed a significant main effect of eventual rank (F3,47 =4.92, p<0.01) and of day of week (F4,47 =3.774, p<0.001), but no significant interaction (Fig. 4B). Post-hoc analysis illustrated that eventual rank 4 monkeys had higher cortisol in the afternoon than both eventual rank 1 (p<0.01) and eventual rank 2 (p<0.05; Fig. 4B) monkeys throughout the week of initial social housing. Eventual rank 1 animals also had significantly lower concentrations throughout this week than eventual rank 3 animals (p<0.05). Additionally, post-hoc analysis showed that afternoon cortisol was significantly higher on Monday compared to every other day of the week (Tuesday through Friday; all p<0.01; Fig. 4B), and Tuesday had significantly higher concentrations compared to Friday (p<0.05). Again, it is important to note that
significant differences between afternoon cortisol concentrations between future rank 1 and future rank 4 animals remained through Friday afternoon (p<0.05; Fig. 4B). Assessment of testosterone concentrations with a two-way repeated-measures ANOVA found no significant main effect for either eventual rank or day of the week. However, an interaction that trended toward significance (p=0.06) was observed (Fig. 4C).

After approximately 3 months and when social hierarchies were stable, a one-way ANOVA demonstrated that there was a significant main effect of social rank on AUC of estrogen (F3,12 =5.32, p<0.05; Fig. 5A). Post-hoc analysis showed that rank 4 monkeys had significantly lower estrogen than both rank 1 (p<0.05) and rank 3 (p<0.01) monkeys (Fig. 5A). Additionally, a one-way ANOVA assessing AUC of progesterone demonstrated a main effect of rank (F3,12 =6.28, p<0.01; Fig. 5B). Post-hoc comparisons revealed that rank 4 monkeys had significantly lower circulating progesterone compared to both rank 1 (p<0.01) and rank 3 monkeys (p<0.05; Fig. 5B). In contrast to estrogen and progesterone concentrations, testosterone concentrations did not vary significantly across social rank (Fig. 5C).

**Experiment 3.** Cognitive assessments of working memory as trait and state variables of social rank. All monkeys were trained on a DMS task involving three delays. There were no differences in baseline performance or delay values prior to social housing (Kromrey et al., 2014). During the first week of social housing, a three-way ANOVA revealed main effects of both eventual rank (F3,165 =5.81, p<0.001) and delay length (F2,165 =7.59, p<0.001) but no main effect of day of week (Fig. 6). Moreover, there was a significant interaction of eventual rank and delay length (F6,165 =3.98, p<0.001). Post-hoc comparisons demonstrated that at the short delay, performance of rank 1 monkeys was
significantly disrupted when compared to all other ranks (p<0.05; Fig. 6A). In contrast, at the medium (Fig. 6B) and long (Fig. 6C) delays performance of eventual rank 4 monkeys was significantly disrupted compared to all other ranks (p<0.05). Finally, a two-way ANOVA of omissions yielded a significant main effect of eventual rank (F3,43 =5.91, p<0.01) and a main effect of day of the week that bordered on significance (p=0.052; Fig. 6D), but no significant interaction. Post-hoc testing revealed that eventual rank 4 animals had significantly more omissions across the week when compared to all other social ranks (p<0.05).

DISCUSSION

The present study extended earlier work on physiological and neurobiological predictors of eventual social rank in cynomolgus monkeys (e.g., Morgan et al., 2000; Czoty et al., 2009a; Riddick et al., 2009). These studies included a more thorough assessment of hormonal profiles, another method of assessing activity and the study of cognitive function during establishment of social hierarchies in 16 female cynomolgus monkeys. As was observed in males (Morgan et al., 2000), there was a direct relationship between body weight and eventual social rank, such that the heaviest monkeys became dominant and the lightest monkeys tended to become the most subordinate. These findings relating body weight to eventual social rank compliment previous conclusions that physical appearance is one of the most important variables in determining social rank (Bernstein and Mason, 1962; Tokuda and Jensen, 1969; Bernstein et al., 1974; Bernstein, 1991; Morgan et al., 2000). Of the two behaviors studied before and after establishing social ranks, neither homecage activity nor a working memory task was predictive of eventual social rank. The former finding is consistent with other studies using different
measures of locomotor activity (Morgan et al., 2000; Riddick et al., 2009). However, homecage activity was significantly increased during the first week of social housing in monkeys that would eventually become subordinate and working memory performance was disrupted in these future subordinate animals. Hormone concentrations involving estrogen, progesterone and cortisol, while not predictive of eventual social rank, significantly changed after stable social hierarchies were established. These findings add to the literature on trait and state variables associated with the social rank.

Consistent with the literature depicting typical menstrual cycles in female cynomolgus macaques, previous studies in these monkeys reported significantly higher concentrations of estrogen during the latter part of the follicular phase and peak progesterone concentrations during the early part of the luteal phase (Kromrey et al., under review). In the present study higher average estrogen concentrations (across the entire menstrual cycle) were associated with monkeys eventually occupying a subordinate rank, specifically rank 3. Studies investigating the relationship between basal estrogen and eventual social rank are scarce because most studies are conducted in well-established social groups. However, a study by Gladue (1991) found estrogen concentrations to be negatively related to aggression in women. Therefore, the increased circulating estrogens may predispose females to avoid confrontation or display less aggression and therefore occupy lower ranks. The other neuroendocrine measures of progesterone, cortisol and testosterone did not predict eventual social rank. The lack of relationship between basal cortisol concentrations and rank is consistent with previous studies in Old World monkeys (Rose et al., 1975; Berstein et al., 1983; Morgan et al., 2000; Czoty et al., 2009a). Similarly, although there is literature supporting the
relationship between testosterone and dominance in human males (Dabbs et al., 1987; Christiansen and Knussman, 1987) and females (Susman et al., 1987), there is no consistent relationship identified in nonhuman primates (see Nader et al., 2012a). Some of these studies have shown a positive relationship between androgens and dominance in established hierarchy groups (Rose et al., 1971; Joslyn 1973; Cochran and Perachio, 1977; Czoty et al., 2009a). However, a greater number has found no correlation (Easton and Resko, 1974; Gordon et al., 1976; Morgan et al., 2000). Overall, with the exception of estrogen concentrations in future #3 ranked monkeys, it appears that circulating concentrations of hormones are not trait markers associated with eventual social rank.

The second part of this study investigated how these behavioral and physiological measures were modified by social housing. Previous studies in our laboratory have shown that although dopamine D2/D3 receptor levels, as assessed with positron emission tomography, did not predict eventual social rank, they were differentially altered depending on the social rank attained following group housing (Morgan et al., 2002; Nader et al., 2012b) and were influenced by menstrual cycle phase (Czoty et al., 2009b). Therefore, it is important to investigate variables at both stages of the social housing process to achieve a greater understanding of the interaction between physiology, neurobiology and social rank. Once socially housed, monkeys who became subordinate performed the majority of submissive behaviors and received the majority of aggressive behaviors while rarely participating in grooming. These behavioral observations reinforce the hypothesis that occupying a subordinate rank (i.e. rank 3 and 4) models chronic social stress.
The current study demonstrated that neuroendocrine measures compliment these behavioral observations, as both morning and afternoon cortisol concentrations were significantly higher in eventual subordinate monkeys compared to dominant monkeys. Although studies of the relationship between rank and cortisol concentration in established groups of nonhuman primates have yielded mixed results, the current findings support a previous study of female cynomolgus macaques in which subordinate individuals exhibited higher levels of glucocorticoids (Shively et al., 1997). Furthermore, these findings match those from a previous study in male cynomolgus macaques, which reported an inverse relationship between cortisol and future social rank, with significantly higher concentrations of cortisol in future subordinate monkeys (Czoty et al., 2009a).

Interestingly, in the Czoty et al. study, the rank differences in cortisol in males were only observed the first two days of social housing, whereas the differences remained in the females throughout the entire initial week of social housing. This is important because these monkeys experienced a persistently high concentration of plasma cortisol for more than 5 days after the introduction to social housing. This chronic activation of the HPA axis diverts energy away from physiological processes that are not required for immediate survival and can therefore have serious deleterious effects on the individual (Seyle, 1976) particularly through suppression of multiple endocrine responses (Harris et al., 2014), including reproductive hormones (Knol et al., 1991; Toufexis et al., 2014). Furthermore, this exaggerated effect underlines the staggering differences between the male and female response to stressors with females reacting more robustly than males (for review see Handa and Weiser, 2014; Ter Horst et al., 2009).
No interaction between eventual rank and total testosterone was observed in these female monkeys. This may be due to the observation that when positive correlations between androgens and dominance are observed in nonhuman primates, it is primarily due to the effects of winning and losing altercations (Mazur and Lamb, 1980; Ellison, 1988), rather than these hormones influencing behavior. Therefore, the relationship will vary with context, group stability and time since the competition occurred (Monaghan and Glickman, 1992; Sapolsky 2005). The present data support this hypothesis, as a very orderly effect of testosterone and eventual rank was observed on Day 1 of social housing when the majority of altercations occurred, yet this effect was not consistent throughout the rest of the week. Importantly, after the first day of social housing, testosterone concentrations were not different in the most dominant versus the most subordinate female monkeys.

Once social hierarchies were stable and had been maintained for three months, follow-up assessments of neuroendocrine measures were obtained to determine whether alterations during the initial week were temporary or more persistent in nature. After 3 months of social housing, both estrogen and progesterone concentrations were blunted in the rank 4 monkeys compared to the other ranks. This alteration of circulating hormones is not surprising as it is well documented that subordinate individuals are often reproductively suppressed (Emlen, 1991; Solomon and French, 1997; Shively et al., 1997; Setchell et al., 2008). Although reproductive hormones may be influenced by multiple factors, including reduced access to resources (Pusey et al., 1997) and/or lack of access to males (Zinner et al., 1994), these factors were controlled in this study. It is most likely that the blunted estrogen and progesterone concentrations in subordinate monkeys
was due to the high levels of chronic social stress resulting from agonistic and aggressive interaction that leads to higher circulating glucocorticoids as observed in other studies (i.e. Dunbar, 1988; von Holst, 1998) or in increased cortisol concentrations as seen in the current study. Future studies will be needed to determine if social rank-related differences are due to altered concentrations of estrogen and progesterone.

After stable social hierarchies had been established, neither testosterone nor cortisol concentrations differed across ranks. These findings are similar to previous studies in male macaques that have probed the relationship between testosterone and social rank (Morgan et al., 2000). Additionally, other groups who have investigated the relationship between social rank and cortisol in established social groups of monkeys have reached similar findings (Stavisky et al., 2001). A limitation to the present study was not assessing HPA axis sensitivity following suppression by the glucocorticoid receptor agonist dexamethasone (DEX), nor adrenal responsiveness to a challenge injection of adrenocorticotropic hormone (ACTH). The study by Czoty et al. (2009a) demonstrated that although social stress-induced elevations in evening cortisol dissipated in male subordinate monkeys by day 3 of social housing, these monkeys remained more sensitive to activation of the HPA axis by ACTH reactivity in the DEX-suppressed state. Therefore, although not yet tested, a similar physiological response may be present in these female subordinate monkeys, whereas sensitivity to acute stressors may remain elevated despite basal cortisol concentrations not differing from dominant animals.

The present study extended behavioral characterizations associated with eventual social rank to include cognitive performance using a working memory task (delayed match-to-sample). At the short delay lengths, impairment in cognitive performance was
observed in future dominant monkeys whereas at the medium and long delay lengths the impairments were seen in future subordinate monkeys. The observed cognitive disruption in future subordinate monkeys was associated with an increased number of omissions in these animals during the initial social housing week. There have been relatively few studies on the relationship between stress and omissions during cognitive testing, however a study conducted by Petrac et al. (2009) found correlations between perceived stress and omissions in human subjects, suggesting that increased environmental stress related to decreased divided attention performance. A similar situation may have occurred in the current study whereby the future subordinate monkeys were unable to focus entirely on the cognitive task because of their unstable social environment, which required constant vigilance.

The interaction between stress and cognitive function during initial social housing is likely due to the overlapping functions of the prefrontal cortex (PFC), which is essential for higher cognitive functions (Fuster, 2000) as well as the processing of emotion (Bechara et al., 2000; Cardinal et al., 2002). Additionally, the PFC is integrated into a negative feedback system that controls aspects of the HPA axis (Herman et al., 2005). Interestingly, Herman and colleagues (2005) found that performance at medium and long delays, but not short delays, was compromised in monkeys with increased HPA activation. Similar findings were observed in a study by Brunner et al. (2006) that demonstrated that humans receiving a high dose of cortisol had impairments on longer memory tasks whereas shorter-term memory and attention remained unaffected. Therefore, it is hypothesized that the functional outcome of increased cortisol on
cognitive performance may be due to selective cognitive disruption at longer (more demanding) delays rather than a generalized cognitive deficit.

Interestingly, the dominant monkeys demonstrated impaired cognitive performance at the short delays but not the longer ones. It could be hypothesized that this impairment was the result of an increase in impulsive responding occurring solely at the short delay. Our current delayed match-to-sample task does not record anticipatory responding, which is typically a measure in tasks where a sustained response on the sample stimulus is required and when that sustained response is not met, an anticipatory response is counted (Jentsch and Anzivino, 2004). However, it is plausible that during the short delay trials, when the match stimuli are presented almost immediately following the initial sample response, the animals are responding quicker than at the longer delays since the response is required straightaway, which would reflect impulsivity (Raiker et al., 2012). Although this difference in response speed may not be large enough to significantly alter the overall response latency (was analyzed in the current studies), future studies will assess response latency at each delay in an effort to parse out this potential involvement of impulsivity.

The present studies addressed, in normal cycling female monkeys, physiological and behavioral variables that may influence the formation of social hierarchies and that may change as a result of occupying a specific social rank. Body weight remains the best predictor of eventual social rank. However, the present study highlights the importance of other behaviors including activity measures and cognition, as well as the influence of circulating concentrations of estrogen, progesterone, testosterone and cortisol. Our findings demonstrate the profound influence of an individual’s social environment on
neuroendocrine, behavioral and functional outcomes associated with many psychiatric disorders as well as other clinical manifestations. Understanding these variables will be important for studying factors related to the etiology and eventual treatment of disease.

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CONFLICTS OF INTEREST: None
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Figure 1. The relationship between the weight of a monkey prior to social housing and the eventual rank of that animal following social housing.
Figure 2. Average daytime locomotor activity prior to social housing (A) and during initial week of social housing (B). Points depict mean (n=4/group) ±SEM values.
Figure 3. Assessment of hormone concentrations as trait markers for eventual social rank. (A) Estrogen (E2), (B) progesterone (P4), (C) morning cortisol, (D) evening cortisol, and (E) total testosterone concentrations prior to social housing as predictors of eventual rank. Points depict mean (n=4/group) ±SEM. * p< 0.05, ** p<0.01.
Figure 4. Assessment of hormone concentrations during the initial week of social rank formation. (A) Morning cortisol, (B) evening cortisol, and (C) total testosterone concentrations during initial week of social housing (Monday-Friday). Points depict mean (n=4/group ±SD values. * p<0.05, ** p<0.01, *** p<0.001.
Figure 5. Assessment of hormone concentrations as state markers for social rank. (A) Estrogen (E2), (B) progesterone (P4), and (C) total testosterone concentrations following social hierarchy establishment (3 months after initial social housing). Points depict mean (n=4/group) ±SEM values. * p< 0.05, ** p<0.01.
Figure 6. Performance on a delayed matching-to-sample task during initial week of social housing compared to prior baseline performance. Dependent measures include accuracy at the short delay length (A), medium delay length (B), long delay length (C) and number of omissions (D). Points depict mean (±SD) values.
CHAPTER IV

EFFECTS OF RANK AND MENSTRUAL PHASE ON ACQUISITION OF COCAINE SELF-ADMINISTRATION, LOCOMOTOR ACTIVITY AND COGNITIVE PERFORMANCE IN CYNOMOLGUS MONKEYS

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Abstract

**Background:** Social environment has been shown to influence cocaine reinforcement. Behavioral studies suggest a direct relationship between estradiol and reinforcing effects of cocaine in females. The current study investigated the contribution of reproductive hormones in socially dominant and subordinate female monkeys to susceptibility to the reinforcing effects of cocaine.

**Methods:** Blood samples were taken to assess estradiol and progesterone concentrations in female monkeys living in groups of four with linear dominance hierarchies. Monkeys were trained under a food-reinforced fixed-ratio (FR) schedule during daily sessions, then given access to ascending doses of cocaine (0.0003–0.1 mg/kg/injection) under the same schedule, with each dose substituted for food reinforcement. Acquisition of cocaine reinforcement occurred when response rates were significantly higher than when saline was made available.

**Results:** Subordinate monkeys appeared more sensitive to the reinforcing effects of cocaine during acquisition of cocaine reinforcement but did not reach statistical significance. Additionally, average circulating estradiol in individual animals was correlated with the dose at which acquisition occurred, with higher estradiol predicting lower doses of acquisition. Furthermore, subordinates self-administered cocaine at a higher response rates compared to dominants in both the follicular and luteal phases of the menstrual cycle. Subordinates also had a higher intake of cocaine in the follicular phase of the menstrual cycle.
Conclusions: These data indicate that the social environment’s influence on an individual’s endogenous hormonal milieu contributes to the reinforcing effects of cocaine. The interactions of socially derived stress, female hormones and cocaine reinforcement must be considered as part of individualized treatment strategies.

Key Words: Estradiol, Progesterone, Social Rank, Cocaine, Female cynomolgus monkey

INTRODUCTION

Drug abuse continues to be a major public health problem, with approximately 1.6 million Americans confirming current cocaine use (SAMHSA 2014). Furthermore, at present there are no FDA-approved treatments for cocaine addiction. Although males are typically cited as having a higher prevalence of cocaine abuse, women are particularly vulnerable to the reinforcing effects of cocaine (Greenfield et al, 2010). In fact, it has been widely documented both in clinical cohorts and preclinical models that females have a different trajectory to cocaine abuse and dependence. Specifically, women have been shown to initiate drug use at an earlier age (Chen and Kandel, 2002), progress from first use to dependence faster (Haas and Peters, 2000) and have more severe addiction symptoms when entering treatment (Westermeyer and Boedicker, 2000; Lynch, 2006; Wagner and Anthony, 2007; Kasperski et al., 2011). It has also been suggested that women who seek treatment exhibit a more severe pattern of drug use-related social, medical and/or psychiatric problems compared to males (Denier et al., 1991; Hernandez-Avila et al., 2004; Weiss et al., 1997). Additionally, when women do attempt to quit, they
are less successful, with lower rates of treatment retention (Becker and Hu, 2008; Carpenter et al., 2006) and higher rates of relapse (Ignjatova and Raleva, 2009); these outcomes are thought to be associated with increased craving (Robbins et al., 1999). Importantly, although these differences pervade multiple stages of drug abuse (i.e. initiation, maintenance, and relapse) female subjects are continually under-represented in both preclinical and clinical research.

Preclinical studies in rodents have shown that phase of estrous cycle plays a role in sensitivity to cocaine’s reinforcing effects. Females are most vulnerable to cocaine’s reinforcing and locomotor effects (Roberts et al., 1989; Quinones-Jenab et al., 1999; Hecht et al., 1999; Feltenstein et al., 2009) as well as resistance to extinction of responding previously paired with cocaine (Kippin et al., 2005; Kerstetter et al., 2008) during estrus. Overall, it appears that estradiol increases, whereas progesterone decreases, the reinforcing effects of cocaine (for review see Bobzean et al., 2014). These findings are further supported by research in ovariectomized (OVX) rodents which has confirmed a key role for estradiol (E2) in acquisition rates of cocaine self-administration and reinstatement of cocaine seeking (Becker, 1999; Lynch et al., 2001; Festa and Quinones-Jenab, 2004; Larson et al., 2005; Frye, 2007). In OVX rats, replacement of E2 restores cocaine self-administration while administration of progesterone, and its metabolite allopregnanolone, attenuates cocaine seeking, cocaine-induced locomotor sensitization and blunts estradiol-augmented increases in cocaine sensitization (Becker, 1999; Lynch et al., 2001; Russo et al., 2003; Niyomchai et al., 2005; Jackson et al., 2006; Anker et al., 2007; Feltenstein et al., 2009). However, these studies primarily utilized subjects who are
either not normally cycling (i.e., OVX and given exogenous hormones) or subjects who have an extensive cocaine self-administration history.

The present study used Old World female cynomolgus macaques which possess an approximate 28-day menstrual cycle phase (Appt, 2004). In addition, these monkeys lived in social groups. Previous studies have shown that social rank can profoundly influence the reinforcing effects of cocaine in both male and female monkeys (Morgan et al., 2002; Nader et al., 2012). In male monkeys, it has been hypothesized that dominant animals were protected from the reinforcing effects of cocaine because they live in an enriched environment, while subordinate monkeys are under chronic social stress. Although we have previously attempted to address the question of whether attainment of a dominant social position in females has similar functional outcomes as males, differences in fluctuating hormones, primarily estradiol and progesterone, were not taken into account (Riddick et al., 2009; Nader et al., 2012). This is an important consideration since circulating estradiol can affect dopamine concentrations (Becker, 1999; Watson et al., 2006), which could ultimately influence cocaine reinforcement.

We previously showed that estradiol and progesterone concentrations were associated with eventual social rank and changed in monkeys that became subordinate in the social group (Kromrey et al. submitted). At present, there are no studies investigating how these hormonal alterations and subsequent fluctuations interact with the reinforcing effects of cocaine. Therefore, the primary objectives of the present study were to: 1) determine how hormonal milieu of females influences vulnerability to acquire cocaine reinforcement; 2) investigate how attainment of a dominant or subordinate social positions relates to vulnerability to the reinforcing effects of cocaine; and 3) assess if
menstrual cycle phase differentially alters cocaine self-administration depending on the monkey’s social rank.

MATERIALS AND METHODS

Subjects. Sixteen socially housed (4 monkeys/pen) adult female cynomolgus macaques (Macaca fascicularis) between 5 and 11 year old served as subjects. All monkeys were fitted with an aluminum collar (Primate Products, Redwood City, California) and trained to sit in a standard primate chair (Primate Products). Monkeys were weighed weekly and feed enough fresh fruit and food (Nestle Purina PetCare Company, St. Louis, Missouri) to maintain healthy body weights as determined by physical appearance and veterinary exams; water was available ad libitum in the home cage which measured 0.71 x 1.73 x 1.73 m (Allentown Caging Inc., Allentown, New Jersey) with removable wire mesh partitions that separated monkeys into quadrants (0.71 x .84 m). During social housing, monkeys were separated and individually housed for 2-4 hours each day to complete self-administration sessions and for feeding. One monkey died of natural causes before completion of cocaine self-administration, bringing the total number of subjects to 15. All animals had a behavioral history of operant responding maintained by sucrose pellets. Menstrual cycle phase was assessed by daily vaginal swabbing (Czoty et al., 2009) and was approximately 28 days. The first day of bleeding was indicative of menses and was counted as Day 1 of the menstrual cycle. We considered days 2-11 the follicular phase and days 18-27 the luteal phase of the menstrual cycle.

To obtain serum for analysis of estradiol and progesterone concentrations, monkeys were seated in a primate chair in a quiet room and trained to sit calmly while a
~3.0 mL blood sample was collected from the femoral vein and then centrifuged (Beckman Coulter, GPR Centrifuge) at 4 °C for 30 min at 3000 rpm. Serum aliquots were stored at -70°C until analysis. Behavioral studies were conducted in both menstrual cycle phases and phasic differences in hormonal concentrations were confirmed by measuring plasma estradiol and progesterone concentrations. Estradiol and progesterone concentrations were performed using a Roche Diagnostics (Indianapolis, IN) Cobas-e411 assay instrument at the Endocrine Services Laboratory at the Oregon National Primate Research Center. The assay sensitivity ranges were 5-4250 pg/ml for estradiol and 0.035-59 ng/ml for progesterone. Intra- and inter-assay variation with the Roche Cobas-e411 is consistently less than 6% for estradiol and progesterone. Estradiol and progesterone concentrations were verified as significantly different between these two phases of the menstrual cycle. Environmental enrichment was provided as outlined in the Institutional Animal Care and Use Committee’s Non-Human Primate Environmental Enrichment Plan. All experimental procedures were performed in accordance with the 2011 National Research Council Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research and were approved by the Wake Forest University Institutional Animal Care and Use Committee.

**Social Rank Determination**

Social status was determined by the outcome of agonistic encounters (Kaplan et al., 1982). From Weeks 2–12 of social housing, two observers separately conducted a total of 22 observation sessions/pen (Kromrey et al., submitted). In these focal group sessions, both initiators and recipients of behaviors were recorded. The monkey in each pen aggressing towards all other monkeys and winning fights was considered dominant to
losers and recipients of aggression. Thus, linear and transitive hierarchies existed in each pen. Eight monkeys were designated as dominant (ranked #1 or #2), and seven were subordinate (ranked #3 or #4).

**Surgery**

Each monkey was prepared with a chronic indwelling venous catheter and subcutaneous vascular port (Access Technologies, Skokie, Illinois) under sterile surgical conditions. Anesthesia was induced with ketamine (10 mg/kg, i.m.) and maintained with isoflurane (~1.5%). Briefly, a catheter was inserted into a peripheral vein to the level of the vena cava. The distal end of the catheter was passed subcutaneously to a point slightly off the midline of the back, where an incision was made. The end of the catheter was then attached to the vascular access port and placed in a pocket formed by blunt dissection. Prior to each drug self-administration session, the back of the animal was cleaned with chlorhexidine acetate solution and 95% ethanol, and the port was connected to the infusion pump located outside the chamber via a 22-gauge Huber Point Needle (Access Technologies). The pump was operated for approximately 3 seconds to fill the port and catheter with saline or cocaine prior to beginning the session. Following every session, each port and catheter was filled with heparinized saline solution (100 U/ml) to prolong patency of the catheter.

**Cocaine Self-Administration**

The self-administration apparatus consisted of a ventilated, sound-attenuating chamber (1.5 x 0.74 x 0.76 m Med Associates, East Fairfield, VT) designed to accommodate a primate chair. Two photo-optic switches (5 cm wide) were located on one side of the
chamber with a horizontal row of three stimulus lights 14 cm above each switch, and a food receptacle was located between the switches. The receptacle was connected with tygon tubing to a pellet dispenser (Gerbarands Corp, Arlington, MA) located on the top of the chamber for delivery of 1-g banana-flavored food pellets (Bio-Serv, Frenchtown, NJ). Additionally, an infusion pump (Cole-Palmer, Inc., Chicago, IL) was located on top of the chamber. Each monkey was trained to respond on the left or right key, under a 30-response fixed-ratio (FR) schedule of reinforcement. Under these conditions, a food pellet was delivered after the 30th response, followed by a 10-sec timeout. Sessions ended after 30 reinforcers or 60 min, whichever occurred first. The light above the response key signaled food availability; only one key was active during a session.

After catheter implantation, food-maintained responding was re-established and saline was substituted for food pellets for at least 5 consecutive sessions and until responding was deemed extinguished (i.e., mean response rate decreased by at least 80% of food-reinforced responding for 3 consecutive sessions with no trends in responding). After re-establishing food-maintained responding, different doses of cocaine hydrochloride (National Institute on Drug Abuse, Bethesda, Maryland; dissolved in sterile .9% saline) were substituted for the food pellets in ascending order from 0.0003 mg/kg/injection increasing in half-log units to 0.1 mg/kg/injection; each dose was available for at least 5 sessions and until responding was deemed stable (response rate mean ± 20% for 3 consecutive sessions with no trends in responding). Sessions ended after 30 injections or 60 min, whichever occurred first. There was a return to food-maintained responding, for at least 3 sessions, between different cocaine doses. The lowest cocaine dose in which response rates were significantly higher than responding leading to saline injections was
defined as the acquisition dose. A cocaine dose was operationally defined as reinforcing by using two-tailed t tests comparing 3-day mean response rates for a given cocaine dose to mean response rates when saline was available.

To better control for menstrual cycle influences on acquisition, each cocaine dose was available between Days 2–10 (early to mid) of the follicular phase and the same dose of cocaine was made available during the mid- to late-luteal phase (Days 19–26) for at least 5 consecutive sessions. The phase at which the next dose was introduced was balanced between subordinates and dominants (i.e. four monkeys of each rank received the doses first in the follicular phase and four in the luteal phase). Food-maintained responding was re-established during the late follicular to early luteal phase when ovulation occurs (typically Days 11–18) and the late luteal to early follicular phase (typically Days 27–menstruation). Following the self-administration session at which the acquisition of cocaine reinforcement criterion had been met, blood was collected for hormonal concentration analysis (described above). Once cocaine self-administration was acquired, the same dose of cocaine was available for the subsequent phase of menstrual cycle. The remaining doses of the cocaine curve were randomly made available until the entire curve was determined.

**Statistical Analysis**

To determine whether there were differences in the rate of acquisition between dominant and subordinate monkeys, a log-rank analysis of Kaplan-Meier survival curves was computed. To evaluate the entire cocaine dose-response curve, the primary dependent variables were response rate (total responses divided by session length) and cocaine
intake (total intake in mg/kg/session). Food-maintained response rates and reinforcers (raw data) were analyzed with separate two-tailed, unpaired t tests. Two-tailed, paired t tests—within dominant and subordinate rank—were performed on response rate and intake measures to determine whether there was an effect of menstrual cycle phase at each dose tested. Because there was at least one cocaine dose at which there was a significant effect of menstrual cycle phase (Table 1), both response rates and intake were analyzed with individual two-way repeated-measures ANOVA for each phase of the menstrual cycle (follicular and luteal) and social rank (dominants and subordinates) followed by post-hoc analysis with all pairwise multiple comparison procedures (Tukey test) at each cocaine dose. To perform the two-way ANOVA, the raw data for intake were square-root transformed due to unequal variances. In all cases, differences were considered statistically significant when p < 0.05.

RESULTS

Effect of hormonal fluctuation across the menstrual cycle on vulnerability to cocaine reinforcement. Menstrual cycle was monitored throughout the study and confirmed with measures of estradiol and progesterone. During the cocaine acquisition phase of this experiment, mean concentrations of E2 and P4 were 91.38 pg/ml ± 9.61 and 34.86 pg.ml ± 7.33 during the follicular phase and 0.35 ng/ml ± 0.1 and 7.54 ng/ml ± 1.58, during the luteal phase. The differences in E2 were significant between phases (p<0.001), as were the P4 differences in follicular and luteal (p<0.001). There were no differences in baseline rates of food-maintained responding between dominant and subordinate monkeys and when saline was substituted for food (Table 2) and there were no group differences in rates of extinguished responding.
Ascending doses of cocaine were sequentially substituted for food in each monkey, and acquisition of cocaine reinforcement was examined. Acquisition of cocaine self-administration was defined as the dose of cocaine which engendered significantly higher response rates compared to response rates when saline was available. For the 15 monkeys studied, 8 acquired in the follicular phase and 7 in the luteal phase, with an equal distribution of dominant and subordinate animals. When assessed independent of menstrual cycle phase, a Kaplan-Meier log-rank test for equality of survival curves indicated that there were no significant differences between the number of dominant and subordinate monkeys that acquired at any dose of cocaine (p=0.26; Fig. 1). Because there were no rank-related differences in acquisition, data were combined across ranks and a significant negative correlation between the AUC of circulating estradiol and the lowest cocaine dose that functioned as a reinforcer was observed (r= -0.71, p<0.01; Fig. 2). That is, the higher the circulating estradiol concentration, the lower the dose of cocaine that was self-administered significantly higher than saline. The correlation between AUC for progesterone and cocaine acquisition was not significant (r= -0.09; p=0.75; data not shown).

*Effects of menstrual cycle phase on the reinforcing strength of cocaine as it relates to social rank.* Examination of complete dose-response curves showed that for response rates during the follicular phase of the menstrual cycle there was a significant main effect of rank [F(1,65) = 16.08; p=0.001], dose [F(5,65) = 5.81; p<0.001], and a significant interaction between rank and dose [F(5,65) = 5.26; p<0.001; Fig. 3A]. Post-hoc comparisons demonstrated that the rank differences in response rate during the follicular phase were significant at the 0.01 mg/kg, 0.03 mg/kg and 0.1 mg/kg doses of cocaine (all
Similarly, a two-way repeated measures ANOVA analyzing response rates during the luteal phase of the cycle revealed a significant main effect of rank \(F(1,65) = 15.66; p=0.002\), dose \(F(5,65) = 8.94; p<0.001\), and a significant interaction between rank and dose \(F(5,65) = 5.39; p<0.001; \text{Fig. 3B}\). Again, post-hoc comparisons showed that the rank differences in response rate during the luteal phase were significant at the 0.01 mg/kg, 0.03 mg/kg and 0.1 mg/kg doses of cocaine (all \(p<0.01\)).

A two-way repeated measures ANOVA on intake and dose during the follicular phase showed a main effect that bordered on significance for rank \((p=0.082)\), a main effect of dose \(F(5,65) = 108.52; p<0.001\), but no significant interaction (Fig. 3C). Post-hoc comparisons revealed that the rank differences in intake during the follicular phase were significant at only the 0.03 mg/kg dose of cocaine \((p<0.05)\). Finally, the same analysis for intake during the luteal phase revealed only a main effect of dose \(F(5,65) = 9.24; p<0.001; \text{Fig. 3D}\).

**DISCUSSION**

The current studies extend earlier work on vulnerability to cocaine reinforcement in socially housed female monkeys to include a more thorough characterization of the role of circulating estradiol and progesterone across the menstrual cycle. While there were no differences in concentrations of either hormone in dominant and subordinate monkeys under baseline conditions, rates of cocaine self-administration and cocaine intake across the menstrual cycle were significantly influenced by menstrual cycle phase. The major finding of the present study was that the higher the circulating estradiol concentration, the more sensitive the monkey was to the reinforcing effects of cocaine. These within-subject
findings are the first to investigate hormonal correlates of intravenous cocaine self-administration and dominance rank in socially housed female monkeys.

Measures of total circulating estradiol and progesterone across the menstrual cycle revealed a significant correlation between estradiol, but not progesterone, and dose at which acquisition of cocaine reinforcement occurred, but not progesterone. These findings are consistent with studies in humans (Evans, 2007; Sinha et al., 2007), nonhuman primates (Mello et al., 2008; Cooper et al., 2013) and rodents (Sell et al., 2000; Lynch et al., 2008; Segarra et al., 2010) that reproductive hormones provide the basis of observed behavioral responses to cocaine (Festa and Quinones-Jenab, 2004). However, this is the first study to assess how individual differences in concentrations of these hormones impact vulnerability to the initial reinforcing effects of cocaine in socially housed female monkeys. There is a known relationship between estradiol and brain concentrations of dopamine (DA); DA being the primary mediator of cocaine reinforcement. Consistent with the present results, studies have shown that estradiol enhances behavioral sensitization to cocaine and the acquisition of cocaine self-administration in female rats (Segarra et al., 2010).

Furthermore, a recent study in rats has demonstrated that estradiol can also enhance intake of cocaine after acquisition of self-administration (Zhao and Becker, 2010). However, the few studies that have probed how estrogen influences cocaine self-administration and drug discrimination in monkeys have largely found either minimal (Mello et al., 2007) or no effect (Mello et al., 2008). These findings are surprising as human studies report menstrual phase differences in the subjective effects of cocaine (Sofuoglu et al., 1999). Our current findings are unique in that we use a sensitive assay to
quantify vulnerability to cocaine reinforcement in normal cycling, socially housed monkeys, which may be a more translational model to the human condition.

The experimental procedure of substituting very low cocaine doses for food was designed to determine the sensitivity of each monkey to the reinforcing effects of cocaine. Starting with very low doses permitted us to quantify sensitivity by directly comparing response rates between cocaine- and saline-contingent responding and generate survival curves depicting cocaine sensitivity between dominant and subordinate monkeys. In an earlier study (Nader et al., 2012), we used this procedure and reported that dominant female monkeys were more sensitive to the reinforcing effects of cocaine compared to subordinate animals. This finding was not replicated in the present experiment. In fact, it appeared that the subordinate monkeys were more sensitive to cocaine reinforcement compared to the dominant female monkeys, which is more consistent with our earlier findings in socially housed male monkeys (Morgan et al., 2002; Czoty et al., 2005). There are several important methodological differences between this study and the earlier study in females that may account for the different findings. Perhaps the most significant difference was that the dose range tested in the current study included sub-reinforcing doses (0.0003 mg/kg/inj) at which no monkeys acquired cocaine the initial time it was made available. In the previous study, 40% of the monkeys acquired cocaine self-administration at the dose presented initially (0.001 mg/kg/inj; Nader et al., 2012). Additionally, the differences in “pre-social housing conditions” between the two studies could be influential. In the Nader et al. (2012) study, the monkeys were individually housed for approximately 18 months before the effects of social housing was examined; in the present study, monkeys were pair-housed prior to
being placed in social groups of four. Future studies will be needed to better determine the influence of prior housing conditions on the socially derived stress and enrichment that accompanying social housing (see Nader et al., 2012).

While there were no significant differences in sensitivity to the lowest dose of cocaine that at which acquisition occurred between dominant and subordinate monkeys, there were significant differences in overall response rates and intake across the entire cocaine dose-response curve once acquisition had taken place. In both follicular and luteal phases, subordinate monkeys had higher response rates compared to dominant monkeys. It is unlikely that pharmacokinetic variables associated with hormonal concentration can account for these findings since it has been shown that there are no differences in cocaine and metabolite levels observed across the menstrual cycle following repeated injections of cocaine (Evans and Foltin, 2006). These findings are consistent with an earlier study in male monkeys showing significantly higher response rates and intake across the entire cocaine dose-response curve in subordinate monkeys (Morgan et al., 2002).

Our results indicating that subordinate female monkeys are more sensitive to cocaine reinforcement are consistent with increased instances of other addictive behaviors observed in animals subject to psychosocial stress such as higher consumption of low-fat and high-fat diets (Wilson et al., 2008; Arce et al., 2010), increased alcohol intake (Bahi, 2013) and increased opiate consumption (Heyne and Wolffgramm, 1998). Alterations in the dopaminergic system may underly this increase susceptibility as individual differences in dopaminergic function have been shown to result in varying degrees of susceptibility to drug abuse (Piazza and Le Moal, 1996; Lucas et al., 1998;
Morgan et al., 2002; Nader et al., 2012). Additionally, it is well accepted that reproductive hormones influence the dopaminergic system (Di Paolo, 1994). Our lab have previously demonstrated that menstrual cycle alters DA receptor availability (Czoty et al., 2009), therefore it is not surprising that we observed significant effects of menstrual cycle phase on cocaine reinforcement. There is considerable evidence in rodent self-administration studies to demonstrate that both phase and exogenous reproductive hormone administration affects cocaine’s reinforcing effects, yet few studies have investigated these effects in non-human primates (Roberts et al., 1989; Hecht et al., 1999; Feltenstein et al., 2009; Mello et al., 2007; Cooper et al., 2013). The studies in which an effect of reproductive hormones on cocaine-maintained behaviors was observed showed that the effects were more pronounced during cycle phases when circulating estradiol concentration are at their highest and progesterone concentrations are at their lowest (Feltenstein and See, 2007). The present findings compliment these preclinical studies as well as several human studies reporting that cocaine’s positive subjective effect ratings are lower during the luteal phase relative to the follicular phase (Sofuoglu et al., 1999; Evans et al., 2002; Sofuoglu et al., 2002; Evans and Foltin, 2006).

Taken together, the present findings indicate that factors that may influence vulnerability to drug abuse – in this study, the relationship between estradiol concentration and lowest dose of cocaine to function as a reinforcer - do not necessarily impact maintenance of continued cocaine use. In the case of the latter, we deomonstrate that social rank was related to differences in sustained cocaine self-administration however hormonal fluctuations were not, as evident by the lack of effect of menstrual cycle phase. We have shown in male monkeys that even when baseline rates of cocaine
self-administration are similar between dominant and subordinate monkeys, the effects of environmental and pharmacological manipulations frequently reveal rank-related differences in sensitivity (Czoty and Nader, 2012, 2013). Whether similar outcomes will occur in socially housed females and if menstrual cycle influences these outcomes, remains to be determined. Therefore, the findings of this set of experiments champions for the inclusion of female subjects in future studies and suggests that outcomes from such studies may be imperative in terms of treatment strategy development and may result in individualized treatment options for women compared to men.

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CONFLICT OF INTEREST: none
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Table 1. The effect of menstrual phase at each cocaine dose tested. Mean (±SEM) response rate and intake at the follicular phase and luteal phase of the menstrual cycle within subordinate and dominant monkeys. *p<0.05 between luteal and follicular at that particular dose.

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<td><strong>Dose (mg/kg)</strong></td>
<td><strong>Follicular [Mean ± SEM]</strong></td>
<td><strong>Luteal [Mean ± SEM]</strong></td>
</tr>
<tr>
<td>0.0003</td>
<td>0.021 ± 0.006</td>
<td>0.023 ± 0.006</td>
</tr>
<tr>
<td>0.001</td>
<td>0.014 ± 0.006 *</td>
<td>0.02 ± 0.006 *</td>
</tr>
<tr>
<td>0.003</td>
<td>0.031 ± 0.014</td>
<td>0.13 ± 0.098</td>
</tr>
<tr>
<td>0.01</td>
<td>0.19 ± 0.13</td>
<td>0.11 ± 0.06</td>
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<tr>
<td>0.03</td>
<td>0.14 ± 0.05</td>
<td>0.3 ± 0.18</td>
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<tr>
<td>0.1</td>
<td>0.2 ± 0.05</td>
<td>0.19 ± 0.05</td>
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<table>
<thead>
<tr>
<th>Subordinates Cocaine Self-Administration and Menstrual Cycle Phase</th>
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<tr>
<td><strong>Dose (mg/kg)</strong></td>
<td><strong>Follicular [Mean ± SEM]</strong></td>
<td><strong>Luteal [Mean ± SEM]</strong></td>
</tr>
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</tr>
<tr>
<td>0.1</td>
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<td>0.23 ± 0.05 *</td>
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Table 2. Baseline response rates and reinforcers in socially housed female monkeys.

<table>
<thead>
<tr>
<th>Social Rank</th>
<th>Response Rate Range (response/sec)</th>
<th>Response Rates [Mean ± SEM]</th>
<th>Pellets (n) Range</th>
<th>Pellets (n) [Mean ± SEM]</th>
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</thead>
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<tr>
<td><strong>Dominant</strong></td>
<td></td>
<td></td>
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<tr>
<td>1 and 2</td>
<td>2.5 - 0.4</td>
<td>1.58 ± 0.25</td>
<td>14.17 - 30</td>
<td>27.21 ± 1.9</td>
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<tr>
<td><strong>Subordinate</strong></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 and 4</td>
<td>2.87 - 0.65</td>
<td>1.43 ± 0.29</td>
<td>24.65 - 30</td>
<td>28.11 ± 0.88</td>
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<table>
<thead>
<tr>
<th>Social Rank</th>
<th>Response Rate Range (response/sec)</th>
<th>Response Rates [Mean ± SEM]</th>
<th>Injections (n) Range</th>
<th>Injections (n) [Mean ± SEM]</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Dominant</strong></td>
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<td></td>
</tr>
<tr>
<td>1 and 2</td>
<td>0.056 - 0.01</td>
<td>0.03 ± 0.006</td>
<td>4.5 - 0.67</td>
<td>2.23 ± 0.47</td>
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<tr>
<td><strong>Subordinate</strong></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 and 4</td>
<td>0.06 - 0.008</td>
<td>0.03 ± 0.008</td>
<td>6 - 0.65</td>
<td>2.47 ± 0.82</td>
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Figure 1. Dominant female monkeys and subordinate monkeys acquire cocaine reinforcement at similar doses. Percentage of dominant (open symbols) and subordinate (closed symbols) monkeys that reached criteria to acquire cocaine self-administration at various doses of cocaine available under a 30-response fixed-ratio schedule of reinforcement.

Figure 2. Relationship between average circulating estradiol and dose at which cocaine first functioned as a reinforcer.
Figure 3. Reinforcing effects of cocaine are greater in subordinate female monkeys compared with dominant animals. Mean (±SEM) rate of responding (responses/sec) when saline or various doses of cocaine were available per session for dominant (ranks #1 and ranks #2, open symbols, n=8) and subordinate (ranks #3 and #4, closed symbols, n=7) monkeys in the follicular (A) or luteal (B) phase of the menstrual cycle. Mean (±SEM) cocaine intake (mg/kg/session) during the follicular (C) or luteal (D) phase of the menstrual cycle. Each dose was available for at least five sessions until responding was stable; data represent the mean of the last 3 days of availability for each monkey. *p<0.05; **p<0.01 between dominant and subordinate monkeys at that particular dose.
CHAPTER V

EFFECTS OF PRIOR COCAINE SELF-ADMINISTRATION ON COGNITIVE PERFORMANCE IN FEMALE CYNOMOLGUS MONKEYS

Sarah A. Kromrey, Robert W. Gould, Michael A. Nader, Paul W. Czoty

The following manuscript was accepted for publication in *Psychopharmacology* January, 2015. Stylistic variations are due to the requirements of the journal. Sarah A. Kromrey performed the experiments, analyzed the data and prepared the manuscript. Robert W. Gould, Michael A. Nader and Paul W. Czoty acted in an advisory and editorial capacity.
Abstract

Rationale: Cocaine use has been associated with cognitive impairments that may contribute to poor treatment outcomes. However, the degree to which these deficits extend into periods of abstinence has not been completely elucidated. Objectives: This study tested whether prior experience self-administering cocaine affected acquisition of two cognitive tasks in 16 adult female cynomolgus monkeys. Seven monkeys had previously self-administered cocaine but had not had access to cocaine for two months at the start of this study. Methods: After monkeys were trained to respond on a touchscreen, associative learning and behavioral flexibility were assessed using a stimulus discrimination (SD) and reversal (SDR) task from the CANTAB battery. Performance on this task was monitored over the subsequent three months. Additionally, working memory was assessed with a delayed match-to-sample (DMS) task. Results: Cocaine-naïve monkeys required fewer total trials and made fewer errors and omissions before acquiring the SD and SDR tasks compared to monkeys who had previously self-administered cocaine; two monkeys in the latter group did not acquire the task. However, this cognitive impairment dissipated over several months of exposure to the task. The number of sessions for touch training and delays required to establish a performance-based curve on the DMS task did not differ between groups. Conclusion: Results suggest that cocaine exposure can impair the ability to learn a novel task requiring behavioral inhibition and flexibility, even after an extended period of abstinence. However, this deficit did not extend to maintenance of the task or to acquisition of a working memory task.
Introduction

Cocaine abuse continues to be a major public health problem with more than 1.6 million Americans confirming current cocaine use (SAMHSA 2014). Attempts to develop pharmacological treatments have been largely unsuccessful. The success of some non-pharmacological approaches, such as cognitive behavioral therapy, may represent a reversal of detrimental effects of cocaine abuse on executive function, which encapsulates the abilities to weigh multiple options, make complex decisions and organize, implement and control a multitude of cognitive functions (Oscar-Berman and Marinković 2007; van der Plas et al. 2009; Sofuoglu et al. 2013). Although some structural and functional alterations that occur due to cocaine abuse can recover during abstinence (Nader et al. 2006; Moeller et al. 2012; Connolly et al. 2013; Morie et al. 2013), relatively little is known about the extent to which cognitive impairments persist after cessation of cocaine use (De Oliveira et al. 2009). Characterizing the relationship between cocaine abuse and cognitive function during abstinence will aid development of therapies to reverse these deficits and to minimize relapse.

Dysfunction in brain dopamine (DA) systems is a hallmark of cocaine exposure and is believed to drive the associated cognitive impairments (Cools and D’Esposito 2011; Ersche et al. 2011; Moeller et al. 2012; Verrico et al. 2013), although it is impossible to determine whether these deficits precede or result from cocaine exposure in humans. Cocaine users display deficits in many aspects of executive function, including
cognitive flexibility and response inhibition (Verdejo-García et al. 2006), which have been attributed specifically to alterations in DA function (Nandam et al. 2013). Cognitive deficits during abstinence have been associated with poorer retention and success in behavioral treatment programs (Volkow et al. 1992; Teichner et al. 2002; Aharonovich et al. 2006; Tomasi et al. 2007; Carroll et al. 2011). However, the time course of recovery of executive function during abstinence is not well characterized. Previous studies reported that memory deficits lessened over periods of abstinence, with cocaine users showing improved cognitive function at 6 months of abstinence when compared to 6 weeks (Di Sclafani et al 2002; Pace-Schott et al. 2008; Hanlon et al. 2011). However, limitations inherent in studies with human subjects make it difficult to track recovery over time. Controlled longitudinal studies in laboratory animals can directly address these questions. Longitudinal studies of cognitive function in monkeys—specifically those that involve repeated drug exposure—have been integral in understanding the development of deficits that accompany chronic drug exposure (e.g. Taffe et al. 2001; Jentsch et al. 2002; Liu et al. 2008; Gould et al. 2012).

Sex differences in sensitivity to cocaine are prevalent in both preclinical and clinical research (O’Brien and Anthony 2005; Anker and Carroll 2011). Because females tend to be underrepresented in clinical and preclinical studies, the extent to which cocaine causes cognitive impairments in females is not well understood. This is concerning because women are particularly vulnerable to cocaine abuse (e.g. Cotto et al. 2010). Women initiate drug use at earlier ages, progress to dependence faster and are more susceptible to the physical, mental and social consequences of abuse (Zilberman et al. 2003; Greenfield et al. 2007b). Furthermore, women are less successful at quit attempts,
with lower rates of treatment retention and higher rates of relapse (Siqueland et al. 2002; Hyman et al. 2008). Importantly, previous studies have provided evidence for sex-dependent brain-behavior relationships in the role of the ventromedial prefrontal cortex in mediating emotional processing, decision-making and executive function (Bolla et al. 2004; Tranel et al. 2005; van der Plas et al. 2009). Preclinical evidence also suggests that female subjects are more sensitive to the abuse-related effects of cocaine across a range of behavioral assays including faster acquisition, higher rates and more pronounced reinstatement of self-administration (Lynch and Carroll 1999, 2000; Carroll et al. 2002; Lynch and Taylor 2005). However, nonhuman primate studies of the effects of cocaine on cognitive function have been conducted almost exclusively in male subjects.

In the present experiments, 16 adult female cynomolgus monkeys were studied. Nine monkeys were cocaine-naïve and seven monkeys had previously self-administered cocaine 5 days per week for three months, but had been abstinent for eight weeks at the initiation of this study. To assess effects of prior self-administration experience on cognition, we examined the time it took to train the monkeys to learn to use a touchscreen, then studied the acquisition of a reversal learning task which included a simple discrimination (SD) followed by the reversal of that discrimination (SDR). These behavioral tasks examined the monkeys’ ability to learn a rule to guide behavior (SD) and to inhibit responding under that rule while learning a new rule (SDR). We used a 3-choice visual discrimination to permit examination of the patterns of errors made to determine if they are perseverative (Arnsten et al. 1997; Jentsch et al. 2002). To assess whether cognitive deficits persisted following acquisition, we assessed performance for three months with re-exposure to the reversal-learning task using novel stimuli. We also
evaluated performance on a delayed match-to sample (DMS) task to measure working memory. In this task, subjects are trained to identify previously presented stimuli after various delay intervals. Because all monkeys had a similar previous experience of lever-pressing reinforced by delivery of a food pellet, we did not expect to observe group differences in touchscreen training. However, we hypothesized that cocaine-experienced monkeys would take longer to acquire the SD/SDR task based on previous results in male rhesus monkeys (Gould et al. 2012). Moreover, we expected to observe that these impairments would be task-specific. Because impairments on the DMS task were shown to resolve within 30 days of abstinence in male rhesus monkeys (Gould et al. 2012), we hypothesized that the delay lengths used to generate performance-based curves would be similar between groups.

**Materials and Methods**

**Subjects.** Sixteen adult female cynomolgus macaques (*Macaca fascicularis*) served as subjects. Seven monkeys had been surgically implanted with an indwelling intravenous catheter as described previously (Czoty et al. 2005) and had experience self-administering cocaine (approximately 150 mg/kg cocaine over 6 months). Eight weeks prior to the start of the present experiments, access to cocaine was discontinued for the cocaine-experienced monkeys and they and 9 additional cocaine-naïve monkeys, who had been self-administering food pellets for three months, began self-administering sucrose pellets 3-5 days per week during under a fixed-ratio schedule. There were no differences between the groups in average age or weight at the start of these experiments (Table 1); weights did not change significantly during the course of the experiments. All monkeys were fitted with an aluminum collar (Primate Products, Redwood City,
California) and trained to sit in a standard primate chair (Primate Products). Monkeys were weighed weekly and feed enough fresh fruit and food (Nestle Purina PetCare Company, St. Louis, Missouri) to maintain healthy body weights (2.6-3.2 kg) as determined by physical appearance and periodic veterinary exams; water was available *ad libitum* in the home cage which measured 0.71 x 1.68 x 0.84 m (Allentown Caging Inc., Allentown, New Jersey). Environmental enrichment was provided as outlined in the Institutional Animal Care and Use Committee’s Non-Human Primate Environmental Enrichment Plan. 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 Wake Forest University Institutional Animal Care and Use Committee.

**Cognitive assessments.** Cognitive testing was conducted 5 to 7 days per week between 9:00 am and 12:00 pm using the Cambridge Neuropsychological Test Automated Battery apparatus (CANTAB; Lafayette Instruments, Lafayette, Indiana) as described previously (Gould et al. 2012). Animals completed a maximum of 200 trials in the stimulus discrimination and reversal (SD/SDR) task and 80 trials in the DMS task. Only one task was assessed in each behavioral session. Total session length of the reversal learning task depended on task performance, as session terminated once reversal criteria were met or a maximum of 200 trials were completed (see below). In the DMS task, each animal completed 80 total trials with three delay lengths (short, medium and long, see below), therefore the total session length varied between animals but lasted one hour on average.

**Experiment 1. Effects of prior cocaine self-administration on acquisition of touchscreen training, a reversal-learning task and a DMS task.**
(1a) Training. Using a touch-sensitive computer screen (Lafayette Instruments; Lafayette, IN) monkeys were trained to touch a square that became progressively smaller across trials. Specifically, there were six sizes of the square, which became smaller after every fourth consecutive touch. Each touch was reinforced with a 190-mg food pellet; touching any other part of the screen resulted in a 10-second timeout (Weed et al. 1999). The primary dependent variable for touchscreen training was the number of daily sessions to reach criteria (two daily sessions in which the monkey reached the smallest square). Data from cocaine-experienced (n=7) and cocaine-naïve (n=9) monkeys were statistically compared using a t-test. Differences were considered statistically significant when $p<0.05$. Once the touch-training criterion was met monkeys were exposed to the SD and SDR tasks.

(1b) SD/SDR task. In the SD task, three shapes (A, B, C) appeared in a horizontal row across the center of the screen. The same three shapes were used for each monkey during this stage of the experiment. A response on one shape (A+) resulted in delivery of a 190-mg food pellet and initiation of a 7-second inter-trial interval (ITI) while responding on either of the other two shapes (B-, C-) resulted in a 10-second timeout, followed by a 7-second ITI. Shapes were randomly distributed throughout the three possible positions on the screen with a maximum of 200 trials per day. Acquisition of the SD was defined as 18 correct responses out of the previous 20 completed trials. Once the acquisition criterion was met, contingencies were altered in the SDR phase so that responding on the previously correct shape was now incorrect (i.e. A-) while a response on one of the previous incorrect shapes now counted as a correct response (i.e. B+). The third shape, which was incorrect in the SD phase, remained incorrect in the SDR phase (i.e. C-). The
same consequences of responding on a correct or incorrect stimulus and the same criterion for acquisition used in the SD phase were used in the SDR phase. Dependent variables included the total number of trials, the number of errors and the number of omissions to acquisition during the SD and SDR tasks. Total trials included correct trials, errors and omissions. Data for errors to criterion underwent a square-root transformation prior to analysis to normalize their distribution (see Wright Jr et al. 2013). Response and pellet retrieval latencies were also recorded. For the SDR task, perseverative errors were determined, defined as responses on the stimulus that had been reinforced in the SD phase (A-). Incorrect responses on the stimulus that had not been reinforced in the SD phase (C-) were termed seeking errors. Two-way analyses of variance (ANOVAs) were conducted using group (cocaine-experienced, cocaine-naïve) and phase of task (SD, SDR) as factors. A two-way ANOVA was also conducted to compare the distribution of errors across the two incorrect stimuli in the SD and SDR phases between groups. Significant main effects were followed by post hoc comparisons using Fisher’s LSD tests. For each dependent variable, there were four comparisons of interest (i.e. the comparison of cocaine-naïve vs. cocaine-experienced groups for both tests, and the comparison for each group across tests). To maintain a family-wise error rate less than 0.05, a Bonferroni correction was applied following Fisher’s LSD analysis. The resulting critical value of p was equal to 0.0125. A similar adjustment was made for analysis of error distribution.

(1c) DMS task. Collection of DMS data began after all animals finished Experiment 1b. In the DMS task, a target image appeared on the screen and when a response was made (i.e. after a 0-second delay), three images appeared. The three images were taken from a
limited stimulus set of six images (i.e. stimuli were not trial-unique). A response on the previously displayed image resulted in delivery of a 190-mg food pellet. A response on either of the two novel images resulted in a 10-second time out and no pellet delivery. Once percent accuracy exceeded 80% for 3 consecutive days with a short (0-sec) delay, delays were gradually increased. Baseline performance was established by increasing delay lengths until similar reductions in percent accuracy were reached in all monkeys. There were three target levels of accuracy in each monkey: >78% accuracy (the corresponding delay was considered the “short” delay), 55%-78% accuracy (“medium” delay) and <55% accuracy (“long” delay). Delays were randomly distributed throughout each session so that there were ~27 trials per delay. Once accuracy remained within these ranges for 5 consecutive days, performance was deemed stable for each monkey and the average short, medium and long delay lengths were calculated. A two-way ANOVA was conducted with group (cocaine-naïve, cocaine-experienced) and delay (short, medium, long) as factors. Significant main effects were followed by post hoc comparisons using Fisher’s LSD tests.

**Experiment 2. Effects of prior cocaine self-administration on maintenance of cognitive performance.** Once all monkeys had acquired the SD/SDR task, they were tested once each week on the SD/SDR task for three months; data from the last week of each month were averaged across monkeys for analysis. On the other four days per week, monkeys responded on the DMS task (Experiment 1c). For the SD/SDR task, shapes were selected randomly from the “CAMCOG 0” list associated with the CANTAB system and presented in non-overlapping sets of three. Sets of shapes were randomized across monkeys and time points, with the stipulation that no monkey saw the same set
twice; the set used for Experiment 1b was not re-used during this experiment. Task completion criteria were identical to those described above. The same dependent variables were analyzed as in Experiment 1b. Data were analyzed using a 3-way ANOVA with group (cocaine experienced, cocaine-naïve), phase of task (SD, SDR) and month (1, 2, 3) as factors, followed by post hoc Fisher’s LSD tests.

Results

Experiment 1. Effects of prior cocaine self-administration on acquisition of touchscreen training, the reversal-learning task and the DMS task.

Training. There were no differences between the 7 cocaine-experienced and 9 cocaine-naïve monkeys in the number of days necessary to complete touchscreen training (7.2 ± 2.6 and 5.9 ± 1.2 days, respectively).

(1a) SD/SDR task. There were no significant differences between groups in response latencies or pellet retrieval latencies during the SD/SDR task (Table 2). Two monkeys in the cocaine experienced group did not reach a criterion level of performance and were excluded from data analysis and presentation. One monkey (C-7431) acquired the SD task after 419 total trials (146 errors and 102 emissions) but developed a side bias once the SDR was implemented. The other 10 monkey (C-7441) took 703 trials to acquire the SD (293 errors and 148 omissions) and after switching to the SDR task, soon began to omit nearly all trials.

Results of analysis of total trials to criterion (Fig. 1, top) revealed main effects of group (cocaine-naïve, n=9 vs. cocaine-experienced, n=5; F1,12= 16.91, p<0.001) and test (SD vs. SDR; F1,12=4.93, p<0.05), and no significant interaction. Post-hoc tests
confirmed the difference between the SD and SDR task collapsed across groups (p<0.05), as well as the effect of group, collapsed across test (p<0.001). Cocaine-naïve and -experienced groups were not significantly different on the SD task but differed significantly on the SDR (p<0.0125). In errors to criterion (Fig. 1, middle) there were also significant main effects of group (F1,12= 11.86, p<0.01) and test (F1,12= 8.85, p<0.05) and no interaction. As with trials to criterion, post-hoc testing confirmed the difference between the SD and SDR task collapsed across groups (p<0.05), as well as the effect of group collapsed across test (p<0.01). Post-hoc comparisons revealed a significant group difference only in the SDR phase (p<0.0125). Finally, there was a main effect of group (F1,12= 8.79, p<0.05) but not test in the number of omissions that occurred prior to reaching criterion for acquisition (Fig. 1, bottom). Post-hoc tests confirmed that the groups differed significantly in omissions made during acquisition of the SDR test (p<0.0125) but not the SD test.

Using three stimuli in the SD/SDR task permitted the determination of whether errors made in the SDR phase were perseverative. During the SD phase (Fig. 2, left), cocaine-experienced monkeys made significantly more errors (main effect of group, F1,12=11.42, p<0.01 and post-hoc difference in errors on the C stimulus, p<0.01), but both groups made a similar number of errors on each incorrect stimulus (no main effect of stimulus B vs. C and no interaction), indicating that, prior to reversal, no bias existed. During the SDR, however (Fig 2, right), a main effect of error type (F1,12=157.20, p<0.001) and a significant interaction (F1,12=5.61, p<0.05) was found, and the main effect of group approached significance (F1,12=4.56, p=0.054). Although both groups made significantly more perseverative than seeking errors, the difference was larger in
the cocaine experienced monkeys. Moreover, the difference in number of perseverative errors between cocaine naïve and –experienced monkeys approached significance (p=0.013).

**(1b) DMS task.** One cocaine-naïve monkey (C-8202) and one cocaine-experienced monkey (C-7434) did not achieve stable DMS task performance with relatively short delays (<10 seconds) within 50 daily sessions; their data were excluded from analysis and presentation. For the remaining monkeys, increasing the delay between the disappearance of the sample stimulus and the presentation of the match and comparison stimuli resulted in delay-dependent decreases in accuracy from near 100% to chance levels (F2,20=88.68, p<0.001; Fig. 3, left). There was no main effect of group and no interaction. The specific delay lengths for individual monkeys are shown in Table 1. Analysis of the delay lengths that made up each accuracy level (short, >78% accuracy, medium, 55%-78% accuracy, long <55% accuracy) for cocaine-naïve (n=8) and cocaine experienced (n=5) monkeys showed no significant main effect of group and no interaction (Fig. 3. right). There was, as expected, a significant main effect of delay length (F2,20=73.36, p<0.001); posthoc analysis confirmed that short, medium and long delays were all significantly different from each other. There no significant differences between groups in response latencies or pellet retrieval latencies during the DMS task (Table 2).

**Experiment 2. Effects of prior cocaine self-administration on maintenance of cognitive performance.** The SD/SDR test was repeated weekly over three months after initial acquisition. In analyzing total trials to criterion (Fig 4, top row), a three-way ANOVA revealed main effects of month (F2,72=3.82, p<0.05) and group (F1,72=9.75, p<0.01) but not test; there were no significant interactions. Post-hoc analyses indicated
that the main effects are explained by the increased trials needed by the cocaine-experienced monkeys to complete the SDR task during month 1. For example, post-hoc tests indicated that cocaine-naïve and cocaine-experienced monkeys only differed in month 1 and only on the SDR task, and that overall month 1 was different from months 2 and 3. Moreover, there was no difference across months for the cocaine-naïve group, but month 1 was significantly different from months 2 and 3 for the cocaine-experienced group. The same pattern of statistical test results was observed for errors to criterion (Fig 4, bottom row); there were main effects of month (F2,72=5.97, p<0.01) and group (F1,72=5.93, p>0.05) but not test. The pattern of results of post-hoc tests was identical to those for trials to criterion, indicating that the significant main effects are explained by the higher number of errors in month 1 in the cocaine-experienced monkeys on the SDR task. Note that acquisition data are included in Fig. 4 for comparison but were not included in statistical analysis. Omissions were very low during the SD task (1.0 ± 0.4 omissions per month across all monkeys) and during the SDR task (1.2 ± 0.5 omissions per month when one cocaine-experienced monkey who averaged 11 omissions per month is excluded).

Discussion

Understanding the extent to which cognitive deficits observed in cocaine abusers persist into protracted abstinence will help guide treatment decisions. Although some research has shown improvements in cognitive tasks in abstinence (Gould et al. 2012; Bell et al. 2013; Morie et al. 2013), other studies demonstrate that deficits in inhibition, cognitive flexibility and verbal memory can persist into at least short term abstinence (for review see Verdejo-Garcia 2004; van Holst and Schilt 2011). These experiments in
Cynomolgus monkeys were designed to assess the effects of prior cocaine exposure and subsequent abstinence on acquisition and maintenance of two cognitive tasks in female monkeys.

In the simple discrimination (SD) task, although cocaine-experienced females required more trials to criterion, committed more errors and made more omissions than cocaine-naïve monkeys on average, these effects did not reach statistical significance. Any differences observed on this first phase of the task were not due to general impairments in ability to respond using the touchscreen since there were no differences between groups in initial reinforcement training with the CANTAB system or in response or pellet retrieval latency. A previous study in male rhesus monkeys (Gould et al. 2012), also showed no significant differences between cocaine-naïve and cocaine-experienced monkeys in acquiring the SD task. Although the difference between groups appears to be larger in the present study compared to Gould et al. (2012), convincing evidence for an effect of cocaine experience on SD performance is lacking. Moreover, results of the present experiments do not provide evidence of sex differences, although firm conclusions are limited by the relatively small sample size.

More trials were required to reach criterion and more errors were made in the SDR phase compared to the SD phase, indicative of a higher cognitive demand in this stage of the task. This supports previous findings utilizing reversal-learning tasks (Jentsch et al, 2002; Gould et al. 2012; Kangas and Bergman, 2014), which require inhibition of a previously established response while learning a new contingency without explicit signals (for review see Bari and Robbins, 2013). Unlike the SD phase, during acquisition of the SDR task cocaine-experienced monkeys required significantly more trials to criterion
compared to cocaine-naïve monkeys. Cocaine-experienced monkeys also made more than twice as many errors on average. These group differences are even more striking in light of the fact that two cocaine-experienced monkeys never learned the reversal task and were thus not included in the data analysis.

The design of the SDR phase of the task permitted analysis of the distribution of errors across the two incorrect shapes, which provides insight into the mechanisms mediating poor cognitive performance following cocaine experience. In the SD portion of the task both groups of monkeys made a similar number of error responses on each non-reinforced shape, indicating that no inherent bias existed. In the SDR phase, however, both groups made significantly more perseverative errors—choices of the stimulus that had been reinforced in the SD task that was no longer correct—than errors on the third stimulus that was never correct. Some responding on the previously reinforced stimulus is to be expected since monkeys must learn that the contingency has changed. On average, cocaine-experienced monkeys made more perseverative errors (but not more seeking errors) compared to cocaine-naïve monkeys, a difference (p=0.013) that approached significance (p<0.0125) after Bonerroni adjustments were made for multiple comparisons. Although it is important to note that perseverative responding was observed in both groups of monkeys, the observation of more perseverative responding in cocaine-experienced monkeys would support the interpretation that cocaine use causes deficits that may be driven by an inability to inhibit previously formed associations in order to decipher the new contingencies. This conclusion would be consistent with an earlier report demonstrating that perseverative errors increased following 14 days of
cocaine treatment in vervet monkeys (Jentsch et al. 2002), and suggest that that these impairments can be present months after drug taking ceases.

In addition to group differences in trials to criterion and perseverative errors, the number of omissions was also significantly different in the SDR phase. An increase in number of omissions resulting from dopaminergic manipulations has been documented in other cognitive tasks, including Go/No-Go tasks and the 5-choice serial reaction time test (Nakamura et al. 1998; Czernicki et al. 2002; Fletcher et al. 2007; Verdejo-Garcia et al. 2007). Increases in rate of omissions can result from an inability to perform the task due to motor deficits or because monkeys did not find the pellets delivered after correct responses to be reinforcing. The lack of group differences in response latencies or pellet retrieval latencies argue against these explanations. Increased omissions may also result from impairments in attention, an aspect of cognitive function that is markedly impaired by cocaine use (Jovanovski et al. 2005; Spronk et al. 2013; Wood et al. 2013). However, impairments of attention would likely be accompanied by increased latencies to respond, which were not observed. A definitive assessment of deficits in attention would require cognitive tasks that directly measure attention, but these were not performed in the present study. Because omissions tended to occur after several incorrect responses were made, it is also possible that higher omissions in cocaine-exposed monkeys are a manifestation of a lack of motivation to engage in the task; cocaine exposed monkeys appeared to be more likely to stop initiating trials after several incorrect responses. Ultimately, it is difficult to determine what factors drive increased omissions in animal models. Whatever the mechanism, there was a clear effect of prior cocaine experience on the rate of omissions. Taken together, these results suggest that cocaine self-
administration produced cognitive impairments in these female monkeys during
avstinence consistent with those observed during and following cocaine exposure in male
rats, monkeys and humans (Jentsch et al. 2002; Schoenbaum et al. 2004; Fillmore and
Rush 2006; Calu et al. 2007; Ersche et al. 2008; Liu et al. 2008; Krueger et al. 2009;

The second cognitive domain that was addressed in this study was working
memory, which we evaluated using a DMS task. A delay-performance curve was
established in the monkeys by introducing varying delay periods between the
disappearance of the sample stimulus and the presentation of the match and comparison
stimuli. This increasing delay length raised the difficulty of the task, which was reflected
in delay-dependent reductions in accuracy. Because the short, medium and long delay
lengths required to produce the respective target performance accuracies did not differ
between groups, we conclude that any deficits in working memory caused by cocaine
self-administration did not persist to this point in abstinence. It is not known whether
deficits existed during or shortly after cocaine self-administration. It is possible that
cocaine intake was too low to produce deficits. It is also possible that cognitive
impairment was present, but alterations in brain function compensated over time (e.g.
Porter et al. 2014). The specificity of these cognitive impairments in abstinence (i.e.,
group differences in discrimination/reversal but not working memory tasks) mirrors
findings in male rhesus monkeys (Gould et al. 2012). It is also possible that the lack of
effect in the present study could be due in part to the extensive training necessary to
establish stable delay curves.
Group differences in performance on the SD/SDR task dissipated when monkeys performed the SD/SDR task weekly. Although some group differences were still apparent at the end of the first month of testing, cocaine-experienced monkeys had shown improvement by then. Performance of the previously cocaine-naïve and cocaine-exposed monkeys was not different, and was near perfect, by the third month of weekly exposure to the task. These data are consistent with a study in male rhesus monkeys (Porter et al. 2013) in which no differences were observed in SD, SDR or DMS tasks after three months of abstinence. Importantly, Porter et al. had previously reported the presence of cognitive deficits in these same subjects (Porter et al. 2011). Taken together, these results suggest that although cognitive deficits may be present in cocaine users and acquisition of certain novel tasks may be more difficult for abstinent cocaine users, performance can improve with practice and/or over time. From a translational point of view these studies provide the encouraging clinical message that cocaine-induced cognitive impairments are reversible if abstinence can be maintained.
References


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a D1 receptor agonist injected into the medial prefrontal cortex.

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Kangas BD, Bergman J (2014) Repeated acquisition and discrimination reversal in the squirrel monkey (Saimiri sciureus). Anim Cogn 17: 221-8


Table 1. Subjects’ ages (years), weights (kg), lifetime cocaine intakes (mg/kg) and the individual delay times in the DMS task (seconds).

<table>
<thead>
<tr>
<th>Subject</th>
<th>Age</th>
<th>Weight</th>
<th>Intake</th>
<th>Short</th>
<th>Medium</th>
<th>Long</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cocaine-naïve</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C-7905</td>
<td>5</td>
<td>4.0</td>
<td>0.0</td>
<td>3</td>
<td>25</td>
<td>45</td>
</tr>
<tr>
<td>C-7902</td>
<td>5</td>
<td>2.8</td>
<td>0.0</td>
<td>3</td>
<td>25</td>
<td>50</td>
</tr>
<tr>
<td>C-7664</td>
<td>6</td>
<td>2.9</td>
<td>0.0</td>
<td>2</td>
<td>45</td>
<td>70</td>
</tr>
<tr>
<td>C-7591</td>
<td>6</td>
<td>3.1</td>
<td>0.0</td>
<td>2</td>
<td>15</td>
<td>40</td>
</tr>
<tr>
<td>C-7558</td>
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<td>3.0</td>
<td>0.0</td>
<td>0</td>
<td>10</td>
<td>30</td>
</tr>
<tr>
<td>C-7870</td>
<td>11</td>
<td>2.8</td>
<td>0.0</td>
<td>1</td>
<td>15</td>
<td>35</td>
</tr>
<tr>
<td>C-7964</td>
<td>5</td>
<td>2.6</td>
<td>0.0</td>
<td>3</td>
<td>15</td>
<td>25</td>
</tr>
<tr>
<td>C-8202</td>
<td>6</td>
<td>2.4</td>
<td>0.0</td>
<td>did not become stable</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C-7460</td>
<td>10</td>
<td>4.0</td>
<td>0.0</td>
<td>0</td>
<td>10</td>
<td>30</td>
</tr>
<tr>
<td>Cocaine-experienced</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C-7438</td>
<td>7</td>
<td>2.6</td>
<td>207.8</td>
<td>1</td>
<td>15</td>
<td>45</td>
</tr>
<tr>
<td>C-7458</td>
<td>7</td>
<td>3.4</td>
<td>170.9</td>
<td>0</td>
<td>10</td>
<td>30</td>
</tr>
<tr>
<td>C-7437</td>
<td>7</td>
<td>3.5</td>
<td>1979.8</td>
<td>0</td>
<td>30</td>
<td>60</td>
</tr>
<tr>
<td>C-7431</td>
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<td>3.2</td>
<td>290.0</td>
<td>0</td>
<td>10</td>
<td>30</td>
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<tr>
<td>C-7457</td>
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<td>2.7</td>
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<td>30</td>
</tr>
<tr>
<td>C-7434</td>
<td>10</td>
<td>2.8</td>
<td>962.0</td>
<td>did not become stable</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C-7441</td>
<td>10</td>
<td>3.3</td>
<td>680.0</td>
<td>not tested</td>
<td></td>
<td></td>
</tr>
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</table>
Table 2. Mean (± SEM) response latencies and pellet retrieval latencies (in sec) in cocaine-naïve (coc-naïve) and cocaine-experienced (coc-exp) monkeys.

<table>
<thead>
<tr>
<th></th>
<th>Response latency, SD</th>
<th>Response latency, SDR</th>
<th>Retrieval latency, SD</th>
<th>Retrieval latency, SDR</th>
</tr>
</thead>
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<tr>
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<td></td>
<td></td>
<td></td>
</tr>
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<td>Coc-naïve (n=9)</td>
<td>2.9 (0.4)</td>
<td>3.4 (0.7)</td>
<td>1.6 (0.5)</td>
<td>1.2 (0.2)</td>
</tr>
<tr>
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<td>2.1 (0.4)</td>
<td>2.8 (0.4)</td>
<td>1.5 (0.3)</td>
<td>1.3 (0.4)</td>
</tr>
<tr>
<td><strong>MONTH 1</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coc-naïve</td>
<td>2.4 (0.2)</td>
<td>2.8 (0.3)</td>
<td>1.2 (0.2)</td>
<td>1.3 (0.2)</td>
</tr>
<tr>
<td>Coc-exp</td>
<td>2.8 (0.5)</td>
<td>3.4 (0.8)</td>
<td>1.0 (0.2)</td>
<td>1.0 (0.2)</td>
</tr>
<tr>
<td><strong>MONTH 2</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coc-naïve</td>
<td>2.0 (0.2)</td>
<td>2.2 (0.1)</td>
<td>1.3 (0.3)</td>
<td>1.2 (0.2)</td>
</tr>
<tr>
<td>Coc-exp</td>
<td>2.8 (0.8)</td>
<td>2.7 (0.8)</td>
<td>1.4 (0.3)</td>
<td>1.1 (0.2)</td>
</tr>
<tr>
<td><strong>MONTH 3</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coc-naïve</td>
<td>2.9 (0.4)</td>
<td>3.4 (0.7)</td>
<td>1.6 (0.5)</td>
<td>1.2 (0.2)</td>
</tr>
<tr>
<td>Coc-exp</td>
<td>2.1 (0.4)</td>
<td>2.8 (0.4)</td>
<td>1.5 (0.3)</td>
<td>1.3 (0.4)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Response latency, sample</th>
<th>Response latency, match</th>
<th>Retrieval latency</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>DMS</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coc-naïve (n=8)</td>
<td>1.4 (0.2)</td>
<td>3.0 (0.4)</td>
<td>1.5 (0.3)</td>
</tr>
<tr>
<td>Coc-exp (n=5)</td>
<td>2.1 (0.4)</td>
<td>2.4 (0.3)</td>
<td>1.0 (0.2)</td>
</tr>
</tbody>
</table>
Figure 1. Performance during acquisition (total trials, errors and omissions to criterion) of the simple discrimination (SD) and reversal (SDR) phases in cocaine-naïve (coc-naïve, n=9) and cocaine experienced (coc-exp, n=5) monkeys. Data for errors was square-root transformed prior to analysis. Bars depict mean (± SD) values. *, p < 0.0125.
Figure 2. Error distribution during acquisition of the reversal-learning task in cocaine-naïve (n=9) and cocaine-experienced (n=5) monkeys. Bars represent mean (± SD) number of errors (square-root transformed) on each non-reinforced stimulus during the SD phase (left) and SDR phase (right). *, p < 0.0125.

Figure 3. Accuracy at (left), and absolute lengths of (right), the delays deemed short, medium and long in cocaine-naïve (n=8) and cocaine-experienced (n=5) monkeys. Points represent mean (± SEM).
**Figure 4.** Trials to criterion (top row) and errors to criterion (square-root transformed, bottom row) during the simple discrimination (SD, left column) and reversal (SDR, right column) phase for the three months following acquisition (A). Points depict mean (± SEM) values in cocaine-naïve (n=9) and cocaine-experienced (n=5) monkeys. Data from acquisition (A) are included for comparison.
CHAPTER VI

DISCUSSION

The overarching goal of the work presented in this dissertation was to determine the hormonal and behavioral variables that influence cognitive performance and vulnerability to cocaine’s reinforcing effects in female cynomolgus monkeys. During the time this research was conducted, a staggering male sex bias existed in both clinical and preclinical research. Initial reforms were undertaken in 1993 when congress passed the National Institutes of Health (NIH) Revitalization Act which required that all NIH-funded clinical research include women and minorities as subjects “in approximately equal numbers of both sexes” unless exclusion was deemed appropriate, such as with trials testing potential drugs for erectile dysfunction. Furthermore, the guideline stated that phase III clinical trials must be designed to allow separate planning, conducting, and reporting of analyses for these groups when prior research has indicated that it may be important. Despite this law, to date, women remain underrepresented in clinical trials despite this law, and even when included, there remains a lack of sex-based reporting of results, thereby abolishing any chance of determining if a therapy has greater benefit in one sex over another. Although this law has stimulated the inclusion of women in clinical research, there has been little to no focus on the importance of including both sexes in basic science or translational research conducted in animals or cell lines.

In an effort to begin addressing some of the issues described above, the NIH recently announced that it will distribute over $10 million in grants to study sex as a biological variable in a diverse array of subjects, from drug abuse to fetal development.
This funding allotment is aligned with the research presented here, to investigate female reproductive hormones in cognitive performance, social rank and susceptibility to the reinforcing effects of cocaine.

Sex differences in physiological and psychological responses to cocaine are well documented both preclinical and in epidemiological studies. Females are known to progressing through the phases of addiction differently than men. However, the downstream mechanisms that result from interactions between estrogen and cocaine on dopamine (DA) systems are not clear (Fattore et al., 2008; Segarra et al., 2010; Bobzean et al., 2014). The DA system mediates many aspects of cognitive function and therefore may interact in sex-specific ways with reproductive hormones and cocaine as well. This may be of particular importance as cocaine users reliably exhibit deficits in executive function across multiple domains integral in facilitating behavioral modification, including working memory and behavioral flexibility (Kubler et al., 2005; Tomasi et al., 2007; Moeller et al., 2010). Moreover, the fact the cognitive performance at the time of treatment initiation has correlated with treatment success (Teichner et al., 2001; Aharonovich et al., 2006; Turner et al., 2009) supports efforts to identify time points at which behavioral treatment strategies may be most effective in female cocaine abusers.

The studies described in this dissertation took a systematic approach to validate the use of normal cycling, socially housed female cynomolgus macaques to study the effects of hormonal fluctuations and stress on cognitive function. Additional studies assessed how these factors impacted susceptibility to the reinforcing effects of cocaine as well as cocaine-induced cognitive deficits. Investigating the interaction between environmental variables, endogenous hormonal milieu and drug self-administration (SA)
is a complex and daunting task, but studies such as these are necessary to provide greater translational utility of the NHP model. Furthermore, it should be pointed out that fewer novel drugs are being approved now than in the past, with most drugs failing in clinical trials. From a pharmacotherapy development standpoint, by considering sex difference earlier in research studies, some of these disappointments could be circumvented and strides could be made towards more personalized medicine.

CHAPTER II: EFFECTS OF HORMONAL FLUCTUATIONS ON COGNITIVE PERFORMANCE IN FEMALE MONKEYS

In order to gain a more comprehensive understanding of how sex hormones influence behavior, the first research question this dissertation assessed whether circulating estrogen and progesterone concentrations interacted with cognition. Specifically, the goal of Chapter II was to determine how phase of menstrual cycle, and resulting fluctuating hormone concentrations, impacts performance on an associative learning and behavioral flexibility task, as well as a working memory. By using precise hormonal analysis through serum collection to validate hormonal state, we were able to make methodological improvements over earlier studies. Our hypothesis was that phases of the menstrual cycle in which estrogen is elevated will result in improved cognitive performance compared to other menstrual cycle phases where progesterone is higher. Importantly, hormonal fluctuations were quantified so that individual hormonal concentrations could be correlated with performance on the tasks where differences were observed.
The role of reproductive hormones on cognitive performance is of interest because sex differences are reliably observed in numerous tasks of cognitive function (Williams and Meck, 1991; Epting and Overman, 1998). The majority of preclinical studies investigating the effect of hormonal fluctuations on cognitive functions are limited by the following: no direct measurements of hormonal concentrations, frequent administration of exogenous hormones rather than assessment of normal fluctuations, testing of only one cognitive task and/or not using a longitudinal within-subject study design. The studies in this chapter addressed these issues in order to gain insight into this multifaceted question.

Associative learning and behavioral flexibility were assessed with a reversal learning test. When the simple discrimination stage of the task, which assessed associative learning, was analyzed with monkeys grouped into four hormonally distinct phases, no effect of menstrual cycle was observed, although a positive correlation was found between the number of total trials and errors to reach acquisition of an individual in the associative learning stage of the task and their circulating progesterone (P4) concentrations. This finding suggested that for the associative learning stage, P4 may exert an impairing effect on performance rather than E2 aiding in this function. Although no studies have been conducted to specifically address the role of progesterone on performance during a reversal learning task, a number of finding in tangential fields suggest this interaction may be crucial for the associative learning stage of the task. Additionally, studies in rodents investigating the contribution of E2 and P4 to multiple hippocampal tasks revealed that although higher concentration of P4 in the hippocampus predicted enhanced performance, higher turnover of P4 to its metabolites (termed
progesterone utilization) in the hippocampus was associated with decreased performance in this task (Ladurelle et al., 2000; Paris et al., 2011).

During the discrimination reversal stage of the task a significant facilitation of performance was revealed during the late follicular phase of the menstrual cycle. This phase of the menstrual cycle was verified to have significantly higher circulating concentrations of estradiol (E2) than the other phases of the menstrual cycle tested. Accumulating evidence indicates that in rodents, E2 enhances DA synthesis (Inagaki et al., 2010), release (Xiao et al., 2003) and turnover (Di Paolo et al., 1985) in areas associated with the mesolimbic pathway, including the PFC (Williams and Goldman-Rakic, 1998). The mesolimbic pathways relays reward-related neuronal and signals between the VTA and the PFC predominantly via dopaminergic neurotransmission (Richardson and Gratton, 1998; Floresco, 2013). This is important to cognitive functions as the DA supply of the PFC is crucial for its maintenance and performance (Rapp et al., 2003). Our current findings of improved cognitive performance on a PFC-dependent task when E2 concentrations are highest indicate that the reproductive hormonal milieu may influence the function of DA neurons in the PFC.

An additional insight into cognition can be gained by tracking perseverative errors during the reversal stage of the task. Distribution of errors can offer insight into the mechanism behind the observed differences in cognitive function at the different phases of the menstrual cycle. Of note, in this study the only phase of the menstrual cycle in which monkeys did not exert significantly more perseverative responses was the late follicular phase. This suggests that hormonal fluctuations at different phases of the menstrual cycle phase may interfere with the ability to disengage from a previously used,
but no longer relevant strategy to obtain reinforcement. In humans cyclic shifts in specific abilities, such as mental rotations (Hampson, 1990) or implicit memory (Maki et al., 2002) have demonstrated estrogen-related ‘deficits’ or ‘improvements’ in learning and memory to estrogen-related biases in strategies for solving certain tasks. By collecting and interpreting the distribution of errors during the two stages of the reversal learning task, we are able to gain insight into the shifts in strategy selection across the menstrual cycle. Furthermore, seeing as these findings are applicable solely to the behavioral flexibility stage of the task, and that estrogens are recognized as powerful modulators of the DA content in the PFC (Sarvari et al., 2014), these findings suggest an interaction between E2 and DA as the crucial mediator for the observed cognitive differences across the menstrual cycle. The link with DA is critical to other studies described in this dissertation related to the behavioral and reinforcing effects of cocaine.

The observed menstrual cycle-related deficits in the reversal-learning task at initial exposure did not extend to subsequent testing of this task. Showing E2-related performance deficits early but not later in training of a cognitive task has also been observed in spatial-learning tasks (Korol et al., 1993; Frye, 1995; Chesler and Juraska, 2000). Moreover, working memory as measured by the delayed matching-to-sample (DMS) task did not show phase-related alterations in performance accuracy. This suggests that performance on hippocampal-dependent tasks may be more stable than PFC-dependent tasks and/or that the extensive training necessary for a stable delay-curve dilutes any phase related differences. Furthermore, some tests of working memory are sensitive to the cognitive enhancing effects of estrogen only after the animals have acquired the tasks and when the cognitive load is high (Bimonte and Denenberg, 1999;
Sandstrom and Williams, 2001). Although we attempted to examine this effect of increased cognitive load with the long delay length, the animals were still routinely exposed to this delay. Therefore, it is possible that had we incorporated a probe session with a novel much longer delay length, we could have observed some of these estradiol-dependent cognitive alterations.

The hypothesis that estradiol and progesterone may influence learning and memory in a task-dependent manner is supported by literature documenting the magnitude and direction of action of E2 on different tasks (for review see Dohanich, 2002; Frick et al., 2010; Barros et al., 2015). With the life expectancy increasing and more options of birth control being made available to women during their reproductive years, as well as hormone replacement therapy for postmenopausal women, the question of cognitive effects of these interventions are frequently raised. Therefore, further investigation into how reproductive hormones influence learning and memory is warranted. Our current study contributes to this literature and offers important insights into how these hormones may influence specific aspects of different cognitive tasks taking into account differing baseline concentrations of hormones and including analysis of cognitive strategy.

The findings from hormone studies demonstrate that acquisition of a novel task may be susceptible to hormonal influence. These findings add to the literature by suggesting that P4 or E2 can differentially influence performance on a task, depending on the brain areas that mediate the task. Specifically, elevated P4 was associated with impaired initial acquisition of the associative learning portion of the reversal learning task (SD), whereas elevated E2 correlated positively with facilitation of initial acquisition of
the reversal portion (SDR). Results from the studies described above are particularly
generalizable to humans because monkeys have a menstrual cycle similar to humans (~30
days). Furthermore, the female monkeys in this study had elevated E2 during the late
follicular phase and elevated P4 during the early luteal phase, which is also observed in
humans. Finally, this new data on the influence of hormones on cognitive performance in
normally cycling female monkeys lays the groundwork for future investigations of the
effects of social rank and drug of abuse on cognition in females.

CHAPTER III: PREDICTORS OF SOCIAL RANK IN FEMALE CYCNOLOGUS
MONKEYS

After observing the interaction between hormonal fluctuations and cognitive
performance, we assessed how circulating E2 and P4 may influence additional behaviors
associated with cocaine abuse. It is understood that vulnerability to numerous diseases
states, including drug addiction, are dependent on multiple factors, including a
susceptible host, a reinforcing agent and a context where the two meet (cf. Mills, 1965).
Therefore, the research within the next set of studies was designed to investigate how
hormones influence a female monkey’s susceptibility to occupy a certain rank in the
social hierarchy (i.e., trait variables), if hormone concentrations changed during social
group formation and how hormone concentrations were altered following stable
occupation of this rank (i.e., state variables).

We hypothesized monkeys that would eventually become subordinate would have
higher circulating cortisol, locomotor activity, and cognitive impairments compared to
dominant monkeys. Alternatively, dominant monkeys would have higher circulating
testosterone during initial social housing. Following hierarchy establishment, subordinate monkeys would exhibit more hormonal disruption (blunted E2/P4) compared to dominant monkeys. These hypotheses were largely drawn from previous findings of studies assessing similar questions in socially housed male monkeys (Morgan et al., 2000; Czoty et al., 2009). Additionally, cognitive performance and locomotor activity were included as outcome measures during the initial week of social housing.

The only measure that was predictive of eventual social rank was greater circulating E2 during baseline in monkeys that would eventually become rank 3 monkeys. However, this heightened E2 is blunted following stable hierarchy establishment to the extent that differences in E2 area under the curve is no longer different between rank 3 monkeys and the dominant ranks. Cortisol concentrations were also elevated in a rank-related manner during the initial week of social housing, with eventual subordinate monkeys displaying higher circulating cortisol compared to dominant ranked monkeys. Finally, there were rank-related cognitive deficits during this initial week with dominant animals performing poorly at short delays of a working memory task, whereas subordinate animals were impaired at medium and long delays. Body weight was predictive of eventual social rank in this cohort of female monkeys. These findings were similar to previous studies in male monkeys in which heavier monkeys were more likely to become dominant (Morgan et al., 2000), but different than previous studies of females (Riddick et al., 2009). There were a number of differences between our monkeys and the study by Riddick et al. (2009). One factor that is pertinent here is that our animals were pair-housed prior to housing in pens of 4 monkeys whereas in the Riddick study animals were individually housed. The experience of living with
another monkey prior to being placed in a group setting may have affected the establishment of hierarchy. We attempted to preemptively answer this question by assessing rank of monkeys when pair-housed to determine if their status in pair-housing predicted eventual rank when housed in groups of 4. However, determining the rank of monkey when pair-housed was not nearly as straightforward as when housed in larger groups. Specifically, reciprocal affiliative behaviors were almost exclusively observed when pair-housed with virtually no aggression documented.

No significant main effects on homecage activity were found in the current study. Direct comparisons between this study and previously published studies cannot be done because novelty-induced locomotor activity was assessed in Morgan et al. (2000) and Riddick et al. (2009), whereas home cage activity was evaluated in the current study by placing Actical monitors on each monkey’s collar. Furthermore, ovarian hormone fluctuations could have influenced activity since it has been documented in rodents that estrus cycle can alter overall locomotor activity (Davis et al., 2008). Although we did not address the relationship of hormonal fluctuations and locomotor activity in the current set of experiments, we did investigate the effect of menstrual cycle phase on locomotor activity in female monkeys (data not shown) and no significant effect of menstrual cycle phase was determined.

One of the major findings of this study is that future rank #3 monkeys had higher circulating E2 than other ranks prior to social housing. It is well known that both E2 and P4 exert structural and functional trophic effects on brain development early in life, and throughout adolescence and adulthood. The overall effect of ovarian hormones on synaptic function seems to be more complex than simple enzymatic up- or down-
regulation; it relies on the brain region-specific expression of progesterone and estrogen receptors, the duration of treatment and possibly also on the dose (or serum/brain levels) of these hormones. By analyzing the fluctuations of serum concentrations across the menstrual cycle, we can gain insight as to the extent of the exposure across each month for individual animals. To our knowledge, measurement of basal circulating estrogen and progesterone as trait measures for eventual social rank has not been conducted previously. Numerous studies have examined how these hormones differ once rank is established. Increased baseline circulating E2 may have predisposed these females to become subordinate by altering their behavior and ultimately influencing their interactions with the other monkeys. Although we did not correlate circulating E2 with any specific subordinate behavior (i.e. lipsmacking or fear grimacing), this hypothesis is supported by one study in humans which reported a negative association between high rank, as judged by peer assessment, and estradiol (Cashdan, 1995).

It has previously been observed in male and female monkeys that cortisol levels were not predictive of eventual social rank (Morgan et al., 2002; Riddick et al., 2009). Our data support these findings and the conclusion that in captive cynomolgus macaques, circulating cortisol prior to social housing is not a trait variable. We observed rank differences in cortisol concentrations during the initial week of social housing with future rank 4 monkeys having significantly higher circulating cortisol compared to rank 1 monkeys even on the final day of serum collection (day 5). A similar study in male cynomolgus monkeys determined that rank differences in circulating cortisol were no longer evident after 3 days of social housing (Czoty et al., 2009). These findings supporting the hypothesis that social housing may be a more stressful experience for
female monkeys. Hypercortisolemia has been documented in subordinate monkeys living in stable social groups in a number of studies (Sapolsky, 1989, 1992; Kaplan et al., 1996; Shively et al., 1997). Additionally, the stability of a group may contribute to circulating cortisol in monkeys of different ranks. A recent study by Qin and colleagues (2013) revealed that in authoritarian groups, cortisol levels were not correlated with social rank, but a negative correlation was found between social rank and cortisol levels in less stringent hierarchies. Our findings support these results as the cortisol differences were most pronounced early in the week. Moreover, as the rank differences remained for the entire week the current findings suggest that the stress associated with establishment of the social hierarchy may be more pronounced in female monkeys compared to males.

In the male study, adrenal responsiveness was assessed using DEX suppression and ACTH administrations following stable hierarchy establishment and it was revealed that although circulating cortisol was not different between ranks, when the system was challenged pharmacologically, rank differences emerged once again with subordinates presenting a larger cortisol response (Czoty et al., 2009). We did not test adrenal responsiveness in the current study but it would be of interest for future assessments so that a more complete picture of how social status in female monkeys related to the HPA axis.

Our hypothesis that future dominant monkeys would have higher circulating testosterone than future subordinates stemmed from human studies investigating androgens in female athletes (Mazur and Booth, 1998; Zilioli and Watson, 2012) and college women (Cashdan, 1995), in those studies dominance was associated with higher concentrations of androgens. Many of these studies observed a ‘competition effect’ (or
winner-loser effect) whereby winners had elevated concentrations of testosterone for a few hours following the competition, while losers showed a decrease in testosterone concentration (Zilioli and Watson, 2014). In other human studies, personality traits such as fearless dominance and self-centered impulsivity predicted greater antagonistic behavior (Geniole et al., 2013). There is evidence in female cynomolgus monkeys living in same-sex groups that dominant females are more aggressive than subordinates (Shively, 1997; Kaplan et al., 2002; Kaplan and Manuck, 2004). Conversely, studies in free-ranging female monkeys have not observed similar correlations between aggression and social rank (Higley et al., 1996). We did not observe significant rank differences in the current study. This could indicate that there may be additional psychological and contextual factors that influence testosterone response besides competition and outcome, that delineate social rank. The differences in these seemingly similar studies are not surprising given that there is extensive evidence that contextual factors (i.e., stability of group, gender composition of cohort, availability of food, etc.) and the inherent characteristics of an individual (i.e., neurobiology, past experiences, temperament) contribute to the social rank of a monkey rather than it being a trait of the individual (Shively and Kaplan, 1991; Kaplan et al., 2002).

Following stable social hierarchy establishment, subordinate monkeys had blunted E2 concentrations compared to baseline, so that the differences between ranks that were previously observed were no longer evident. This observation of differences in E2 concentrations according to rank in socially housed monkeys has been observed in previous studies reporting lower E2 concentrations in subordinate monkeys across a number of species (Abbott et al., 1981; Kaplan et al., 1991; Saltzman et al., 1998; Kaplan
et al., 2010). Reduced E2 may indicated impaired ovarian function. The long-term effects of impaired ovarian function may adversely affect a females’ health (Kaplan and Manuck, 2008). In fact, ovarian insufficiency has been linked to bone loss, cognitive impairment, movement disorders, cardiovascular disease and premature death (Sowers et al., 1998; Rocca et al., 2006; Gallagher, 2007). Ovulatory and/or neurohormonal disruptions in women is difficult to ascribe solely to psychological stress due to the frequent co-occurrence of metabolic disruptions (Berga, 1996; De Souza et al., 2003; Loucks and Thuma, 2003; Williams et al., 2007). However, reproductive dysfunction of psychogenic origin in women is often ascribed to stress imposed by the environment (Drew, 1961; Berga, 1996) and is called functional hypothalamic anovulatory syndrome, which occurs along a continuum from mid-luteal phase deficits to anovulation and amenorrhea (Ginsburg, 1992; Berga, 1996). Furthermore, the role ovarian endocrinology plays in modulating a woman’s risk to diseases such as multiple sclerosis (D’hooghe et al., 2013), cardiovascular disease (Kallen and Pal, 2011) and postpartum depression (Saleh et al., 2012) has been a recent research interest. Additional research into the long-term effects of hormonal dysfunction observed in our subordinate monkeys is vital to understanding the health risks associated with the menstrual dysfunction that occurs in women.

The final assessment in this study involved the effect of social hierarchy establishment on cognitive performance. As hypothesized, cognitive impairments were observed at the medium and long delays in future subordinate animals. Furthermore, these cognitive deficits were reflected in an increased number of omissions. Subordinate monkeys demonstrated both increased circulating cortisol and cognitive impairments
during the initial week of hierarchy establishment, indicating a possible interaction between stress induced cortisol and cognitive function is likely responsible. Cortisol can affect cognitive performance acutely through the activation of receptors located in the PFC, hippocampus and amygdala (Lupien et al., 2007). Moreover, higher cortisol release has been related to poor cognitive performance in healthy humans in a multitude of studies (Hodgson et al., 2004; Li et al., 2006; Beluche et al., 2010; Evans et al., 2011, Franz et al., 2011). Although animal studies have shown that low long-term cortisol exposure may negatively affect cognition (Stienstra et al, 1998; Wossink et al., 2001; Finsterwalk and Alberini, 2014), this is the first study to document short-term, within-subject cognitive impairments and cortisol dysregulation in a nonhuman primate model of social stress.

Cognitive impairments were observed in rank #1 monkeys as well, although these impairments were only evident at the short delay of the task. Accuracy during the short-delay interval is well above chance level and is consistent with accuracy levels observed in other studies using no delay (D’Amato et al., 1985; Truppa et al., 2014). Important components of cognition include the ability to attend, to learn and to remember. Although impairment at any one of these components results in disrupted performance, the specific component where the impairment occurs (i.e. attending or recall) is also important. The short delay represents a very early stage of visual-memory processing and decreases in accuracy at this stage have been attributed to distractibility at times due to the environmental context in previous research (Buccafusco, 2008; De Lillo et al., 2011). In light of this, it is possible that the decrease in accuracy at the short delay is due to the need to allocate cognitive resources to alternative processes, such as increased contextual
vigilance, which may have become essential during this period of hierarchy establishment. Because at this short delay, the animals must respond on a ‘match’ stimulus so quickly after the ‘sample’ stimulus, it is possible that they are rather attending to aspects of their environment at this time. This could account for the observed detrimental effect on only the short delay trials, as the longer delays allow them additional time between ‘sample’ and ‘match’.

Overall, the results in Chapter III indicate that endogenous reproductive hormones may influence eventual occupied social rank, with higher circulating E2 predicting a subordinate rank. Additionally, cognitive impairments in working memory were observed in all the ranks, although rank differences were observed depending on the delay interval. These findings suggest that the mechanisms underlying the observed cognitive dysfunction may differ between ranks. Future studies in NHPs should address how long-term social rank occupation interacts with cognitive performance. Finally, a hormonal profile was observed in subordinate monkeys following social housing with increased cortisol concentration short-term and reduced E2 and P4 concentrations long-term. The disease risk associated with this altered hormonal profile would be of interest for future studies.

**CHAPTER IV: EFFECTS OF RANK AND MENSTRUAL PHASE ON ACQUISITION OF COCAINE SELF-ADMINISTRATION, LOCOMOTOR ACTIVITY AND COGNITIVE PERFORMANCE IN CYNOMOLGUS MONKEYS**
The next set of studies conducted in this dissertation investigated how circulating hormones, and social rank following stable hierarchy establishment influence vulnerability to the reinforcing effects of cocaine. *We originally hypothesized that subordinate monkeys would be more vulnerable to the reinforcing effects of cocaine. Additionally we proposed that all monkeys would demonstrate an increased SA profile during the follicular phase of the menstrual cycle compared to the luteal phase.* Our major findings from this chapter were that 1) subordinate animals did not acquire cocaine SA at significantly different rates, but they did SA at higher rates and intake during the follicular phase of the menstrual cycle, 2) higher circulating E2 predicted acquisition at a lower dose of cocaine. The current study was a crucial addition to previous studies conducted by our group investigating how social environment influences vulnerability to the reinforcing effects of cocaine (Morgan et al., 2002; Nader et al., 2012). Contrary to the Nader et al. (2012) study, acquisition of cocaine reinforcement did not significantly differ as a function of social rank in this cohort of females. However, the average dose that monkeys acquired cocaine SA was different, with subordinate monkeys acquiring at a lower dose (0.003 mg/kg) than dominant monkeys (0.01 mg/kg). This difference in mean dose of acquisition suggests a potential difference between vulnerability to the reinforcing effects of cocaine due to social rank. Therefore, it is not surprising that significant social rank-related differences in response rates and intake across the entire dose-response curve were revealed. In both the follicular and luteal phases of the menstrual cycle, subordinate monkeys had higher response rates compared to dominant monkeys. These findings with subordinate females were similar to a previous study in
male monkeys which demonstrated higher response rates and intake in subordinate males compared to dominants (Morgan et al., 2002).

In light of the findings from Chapter IV that female monkeys who become subordinate had higher circulating cortisol, which was similar to findings in male monkeys (Czoty et al., 2009), it can be assumed that both of these groups of subordinate monkeys are in a state of chronic social stress. Although cocaine SA was not studied in the male monkeys in the Czoty et al., 2009 study, the Morgan et al. (2002) study revealed that no tested dose of cocaine functioned as a reinforcer in dominant male monkeys. This is in stark contrast to both our current findings and the results from Nader et al. (2012) in which all female subjects acquired cocaine SA regardless of rank. However, it should be noted that compared to males, females reliably exhibit a greater and longer-lasting behavioral, physiological and molecular stress response to social and non-social stressors (Handa et al., 1994). Furthermore, women are twice as likely as men to develop major depressive disorder (Schuch, 2014) many of whom may present with a comorbid substance use disorder (Kessler et al., 2005). Therefore, the effects of chronic social stress on the mesolimbic reward circuitry that may be underlying the differing vulnerability to the reinforcing effects of cocaine between female dominant and subordinate monkeys in the current study, may also speak to more pronounced effects in these female monkeys regardless of rank compared to male socially housed monkeys. Furthermore, this reflection of the human condition in our model of chronic social stress in subordinate female monkeys supports its relevance as a useful tool for characterizing individual differences associated with stress and substance use behaviors.
Another major finding from this chapter was an interaction between E2 and the reinforcing effects of cocaine. Circulating E2 concentrations correlated with the dose at which cocaine acquisition occurred. Specifically, monkeys with higher circulating E2 were more likely to acquire cocaine SA at lower doses of cocaine. This result suggests a modulatory effect of E2 and supports several lines of evidence demonstrating that E2 affects components of DA transmission including striatal DA turnover and release (Becker and Beer 1986, Di Paolo et al., 1985) and density of striatal DA uptake sites (Morissette et al., 1990). E2 has also been shown to promote the sensitivity of VTA DA neurons to cocaine (Zhang et al., 2008) and to enhance cocaine-stimulated striatal DA release (Peris et al., 1991). These effects of E2 on DA activity could be responsible for the observed vulnerability to cocaine reinforcement. Studies in women typically assess hormonal influence on the reinforcing effects of cocaine according to menstrual cycle phase, rather than by collecting repeated blood samples to gain insight into overall hormonal exposure. In those studies, women had greater subjective response to cocaine in the follicular phase of the menstrual cycle when estrogen levels are high (Sofuoglu et al., 1999; Evans et al., 2002; Evans and Foltin, 2006) and DA D2/D3 receptor availability is low (Czoty et al., 2009).

Taken together, we and others provide evidence indicating that the reinforcing effects of cocaine are powerfully influenced by a women’s hormonal milieu. This is particularly important considering that in Chapter II we demonstrated that during the same menstrual cycle timeframe, when E2 is high, more errors and perseverative responding were. These findings suggest that elevated E2 concentrations at certain phases of the menstrual cycle may contribute to vulnerability to the abuse cocaine by mediating
both the reinforcing effects of cocaine as well as impaired cognitive function. In light of the fact that P4 is increased during the luteal phase of the cycle, it is possible that instead of E2 potentiating the reinforcing effects of cocaine, P4 is attenuating them. A recent study by Fox et al. (2013) investigated the effect of exogenous P4 on drug craving and cognitive performance and found that P4 decreased cue-induced craving and improved cognitive performance. Although attenuation of reinforcing effects with P4 could explain the menstrual cycle phase effects observed in the current studies, it does not explain the relationship between average circulating E2 and lowest reinforcing dose of cocaine. Therefore, further sex-related research is warranted to fully determine the effects of E2 on cognitive function and the stress system in cocaine using populations.

Because the rate of acquisition did not replicate the results of the previous investigation into female social rank and vulnerability to the reinforcing effects of cocaine (Nader et al., 2012), a thorough assessment of potential differences in the study design and subjects was necessary to parse out potentially important differences in experimental details. One of the greatest differences between the studies was the range of cocaine doses tested. The Nader et al. (2012) study examined self-administered at doses of 0.001-1.0 mg/kg. At the first dose available (0.001 mg/kg) 38% of the monkeys (50% of the dominants and 25% of the subordinates) SA that dose of cocaine (Nader et al., 2012) which makes it difficult to conclude that that was the lowest reinforcing dose of cocaine for these monkeys- no lower dose was assessed that was not reinforcing. In an effort to make sure the first dose tested in each monkey was low enough to be “saline like”, the lowest dose used in the current acquisition study was 0.0003 mg/kg/injection. We demonstrated that not only was 0.0003 mg/kg not reinforcing to any monkeys during
the initial exposure, the next highest dose of 0.001 mg/kg did not function as a reinforcer in the dominant monkeys either. This is in stark contrast to the Nader et al. (2012) study where this same dose (0.001 mg/kg) functioned as a reinforcer in 40% of the dominant monkeys and suggests that subtle differences in experimental design, such as order of exposure to each dose 0.0003 mg/kg prior to 0.001 mg/kg influenced the behavioral effects of the doses.

Although instrumental training with a natural reinforcer (i.e., food, sucrose pellets) prior to the onset of drug SA is common and acts to facilitate acquisition (Bongiovanni and See, 2008), the impact of repeated extinction of this training has not been specifically tested. Therefore, it is possible that by testing each dose of cocaine twice, by starting out at lower doses and by requiring a more stringent extinction criterion (less than 20% reinforcers earned compared to food maintained responding in the current study compared to ‘deemed extinguished’ which in some cases was above 15 injections in the older studies) the behavior of these monkeys was driven more by the reinforcing effects rather than additional external cues that may have influenced results in the previous study. If this theory is correct, it would suggest that the dominant monkeys in the previous study (Nader et al. 2012) may have SA the lower doses of cocaine for reasons besides the reinforcing effects of cocaine, such as context-induced seeking of food pellets, although without additional studies this conclusion remains speculative.

Another important variable to consider was that the monkeys in the current study were pair-housed prior to social housing whereas the monkeys in the Nader et al. (2012) study were individually housed. Although the present cohort of monkeys were not assigned to groups with monkeys that they were previously paired with, it is possible that the prior
experience of living with another monkey contributed to their response to being part of the new social group. Additionally, the animals in the current study were younger and bred domestically compared to the older, foreign born monkeys in the Nader et al., (2012) study. Understanding how these variables – individual vs. pair housing prior to housing in groups of 4, studying very low cocaine doses before higher doses, age at the start of the study, whether the monkey was feral or domestic –mediate the interactions between socially factors and vulnerability to cocaine abuse will be critical for future studies.

The present study demonstrates that the effect that social context has on cycling hormone concentrations can produce a susceptible phenotype to the reinforcing effects of cocaine. These findings compliment a plethora of data that cite evidence for gender differences in cocaine reinforcement in rodents (Lynch et al., 2006; Roberts et al 1989), monkeys (Broadbear et al., 1999; Mello et al., 2007) and humans (Fattore, 2008) and point to female sex hormones as the primary mediator for these observed differences. As mentioned previously, the difference in number of female monkeys who SA at least one dose made available in the present study compared to the males in the Morgan et al. (2002) study, indicates a higher vulnerability of females to the reinforcing effects of cocaine regardless of social rank and emphasizes the importance of cocaine abuse research utilizing both male and female subjects. These preclinical findings may reflect the human condition of increased responsiveness to drug-conditioned stimuli compared to males, which may contribute to women’s increased tendency for relapse (Elman et al., 2001; Robbins et al., 1999; Sterling et al., 2004). The downstream mechanisms that result from interactions between estrogen and the effects of drugs of abuse on the DA system
are just beginning to be explored. We propose that sex differences in addiction are due to neural systems which mediate positive and negative reinforcement that are differentially modulated by ovarian hormones.

CHAPTER V: EFFECTS OF PRIOR COCAINE SELF-ADMINISTRATION ON COGNITIVE PERFORMANCE IN FEMALE CYNOMOLGUS MONKEYS

One of the key challenges in human cocaine dependence research is determining a way to predict who is at risk of relapsing during treatment. Human studies have demonstrated that cognitive performance prior to entering treatment is one of these predictors. Although cognitive deficits in tasks measuring attention, behavioral flexibility and working memory associated with chronic cocaine use in humans are well documented (Hester and Garacan, 2004; Kubler et al., 2005; Tomasi et al., 2007; Woicik et al., 2009), these studies largely include only male subjects. Due to the pronounced sex differences at most stages of the addiction cycle, the need for assessment of cognitive function in female subjects during abstinence is glaring. Therefore, the next compilation of studies (Chapter V) were designed to compare behavioral flexibility and working memory between cynomolgus monkeys with previous experience SA cocaine that were 8 weeks abstinent and age-matched, cocaine-naïve monkeys. We hypothesized that following abstinence, monkeys with a chronic SA history would demonstrate impaired behavioral flexibility compared to age-matched, cocaine-naïve monkeys. However, impairments in working memory performance were not expected to be apparent during this same timeframe.
Significant impairments in reversal learning were evident in monkeys with a cocaine SA history compared to drug-naïve monkeys. Although direct measures of the DA system were not assessed in the current studies, previous human and NHP studies have shown impaired executive function on tasks that are mediated by areas known to be influenced by dopaminergic function (Beaulieu and Gainetdinov, 2011; Gould et al., 2012) and this impaired executive function is potentially to be driven by a hypodopaminergic state (Bolla et al., 1998; Volkow et al., 1996, 2004). In support of this, imaging studies revealing that cocaine SA is associated with decreased DA D2-like receptor availability (Volkow et al., 1993; Nader et al., 2006). Furthermore, a direct correlation between DA D2-like receptor availability in the striatum and reversal learning performance in monkeys was reported recently (Groman et al., 2011). Additional evidence comes from NHP studies demonstrating that cocaine exposure, both noncontingent and SA, impairs reversal learning and set-shifting in monkeys (Jentsch et al., 2002; Porter et al., 2011; Gould et al., 2012).

The role of the DA system in the observed cognitive deficits displayed by the cocaine-experienced monkeys is further highlighted by the differences between groups of perseverative responding. Monkeys with a SA history resorted to perseverative responding on the previously rewarded stimulus, whereas naïve monkeys were more likely to evenly allocate their responses between the no-longer rewarded stimulus and the previously non-reinforced stimulus. Perseverative responding is thought to be modulated by DA neurotransmission (Cools et al., 2007; Clarke et al., 2011). This inability to switch responding between the stimuli based on their changing relationship with reward is a prominent symptom in cocaine abuse, as well as obsessive-compulsive disorder and
schizophrenia (Jentsch et al., 2002; Everitt and Robbins et al., 2005; Ersche et al., 2008; Clarke et al., 2008). Importantly, a recent study has shown that DA agonist treatment can normalize this perseverative responding and may indicate novel avenues to pharmacologic treatment of stimulant abuse (Ersche et al., 2011).

As hypothesized, similar delay-dependent reductions in working memory were observed in cocaine-naive and cocaine-experience monkeys. This is consistent with a previous study in male rhesus macaques that assessed performance on a delayed match-to-sample task during ongoing cocaine SA (Gould et al., 2012). This study also found that following 30 days of abstinence performance improved in the cocaine-experienced animals but remained unchanged in the cocaine-naive males. Therefore, it is possible that cognitive deficits may have existed at some point during the 8 week period of abstinence in our study and the time point we selected was too late to detect such impairments. Therefore, future studies should address the within-subject cognitive profile of monkeys prior to, during and following cocaine SA in an effort to better understand the alterations associated with chronic cocaine use that may impede behavioral treatment strategies.

The data in Chapter V demonstrated that deficits exist in only one of the two cognitive domains assessed. We hypothesized that the lack of effect of drug history on working memory could be due to multiple factors. First, these domains are mediated largely by different brain areas. These areas may be either differentially affected by cocaine exposure and/or recover at different rates from this chronic cocaine intake. Our study design did not allow us to tease apart which of these mechanisms underlies these results because we did not take cognitive measures prior to abstinence. Alternatively, it could be presumed that there would not have been differences in working memory.
between groups at either the immediate or 8 week abstinent time points because a previous study by Gould et al. (2012) demonstrated no differences in working memory performance during active cocaine SA. Additionally, the amount of training required for the two tasks may have influenced their susceptibility to disruption. Differences in the performance on the reversal-learning task were observed solely during the first exposure. Once monkeys had performed the task multiple times, these group differences were no longer observable. Due to the fact that the dependent variables of the reversal-learning task (i.e. number of trials, errors and omissions to acquisition) can be collected in a short number of days, data can be collected the initial time a monkey is exposed to the task. However, the delayed match-to-sample task requires extensive training to meet the performance requirements at multiple delays and therefore the task performance may be more resilient to disruption. Overall, the results of the studies in Chapter V are encouraging to treatment-seeking cocaine abusers considering that treatment goals are first and foremost remaining abstinent but secondarily to integrate back into normal society through social and employment settings which require high levels of cognitive function.

The findings within this dissertation offer important insight into the hormonal influences on cognitive performance, social status and the reinforcing effects of cocaine in a female NHP model of social stress and cocaine abuse. These results argue for the inclusion of female subjects in models of human disease states. Although it has always been assumed that what is observed in males will apply to females, the results of these studies indicate that endogenous hormonal fluctuations may substantially influence females in numerous areas including cognitive performance and cocaine SA. Seeing as
sex hormones are known to influence symptoms in human diseases ranging from multiple sclerosis to epilepsy (Nicot, 2009; Veliskova and DeSantis, 2013), our findings are not surprising. Furthermore, sex differences in incidence, prevalence, symptoms, age at onset and severity have been widely documented in autoimmune diseases such as rheumatoid arthritis and lupus (Pennell et al., 2012; Schwartzman-Morris and Putterman, 2012), psychological disorders, including major depressive disorder, schizophrenia, autism and attention deficit hyperactivity disorder (Hasson and Fine, 2012; Goldstein et al., 2013; Schuch, 2014; Van Wijngaarden-Cremers et al., 2014). These documented sex differences in mental health might have a bearing on drug use and treatment.

Despite the obvious and documented sex differences to experimental outcomes, male research subjects continue to dominate biomedical studies. The studies in this dissertation exemplify the profound effect fluctuations of E2 and P4 across the menstrual cycle have on cognitive performance of a novel task. Furthermore, these experiments add important insight into the current literature on how environmental and social context influence self-administration and cognitive performance by extending the research to include female subjects and demonstrating that in females, both social rank and menstrual cycle phase can exacerbate susceptibility to the reinforcing effects of cocaine. Considering what is known about individual differences in the effects of environmental stimuli (Czoty and Nader, 2012) and potential pharmacotherapies (Czoty and Nader, 2013) on cocaine choice in socially housed male monkeys, the results of the current studies underscore the need for a similar investigation in female socially housed monkeys. Additionally, due to the significant effect of menstrual cycle phase on cocaine SA, the results of these studies add to emerging data suggesting that cessation
interventions for female substance users may result in different outcomes based on gonadal hormones at quit date (Mazure et al., 2011). Specifically, quit attempts may be more successful if attempted in the luteal phase of the menstrual cycle. Overall, the studies within this dissertation contribute vital information regarding a much needed female NHP model of cognitive function, social stress and cocaine abuse. Importantly, these studies form the neurobehavioral basis for further exploration into variables that make females more vulnerable to developing and sustaining addiction compared to males.
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271
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Mentors: Michael A. Nader, Ph.D and Paul W Czoty.

Ruth L. Kirschstein National Research Service Award (NIDA)

Social status and hormones on cognition and cocaine abuse in female macaques

The goal of this award is to receive training in self-administration, hormonal radioimmunoassay and cognitive assessment in socially housed non-human primate. Concurrently, this project is aimed at understanding the effects of social status on cognitive performance, endogenous hormonal fluctuations and psychostimulant sensitivity. Female cynomolgus macaques are used to explore a potential mechanism for the sex differences observed in cocaine self-administration.

Role: PI
COMPLETED RESEARCH SUPPORT

T32 DA007246-20 Kromrey 08/15/2011 – 06/30/2013

Mentor: Michael A. Nader, Ph.D and Paul W. Czoty, Ph. D.

NIDA Neuroscience of Drug Abuse Training Grant – predoctoral fellowship

The goal of this award is to receive graduate training in self-administration, and basic biochemical techniques. Concurrently, this project is aimed at documenting the cognitive effects of cocaine following acute and chronic exposure as well as abstinence from the drug.

Role: Graduate student