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Sex differences in the conditioned rewarding effects of cocaine: Role of hormonal mechanisms

By

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A dissertation submitted to the Graduate Faculty in Psychology in partial fulfillment of the requirements for the degree of Doctor of Philosophy, The City University of New York

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Abstract

Sex differences in the conditioned rewarding effects of cocaine:

Role of hormonal mechanisms

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Scott J. Russo

Advisor: Professor Vanya Quinones-Jenab

Sex dependent neurochemical changes after cocaine administration could affect psychomotor activation and may be critical in the drug's rewarding/reinforcing properties. The aim of this proposal is to determine whether there are sex differences in cocaine-induced conditioned place preference (CPP), and the extent to which gonadal and adrenal hormones modulate cocaine CPP in male and female rats. To address these questions, side-by-side comparisons were conducted to determine the effects of conditioning length, cocaine dose and adrenalectomy on cocaine CPP in male and female rats. Female rats demonstrated cocaine CPP after 4 pairing sessions, while male rats required 8 pairing sessions to develop CPP for cocaine. Also, female rats developed CPP at cocaine doses of 5 and 10 mg/kg while male rats required higher cocaine doses (20 mg/kg). Overall, females had higher blood serum levels of corticosterone. Furthermore, a dose-dependent effect on serum levels of corticosterone was observed only in female rats, where rats conditioned with 20mg/kg cocaine had significantly higher serum levels of corticosterone than rats conditioned with 5mg/kg cocaine. However, adrenalectomy did not affect CPP for cocaine in either sex. These results suggest that a female's higher

sensitivity to cocaine's rewarding effects is not completely mediated by the Hypothalamic-Pituitary-Adrenal (HPA) axis.

Therefore, sex differences in the acquisition and/or expression of cocaine CPP may be modulated by other mechanisms, such as the HPG axis. Although both intact and gonadectomized male and female rats showed a significant CPP for cocaine, ovariectomy attenuated the magnitude of preference. These alterations coincided with a decrease in serum levels of corticosterone. In ovariectomized rats, pretreatment with progesterone inhibited cocaine CPP while estrogen plus progesterone potentiated the magnitude of CPP. Additionally, gonadectomy and ovarian hormone replacement in female rats affected monoamine levels and turnover ratios in the ventral tegmental area and nucleus accumbens shell. While no effects of castration were observed ovariectomy decreased levels of dopamine and serotonin in the ventral tegmental area. In females, progesterone replacement increased levels of serotonin and dopamine in the ventral tegmental area while estrogen plus progesterone replacement increased dopamine levels in the nucleus accumbens. These results indicate that ovarian hormones may influence cocaine reward by altering monoaminergic systems.

Since progesterone has been shown to block cocaine's rewarding properties in ovariectomized female rats, it may also inhibit cocaine CPP in intact male and female rats. Unlike ovariectomized rats, chronic progesterone did not inhibit cocaine CPP in intact male or female rats. Alternatively, acute injections of progesterone 4 hours before the conditioning phase blocked cocaine CPP in females, which suggests that progesterone could be used as a potential therapy for drug abuse in females. Our results show that

gonadal hormones modulate the rewarding properties of cocaine in females through neurochemical alterations in the brain's reward circuit. Thus, sex differences in the hormonal regulation of cocaine reward may contribute to the current sex disparities in overall cocaine use and rates of relapse.

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I. Overview:

Cocaine is an active alkaloid found in the leaves of Erythroxylon coca, a tree indigenous to Peru and Bolivia. The drug has been used as a psychostimulant among the people of Colombia for 2,500-5,000 years [1]. In the United States however, its use remained relatively limited until the late 1800s [1]. Around this time cocaine began to see heightened popularity when a young physician by the name of Sigmund Freud advocated its use as a treatment for a multitude of personality disorders and even morphine addiction [2]. It wasn't long before reports of cocaine addiction threatened its newfound popularity and by 1894, the American Medical Association was beginning to question its use [1]. Finally, in 1914 the use of cocaine was banned by the Harrison Narcotic act [1].

Since the ban of cocaine as a potentially addictive substance, its use has fluctuated affecting all classes and races of people. Today, an estimated 20% of Americans have had the opportunity to use cocaine. Among those who eventually use cocaine, approximately 33% are woman. Although, males are more likely than females to have an initial opportunity to use drugs, there seems to be no differences in the progression to intense drug use following the initial use [3;4]. Thus, sex differences in the pattern of drug abuse may be circumstantial thereby providing males with greater opportunities to progress from initial to habitual use [3;4]. Alternatively, sex differences in the physiological response to cocaine may influence the desire to engage in drug taking

behaviors. Gonadal hormones have a profound effect on brain function and a female's hormonal state at the time of initial cocaine use may influence the subjective or behavioral response to the drug. Thus, increased vulnerability during certain stages of a female's menstrual cycle, could affect the progression to habitual use and/or drug-induced alterations after repetitive cocaine exposure.

Recent evidence has shown that there are sex differences in the behavioral and neurochemical response to cocaine in humans [5-7] and animals [8-10]. Although progress has been made in understanding the clinical basis of cocaine abuse, more extensive studies are needed at the neurochemical and behavioral levels, elucidating the mechanisms underlying sex differences in these responses. Sex differences in Hypothalamic-Pituitary-Gonadal (HPG) or Hypothalamic-Pituitary-Adrenal (HPA) axis activation may influence alterations in one or more of these components of the monoamine system. Thus, sex dependent neurochemical changes after cocaine administration could affect psychomotor activation and may be critical in the drug's rewarding/reinforcing properties. The aim of this proposal is to determine whether there are sex differences in cocaine-induced conditioned place preference (CPP), and, the extent to which gonadal and adrenal hormones modulate cocaine CPP in male and female rats.

II. General effects of cocaine in the CNS: Behavioral and cellular mechanisms

A. Cocaine's effects on the monoamine system:

Cocaine acts by binding directly to monoamine transporters thereby preventing monoamine re-uptake into the neuron and increasing the concentration of these neurotransmitters at the synapse [11;12]. Studies have shown that cocaine increases synaptic levels of dopamine, norepinephrine, and serotonin, in the ventral tegmental area (VTA), nucleus accumbens (NAc), and medial prefrontal cortex, areas important in the behavioral effects of cocaine [13;14]. The effects of acute cocaine on dopamine D1 receptor expression are inconsistent, with reports of increases, decreases and no change [15-22]. Alternatively, chronic cocaine administration up-regulates D1 receptor expression [16]. In post mortem tissue taken from cocaine overdose patients, serotonin transporters (SERT) in the striatum, substantia nigra, amygdala, and adjacent paralimbic cortical areas were up regulated [23]. Although the effects of cocaine on α1b-adrenergic receptors are not well established norepinephrine transporter (NET) is up regulated after chronic cocaine [24]. Although, most agree that dopamine has a profound effect on the behavioral response to cocaine, Uhl et al. [25] has suggested that dopamine, serotonin, and norepinephrine all contribute to these responses.

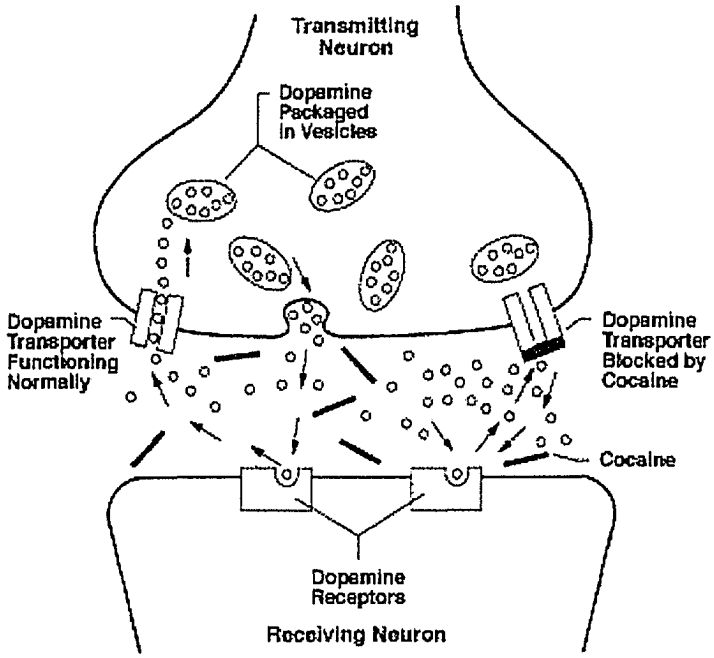

Figure 1: Model of how cocaine affects the monoamine system. Cocaine binds directly to monoamine transporters (including dopamine as well as serotonin and norepinephrine) and blocks the reuptake of these neurotransmitters at the synapse.

B. Cocaine's effects on reward, locomotor activity, and monoamines:

The mesocorticolimbic monoamine system, including the VTA and projections to and from the medial prefrontal cortex and NAc (Figure 2) has a primary role in mediating the rewarding and psychomotor stimulant properties of cocaine [12;26;27]. Early theories of drug abuse contend that cocaine's effects on dopamine neurons in this circuit are necessary for cocaine-induced reward and locomotor activity [28-30] [31]. It has been shown that dopamine lesions of the NAc block drug-induced conditioned place preference (CPP) [32]. Pharmacological manipulation of the dopamine system has produced conflicting results. Antagonists of the dopamine D2 receptor attenuate the locomotor response to cocaine, but have no effect on cocaine-induced CPP [33]. On the

other hand D1 antagonists are potent in blocking both cocaine-induced CPP [33;34] and locomotor behavior [35-37].

Manipulations of the serotonin system also result in profound differences in the behavioral response to cocaine [38-40]. McMahon & Cunningham [40] showed that antagonists of the serotonin (2a) receptor attenuated cocaine-induced locomotor behavior and cocaine discrimination. Likewise, inhibition of serotonin (2a) receptor decreases cocaine-seeking behavior [39]. Although the effects of norepinephrine on cocaine-induced behavioral responses are not as well established, α1b-adrenergic receptor knockout mice have a decreased locomotor response to cocaine, weaker sensitization responses, and lower cocaine intake [41].

Recently, the role of serotonin, dopamine and norepinephrine in regulating the rewarding properties of cocaine has received strong support with the characterization of SERT, DAT, and NET knockout mice. Unlike single mutations of DAT and SERT, where cocaine-induced CPP is preserved [42], a double SERT and DAT knockout completely abolishes cocaine-induced CPP [43]. Alternatively, a combined NET and SERT knockout enhances cocaine-induced CPP. Uhl et al. (2002) has suggested that a balance between cocaine's actions on these three transporter systems regulate cocaine's rewarding and aversive properties.

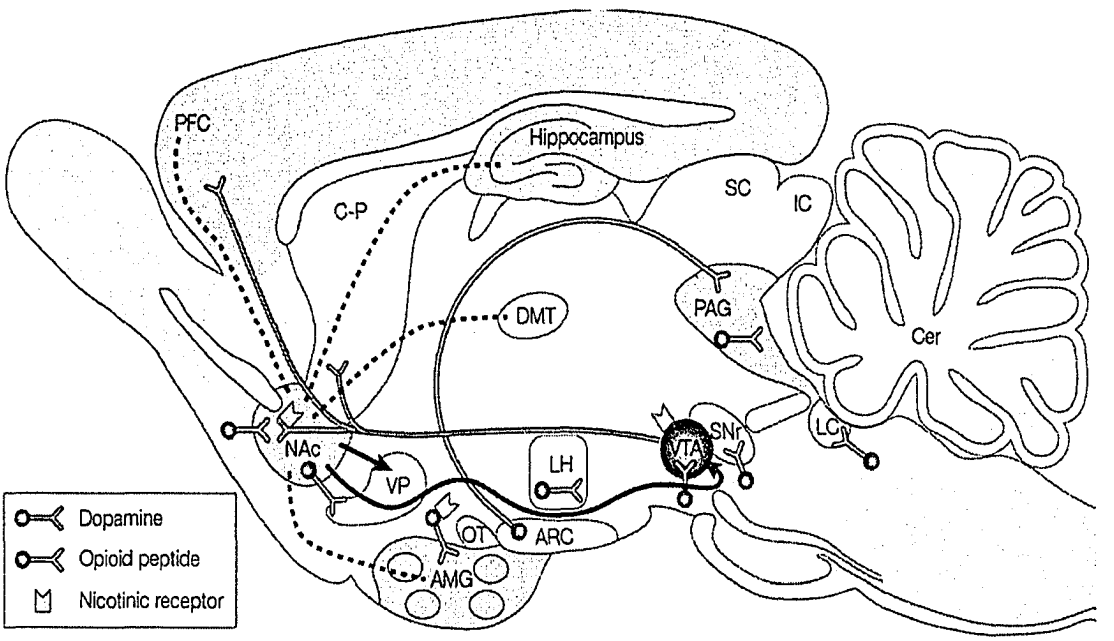

Figure 2: Neural circuitry regulating the addictive properties of drugs of abuse. Dopaminergic projections to and from the VTA, NAc, prefrontal cortex, and other limbic structures are thought to be an important substrate for the rewarding properties of cocaine [26]

III. Sex differences in the behavioral, and hormonal response to cocaine

A. Sex differences in the behavioral response to cocaine:

Increasing reports have shown that there are gender differences in the behavioral and subjective response to cocaine [5-7]. For example, Lukas et al. [5] reported that male subjects achieve a faster and higher peak of plasma cocaine levels with more episodes of euphoria or "good feeling" than woman. Alternatively, work by Mendelson's group reported no differences in the subjective response or plasma cocaine levels between the

sexes [44]. This may be due, in part, to the administration paradigm, since subjects in the study by Lukas et al. [5] self-administered a dose of intranasal cocaine while subjects in Mendelson's study were administered an injection of cocaine by the experimenter. Although there are no gender differences in drug taking behaviors such as the time spent using, total amount used, and money spent, a study has shown that men remain abstinent longer than woman [7]. Therefore, shorter abstinence periods in females may be related to an increased sensitivity to drug-conditioned stimuli and increased craving [6].

Recent advances using animal models of cocaine addiction have helped us to understand the biological basis of sex differences. In rodents, females' show enhanced stereotypic and locomotor behaviors following acute [45;46] and chronic [46] cocaine administration. However, sex differences in the response to drugs of abuse may only be present in certain aspects of drug-related changes in the animal. Craft and Stratmann [47] suggest that there are few sex differences in the discriminative stimulus effects of cocaine, but significant differences in the locomotor response. Lynch & Carroll [48] showed that female rats acquire both cocaine and heroin self-administration more quickly than do males, and on average, mean drug intake is slightly higher in females. Furthermore, reinstatement of self-administration following a cocaine priming injection is quicker in female than in male rats [48]. These findings are important in understanding sexual dimorphisms in the patterns of drug intake and rates of relapse.

B. Mechanisms regulating sex differences in response to cocaine

1. Sex differences in cocaine-induced HPG activity:

Direct modulation of the HPG axis by cocaine may influence the behavioral and/or neurochemical response in male and female rats. Cocaine has been shown to affect HPG activation at all levels of the feedback loop. In the hypothalamus, cocaine modulates gonadotropin-releasing hormone (GnRH) [49;50]. Likewise, the release of leutinizing hormone (LH) from the pituitary is increased in response to cocaine and is dependent upon the stage of the cycle in females [51;52]. Cocaine also affects LH secretion throughout the rat estrous cycle. During proestrus, levels of LH decrease after cocaine administration [53]. LH/FSH modulation by cocaine may be due in part to an indirect effect of cocaine on GnRH neurons in the hypothalamus [54].

At the level of the gonads, cocaine influences plasma progesterone in intact [55] and pregnant females [56;57]. During proestrus, cocaine dramatically increased plasma levels of progesterone greater than during any other stage of the cycle [58]. Interestingly, cocaine's modulation of plasma progesterone is transient and levels return to those seen in controls after three hours [59]. Since cocaine treatment in ovariectomized rats given progesterone, does not alter plasma levels of progesterone, increases in intact rats are probably due to an increase in the secretion rates of progesterone rather than an acceleration of its biotransformation product [55]. In males, progesterone is increased by cocaine [57;58], amphetamine [60] and marijuana [61]. During the follicular phase, but not in the midluteal phase of the rhesus monkey, cocaine increases plasma levels of estradiol [51]. In contrast, no changes in estradiol levels have been reported after acute cocaine administration in female rats [62]. This discrepancy in the neuroendocrine

response to cocaine in rats and monkeys needs further investigation. In male rats, following "binge" [58] or acute [58;63]cocaine administration, testosterone is significantly decreased. Interestingly, Berul and Haclerode [63] hypothesized that cocaine's effect on testosterone levels may act on both the synthesis and secretion, since LH is not increased above control levels.

2. Gonadal hormones modulate the behavioral response to cocaine:

Since gonadal hormones have profound effects on CNS plasticity, cocaine-induced alterations of the HPG axis (via LH/FSH, progesterone, estrogen or testosterone plasma levels) may influence neuronal activation, which in turn controls behavioral responses. Therefore, sex differences in the hormonal profile of male and female rats may play a key role in the cascade of events that occur following cocaine administration. Taken together, the regulation of the HPG axis by cocaine may underlie differences during the female cycle and play a pivotal role in the observed sex differences in the neurophysiological effects of cocaine.

Endogenous female hormone profiles during the different stages of the menstrual cycle result in significant behavioral and neurochemical differences. Women in the follicular phase show higher peak plasma cocaine levels than during the luteal phase [5]. In rats, the estrous cycle influences an animal's motivation to self-administer cocaine [64]. Furthermore, rats during estrus experienced greater activity and greater intensity of stereotyped and locomotor activity than during other stages of the cycle [65]. There are also significant differences in the behavioral response to other psychomotor stimulants during the stages of the estrous cycle. Amphetamine-induced stereotypy and locomotor

behavior is augmented during estrus [66-68]. In order to avoid complication by the hormonal fluctuations associated with the estrous cycle, gonadectomized rats provide a model system to investigate the influence of gonadal hormones on cocaine-stimulated behaviors.

Both castration and ovariectomy affect the behavioral response to cocaine [59;69;70]. Ovariectomy decreases cocaine-induced enhancements of locomotor but not stereotyped behaviors [59]. Following binge or single dose cocaine administration, estrogen and progesterone suppress cocaine-induced locomotor behavior, while estrogen and progesterone alone have no effect [71]. There is also a temporal interaction between estrogen and progesterone on locomotor behaviors. We have previously shown that cocaine-induced behavioral activation is dependent upon the time in which progesterone is administered after estrogen [72]. In male rats, testosterone lowers stereotyped activity such as head swaying, exploratory behavior and uncoordinated body movements [73], and the locomotor response to cocaine [74]. Overall, these studies suggest that gonadal hormones may play a pivotal role in the behavioral response to cocaine.

3. Gonadal hormones modulate mesocorticolimbic monoamines:

Current literature shows that gonadal hormones (estrogen, progesterone, and testosterone) regulate monoamines in various ways [75-78]. Therefore, sex and estrous-related differences, which have been reported in the dopamine system, may be related to differences in hormonal regulatory mechanisms [8;66;79]. Robinson, et al. [80] showed that electrical stimulation of the mesolimbic dopamine system elicited sex and estrous cycle dependent differences in rotational behavior. Furthermore, Becker & Cha [66]

reported that females have increased release of dopamine in the striatum after an acute injection of amphetamine compared to males. In females, monoaminergic activity is altered by cyclical changes in gonadal hormones throughout the various stages of the estrous cycle [79;81;82].

Ovariectomized rats show a profound attenuation of monoaminergic neurotransmission in various brain regions. Ovariectomy results in decrements of dopamine receptors in the striatum [83;84] and substantia nigra [84], but not in the globus pallidus or accumbens [84]. Ovarian hormone replacement enhances dopamine receptor expression in ovariectomized female rats. Bazzet & Becker [85] reported differential responses in dopamine receptor levels after estradiol replacement. Furthermore, chronic estradiol replacement increases dopamine uptake sites in the striatum [79]. Cyr et al. [86] showed that ovariectomy-induced decreases in serotonin (2A) receptor densities could be up regulated to control levels following replacement with estrogen. Progesterone has been shown to enhance the metabolism of both serotonin and dopamine [87]. Previous work in our laboratory shows that cocaine affects serotonin levels in the prefrontal cortex and is modulated by ovarian hormones [88]. Although the direct relationship between the HPG axis, monoamines and cocaine reward is not known, it's possible that gonadal hormones modulate CPP via monoaminergic mechanisms.

4. Sex Differences in the HPA response to cocaine:

It is well established that females release greater levels of adrenocorticotropic hormone (ACTH) and corticosterone in response to environmental stressors when compared to males [89;90]. Interestingly, gonadal hormones are a key factor in the

reported sex differences in HPA activity. Vamvakopoulos et al. [91] showed that the corticotropin releasing factor gene contains a promoter region, which binds the estrogen response element. Further evidence showed that increased HPA activity in response to stress in females could be abolished following ovariectomy. Ovariectomy also decreases cocaine-induced release of corticosterone in females, while castration has no effect [92]. As described in Figure 3 below, interactions between the HPA and HPG axes in male and female rats may influence the behavioral response to cocaine and play an important role in sex differences in these responses.

5. Adrenal hormones modulate behavioral responses to cocaine:

Environmental stressors that activate the HPA axis play a role in the onset of many psychological disorders including drug addiction. Cocaine causes activation of the HPA axis, which is characterized by an induction of both ACTH and corticosterone [93-95]. In turn, the HPA affects the acquisition and reinstatement phase of cocaine self-administration [96-98]. Furthermore, glucocorticoids have been shown to enhance behavioral sensitization to psychostimulants [99-101], while inhibition of corticosterone by metyrapone decreases cocaine-induced locomotor behavior and reinstatement of cocaine self-administration [102]. The implications of stress in the potentiality for drug use along with sex-related differences in HPA activation suggests that glucocorticoid function may underlie sex differences in the behavioral response to cocaine.

6. Adrenal hormones modulate mesocorticolimbic monoamines:

The role of adrenal hormones in regulating the monoamine system has also been shown. Adrenalectomy suppresses dopamine levels in the striatum [84;103] and the shell

of the NAc [103]. Moreover, adrenalectomy has been shown to decrease the induction of Fos proteins in the striatum following D1 agonist binding [104]. Both stress and glucocorticoid replacement induces dopamine release in the nucleus accumbens (NAc) and striatum [103]. In turn, microinjections of a dopamine agonist and cocaine in the NAc, striatum and medial prefrontal cortex increase plasma levels of corticosterone [105]. Taken together, these results suggest that sex differences in the regulation of monoamines by adrenal hormones may influence the behavioral response to psychostimulants.

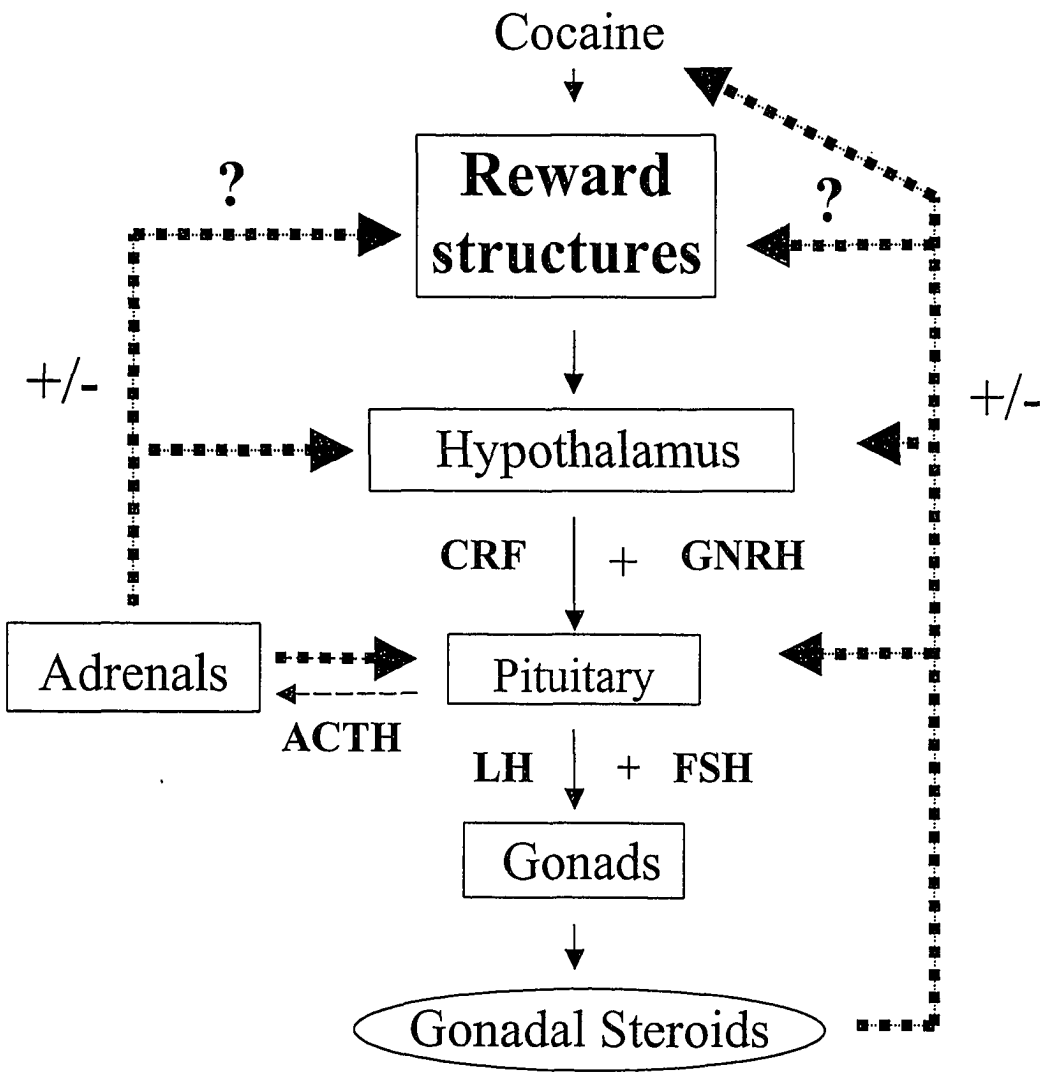

Figure 3: Hypothetical model of the interactions between cocaine and the HPA and HPG axis. Sex differences at all levels of this feedback loop may explain sex differences in the behavioral and neurochemical response to cocaine [modified from [10]].

V. Assessment of reward-related behaviors

A. Reward vs. reinforcement:

Associative learning, which measures reward, requires that a stimulus itself has an appetitive nature [106]. Therefore, repeated pairings of a conditioned stimulus (CS) with an unconditioned stimulus (US) will eventually induce a conditioned response (CR) in the absence of the US. Alternatively, using instrumental learning paradigms (a measure of reinforcement), an animal is trained to perform a specific motor response in order to achieve an outcome [107]. Requirements of operant responding are that the outcome itself is rewarding to the animal. Under these circumstances, the frequency of responding increases as the strength of the rewarding stimulus increases [106].

B. Natural and brain stimulation reward/reinforcement:

According to Wise [108] the brain's reward circuit evolved to process information about natural rewards such as sex and food. It is well established that sex, food, and exercise can produce goal-directed and reward-related behaviors in humans and animals [108-112]. Studies have shown that males will develop a conditioned place preference for an environment previously paired with a receptive female [113] and food [114]. Furthermore, animals will press a lever for access to a running wheel [115], receptive female [116] and food [109]. Brain stimulation reward (BSR) has also been used extensively to measure reinforcement in laboratory animals [117-120]. With this paradigm an animal can be trained to press a lever, which delivers a current through an electrode implanted in various CNS structures. Though first demonstrated by Olds & Milner in the septal area [121], animals will press a lever and self stimulate in the VTA [122;123], NAc [124;125], prefrontal cortex [126] and hypothalamus [122;124;125;127].

Interestingly, drugs of abuse produce reward through similar actions on these highly evolved CNS structures [108;128].

C. Models of drug reward/reinforcement:

1. Drug self-administration:

Models of drug self-administration indicate that an animal will work for an infusion of cocaine [129]. With this procedure an animal is surgically implanted with a catheter directly into the jugular vein [130]. An infusion pump is connected to the catheter, which is activated when the animal presses a lever located within the operant box. As stated by Bardo & Bevins [131] self-administration is a measure of reinforcement, in which the drug increases the probability of the operant response.

2. Drug-induced CPP:

CPP is a model used to assess drug reward by establishing contiguous associations between environmental stimuli and a drug's rewarding properties [131]. An animal is then tested for preference with environmental stimuli previously associated with drug administration. The benefit of using this paradigm is the ease, sensitivity, and linear correlation between dose and response [132;133]. This technique also enables one to determine positive and aversive properties of drugs, while allowing experimental control of drug associations [132;133].

3. How these models complement each other:

Although models of CPP need to be distinguished from self-administration, there is large degree of overlap between compounds shown to induce both behaviors. These

include cocaine [32], morphine [132;134], amphetamine [135;136], alcohol [137;138], nicotine [139], cannabinoids [140;141] and 3,4 methylenedioxy-methamphetamine (MDMA) [142]. Likewise, compounds such as dopamine, opioid and cholinergic receptor antagonists, as well as antidepressants are not self-administered nor do they induce CPP [131]. Despite these similarities, compounds such as lysergic acid diethylamine (LSD) and buspirone induce CPP but are not self-administered and pentobarbitol and phencyclidine are self-administered but do not induce CPP [131]. In addition to these findings, recent studies have demonstrated a more convincing distinction between CPP and self-administration. Bardo & Bevins [143] showed that individual differences in the magnitude of amphetamine CPP and rates of amphetamine self-administration are not correlated. Moreover, neuropharmacological mechanisms, which underlie CPP and self-administration, are different. Studies have shown that D2 dopamine receptor antagonists attenuate cocaine self-administration [144], but not cocaine CPP [32]. Although certain dissociations exist between both methods of drug assessment, data from these studies complement each other and add to our overall understanding of drug abuse.

VI. Sex differences in and hormonal regulation of reward-related behavior:

Sex differences have been reported in a variety of reward-related behaviors. It has been shown that females consume greater amounts of alcohol than males [145]. Middaugh & Kelley [146] extended these findings by showing that sex differences in ethanol consumption are related to lever press response demands and reward accessibility. Further studies revealed significant sex differences in the acquisition of cocaine and heroin self-administration, showing that females acquire cocaine self-

administration quicker and show higher rates of responding [147]. Taken together, females may be more sensitive than males to the rewarding properties of drugs of abuse, and this may be related to differences in the hormonal mechanisms that control reward.

As shown in Table 1, estrogen and progesterone produce widespread effects on many types of natural and drug reward-related behaviors. In cycling females, drug self-administration [64;148], sexual receptivity [149;150], wheel running [151] and BSR [117-119] peak between the night of proestrus and the morning of estrus. Although the effects of gonadectomy are not as well established, estrogen replacement has been shown to increase drug self-administration [152], sexual receptivity [153], food reinforced T-maze alternations [154], wheel running [155] and BSR [119;120]. Moreover, when estrogen and progesterone are co-administered, they synergize to produce more intense lordosis responses [156] and increase the frequency of BSR [119].

As shown in Table 1, there is a lack of understanding about the role of progesterone in reward-related behaviors. Although it has been shown that progesterone enhances estrogen-induced lordosis in females, there are few studies aimed at determining the role of progesterone alone. However, some studies in rats and humans have shown that progesterone may have inhibitory effects on reward [157;158]. In particular, progesterone decreases male lordosis behavior [157] and the subjective effects of cocaine in humans [158]. Furthermore, Roberts et al. [64] reported that females in diestrous (when progesterone levels are high and estrogen levels are low) self-administer cocaine with less frequency than during other stages of the estrous cycle while females during estrus (when both estrogen and progesterone levels are high) administer cocaine

with greater frequency. In other areas of research there are reports that progesterone inhibits the psychomotor response to cocaine [159] and performance on learning and memory tasks [160;161]. Taken together, it is possible that exogenous progesterone in male and female rats, and fluctuations of progesterone during the female cycle may have inhibitory effects on cocaine-induced reward.

Table I: This table includes a comprehensive list of gonadal hormone and estrous cycle effects on natural and drug reward and brain stimulation reward.

Hormonal state	Types of reward/reinforcers	Results	Reference
Gonadectomy	Drug reward/reinforcement	no effect on ethanol self-adminstration	*Roberts et al., 1998b*
	Brain stimulation reward	N/A	
	Natural rewards/reinforcers	↓ food reward	*van Hest et al., 1989; Mello et al., 1986*
Estrous cycle	Drug reward/reinforcement	↑ cocaine self-adminstration during estrous	*Roberts et al., 1989; Lynch & Caroll, 2000*
		no effect on nicotine self-administration	*Donney et al., 2000*
		↑ ethanol self-adminstration during estrous *	*Roberts et al., 1998a*
	Brain stimulation reward	↑ self stimulation on the evening between proestrus and estrus	*Prescott R.G.W., 1966; Steiner M., 1981; Steiner et al., 1982; Bless et al., 1997*
	Natural rewards/reinforcers	↑ wheel running on evening between proestrus and estrus	*Steiner et al., 1982*
		↑ lordosis on evening between proestrus and estrus	*Wang, 1923*
Estrogen	Drug reward/reinforcement	↑ the acquisition of cocaine self-administration in females	*Lynch et al., 2001*
		↑ alcohol self-adminstration in monkeys	*Mello et al., 1986*
		↑ the acquisition of heroin self-administration in females	*Roth et al., 2002*
	Brain stimulation reward	↑ self-stimulation of the medial forebrain bundle	*Meyerson et al., 1969;*
	Natural rewards/reinforcers	↑ lordosis in male and female rats	*Davidson et al., 1968;*
		↑ Sexual receptivity	*Cushing & Hite, 1996*
		↑ wheel running	*Fahrbach et al., 1985;*
		↑ food reinforced T-Maze alternations	*Fader et al., 1998*
Progesterone	Drug Reward	no effect on ethanol self-administration in females	*Janak et al., 1998*
	BSR	N/A	
	Natural rewards/reinforcers	▼ lordosis in male rats	*Chabli et al., 1989*
Estrogen+Progesterone	Drug Reward	N/A	
	BSR	↑ self-stimulation reward compared to estrogen alone	*Bless et al., 1997*
	Natural rewards/reinforcers	↑ lordosis in female rats compared to estrogen alone	*Boling & Blandau, 1939; Beach, 1942*

* only when animals' cycles are synchronized.

Some have suggested that gonadal hormones alter reward-related behaviors through modulation of dopaminergic/serotonergic neurotransmission in midbrain structures [162]. In male rats, peripheral and intra-accumbal injections of testosterone produce CPP that is blocked by dopamine receptor antagonists [163]. Although the role of estrogen and progesterone in cocaine CPP are not well known, it has been shown that these hormones have a dramatic effect on sexual receptivity and consummatory behaviors [164;165]. Moreover, hormonal regulation of dopaminergic/serotonergic mechanisms in the midbrain is considered to be an essential component of sexual behavior [150;166;167]. Although the effects of gonadal hormones on cocaine reward are not fully understood, we postulate that similar to other reward-related behaviors, these hormones may play a key role in cocaine CPP. Furthermore, sex differences in cocaine reward may result from distinct hormonal mechanisms in male and female rats, which modulate monoamines within the brain's reward circuit.

VII. Significance and aims of this work:

Sex differences in the rewarding/reinforcing properties of cocaine may play a crucial role in the behavioral response to cocaine. As described above, the HPG and/or HPA axis may influence cocaine-induced CPP by acting upon monoamines within the brain's reward circuit. We postulate that interactions between the HPG and HPA axes might affect reward mechanisms by regulating mesocorticolimbic monoamine systems (Figure 2). Therefore, hormonal profile during conditioning may predict the reward potential of cocaine. This has serious implications in the potentiality of drug abuse in both sexes, and may contribute to sex differences in the rewarding effects of cocaine.

To test these postulates the following specific aims are proposed:

Specific Aim One: We will determine if there are sex differences in cocaine-CPP and the extent to which endogenous adrenal hormones modulate cocaine CPP in either sex.

Specific Aim Two: We will determine if endogenous gonadal hormones modulate cocaine CPP in male and female rats, as well as the role of ovarian hormones in the regulation of cocaine CPP in ovariectomized females. Finally, neurochemical measurements will be taken to determine if changes in dopamine and serotonin in the VTA and NAc underlie the hormonal effects on cocaine CPP.

Specific Aim Three: We will determine if progesterone modulates the acquisition and/or expression phase of cocaine CPP in intact male or female rats.

Chapter 2: Sex differences in the conditioned rewarding effects of cocaine.

I. Introduction

Previous studies suggest that there are sex differences in cocaine-induced behavioral activation. For example, female rats have markedly enhanced stereotypic and locomotor responses [92;168], show higher rates of self-administration [148], and have an exaggerated corticosterone response to acute and chronic cocaine [46]. While it is not clear what specific mechanisms underlie these sex differences, it has been hypothesized that the HPG and HPA axes may, in part, modulate the behavioral and endocrinological responses to cocaine [10]. Specifically, in female rats, ovariectomy attenuates locomotor activity and HPA responses to cocaine [169]. Likewise, after estrogen and progesterone replacement, cocaine-induced locomotor activity and self-administration are affected (i.e., estrogen enhances rates of self-administration while estrogen + progesterone suppresses ambulatory behaviors) [71;152].

Conditioned place preference has been used previously to determine the rewarding effects of drugs in experimental rats by establishing contiguous associations between drug administration and environmental stimuli [131;133]. It has been shown show that women are more sensitive to drug-conditioned stimuli and report a greater desire to use cocaine [6]. However, it is not known if the previously reported sex differences in overall cocaine use and reported subjective effects are based upon sex differences in the ability to develop these contiguous associations. Our studies used a place preference conditioning procedure in male and female rats to determine if there are sex differences in the acquisition and/or expression of place preference for cocaine.

Additional studies were conducted using adrenalectomized rats to determine whether adrenal hormones contribute to the acquisition and/or expression of cocaine-induced CPP and account for sex differences in these responses.

II. Methods

Animals:

Eight-week-old intact, adrenalectomized (ADX) and SHAM female and male Fischer rats (Charles River, Kingston, NY) were single-housed in a standard cage with free access to food and water and maintained on a 12-hour light/dark cycle (lights on at 9:00 A.M.). ADX animals were maintained on water supplemented with 0.9% sodium chloride. One week after arrival, animals were randomly assigned to either saline- or cocaine-treatment groups and conditioned as described below (section 2.3). After testing, rats were decapitated following a brief (20 seconds) exposure to CO_2. Trunk blood was collected, centrifuged at 3,000 rpm for 15 minutes at 4°C, and frozen at -80°C until used. Animal care was in accordance with the *Guide for the Care and Use of Laboratory Animals* (NIH publication 85-23, Bethesda, MD) and approved by the Institutional Animal Care and Use Committee at Hunter College. All chemicals were purchased from Sigma Chemical Co. (St. Louis, MI).

CPP Apparatus:

Place preference cages, purchased from Med Associates (Georgia, VT), consisted of a rectangular box with three distinct chambers. The center neutral gray chamber was 12 cm long, with a smooth PVC floor. The two conditioning chambers were 28 cm long

and had different visual and tactile cues [one was black with a stainless steel grid rod floor and the other was white with a stainless steel mesh floor]. The chambers were separated by computer-automated guillotine doors to allow access to all three chambers.

CPP Procedure:

The conditioning procedure was conducted as described previously [170-175]. For pre-conditioning, rats were placed into the neutral gray area (for a 5-minute acclimation period) and then allowed free access to all three chambers for 15 minutes. Rats were then randomly assigned to either cocaine/saline conditioning groups or saline/saline control groups. The second phase consisted of 4 (2 cocaine/saline pairings) or 8 (4 cocaine/saline pairings) days of conditioning. Thirty minutes after lights were on, rats (n = 5-10 animals/group) received i.p. injections of saline (0.9%) or cocaine (doses ranging from 5 to 20 mg/kg dissolved in 0.9% saline) in the conditioning chambers. On conditioning days 1 and 3 (for 4-day paradigm) or 1,3, 5, and 7 (for 8-day paradigm), rats were injected with cocaine and immediately confined to one chamber for 30 minutes. On alternate days, rats were injected with saline and immediately confined to the opposite chamber for 30 minutes. Control rats received saline in both chambers on alternate days. On the testing day, rats were placed into the neutral gray chamber (for a 5-minute acclimation period) and then allowed 15 minutes with free access to all three chambers in a drug-free state. Time-spent, total locomotor activity (sum of all horizontal counts), and number of entrances (multiple beams broken as animal enters the chamber) and explorations (single beam broken in adjacent chamber without entry into the chamber) were recorded using a computerized photo-beam system run with MED-PC software.

Corticosterone Radioimmunoassay:

Serum levels of corticosterone were analyzed using Coat-A-Count Radioimmunoassay kits (Diagnostic Product Corporation, CA). Intra-assay coefficient of variance averaged less than 10%. Results were determined using a log-logit analysis within Graph Pad Prism (Graph Pad Software, Inc., CA).

Data Analysis:

Dependent measure t-test analyses within each group were used to determine statistically significant differences between time spent, entrances, and explorations in each chamber. Two-way ANOVAs were used to analyze CPP scores (time spent in cocaine-paired chamber minus time spent in saline-paired chamber), locomotor behavior, and serum levels of corticosterone [Drug x Length of Conditioning (4 vs. 8 Days), Drug x Group (SHAM vs. ADX), and Drug Dose (0, 5, 10, or 20mg/kg) x Sex (Male vs. Female)].

III. Results

Throughout the study, control rats receiving saline treatment in both conditioning chambers did not exhibit significant differences in the time spent in each chamber, confirming the unbiased nature of the procedure. Average time spent in each chamber was 295.6 ± 11.7 sec. and 256.7 ± 11.0 sec., respectively. Moreover, no differences in the number of explorations or entrances were observed in control rats. The average number of entrances and explorations in each chamber were 58.3 ± 3.4 and 56.1 ± 3.7 and 78.9 ±4.3 and 74.0 ± 5.3, respectively.

Sex differences in the acquisition of cocaine CPP and corticosterone:

Figure 4 shows the effects of the length of conditioning on cocaine-induced CPP in male and female rats. Female rats developed a significant CPP for cocaine after 4 ($t(4) = 5.78$, $p < 0.05$) and 8 ($t(4) = 3.96$, $p < 0.05$) conditioning sessions. However, male rats developed a significant CPP for the cocaine-paired chamber only following 8 conditioning sessions ($t(4) = 3.81$, $p < 0.05$). There were no significant differences in the magnitude of CPP scores between any of the groups ($p > 0.05$).

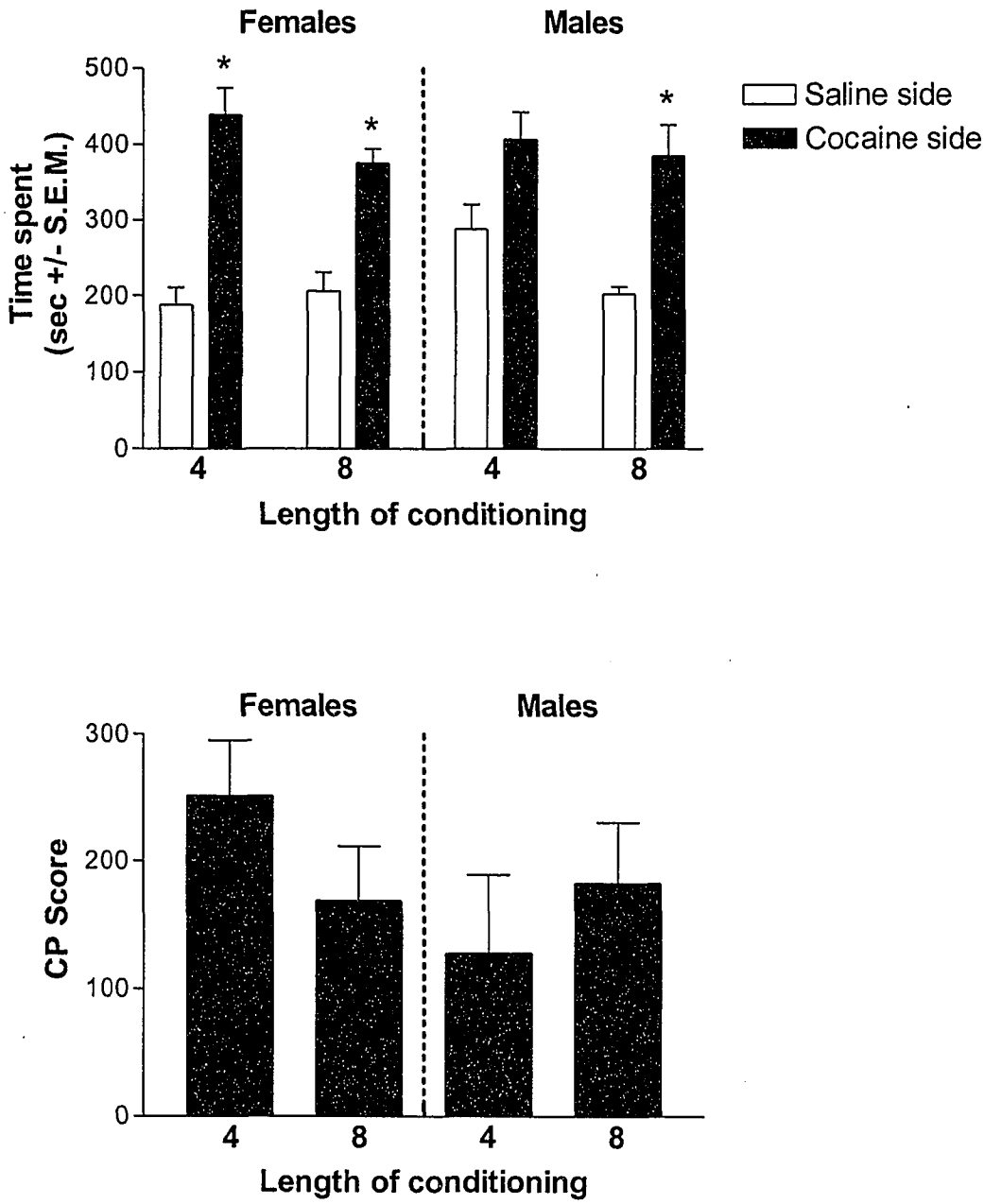

Figure 4: Sex differences in conditioned place preference for cocaine (10mg/kg) following 4 vs. 8 days of conditioning in female and male rats. Time spent in the saline-paired (white bars) and cocaine-paired (black bars) chambers **(A)** and CPP scores (time in cocaine side minus time in saline side) **(B)** on the test day, represented as mean (± SEM). * Indicates statistically significant differences at $p < 0.05$ ($n = 5$-10). [Abbreviations: SAL=Saline; COC=Cocaine]

After 4 conditioning sessions, female rats entered and explored the cocaine-paired side more often than the saline-paired side [$t(4) = 2.77$, $p < 0.05$, and $t(4) = 2.77$, $p < 0.05$, respectively; see Table II]. However, no significant differences were observed in the number of entrances and explorations in female rats after 8 conditioning sessions or in males after 4 or 8 conditioning sessions ($p > 0.05$). As shown in Table II, females had overall higher serum levels of corticosterone than those of male rats ($F(1,32) = 64.95$, $p < 0.01$).

Table II. Serum levels of corticosterone after 4 or 8 days of cocaine conditioning, different doses of cocaine, and adrenalectomy in male and female rats.

EXPERIMENT	Group		Drug	Corticosterone
LENGTH OF CONDITIONING	Females	4 days	Saline	150.8±64.1
			Cocaine	227.0±68.5
		8 days	Saline	383.6±60.3
			Cocaine	242.4±94.2
	Males	4 days	Saline	91.5±28.5
			Cocaine	53.9±16.7
		8 days	Saline	53.9±16.7
			Cocaine	115.7±11.1
DOSE RESPONSE	Females	4 days	Saline	281.2±44.0
			Cocaine (5)	229.6±17.7
			Cocaine (10)	350.0±52.9
			Cocaine (20)	***367.0±29.2**
	Males	4 days	Saline	154.7±37.8
			Cocaine (5)	106.7±28.8
			Cocaine (10)	159.6±27.1
			Cocaine (20)	181.4±27.9
ADX vs SHAM	Females	SHAM	Saline	243.9±25.1
			Cocaine	244.4±23.6
		ADX	Saline	7.5±7.1
			Cocaine	13.9±5.4
	Males	SHAM	Saline	70.9±9.3
			Cocaine	84.7±14.5
		ADX	Saline	3.1±2.9
			Cocaine	3.9±3.6

Serum levels of corticosterone are expressed as mean ng/ml (± SEM). * In bold indicates statistically significant differences between doses at $p < 0.05$. [ADX=Adrenalectomy; SHAM=SHAM surgery].

Sex differences in the effects of cocaine dose on CPP and corticosterone:

To determine if sex differences in the development of CPP were based on the cocaine dose used, we examined the effects of various doses on place preference in both sexes (Figure 5). In male and female rats, there was a dose-dependent effect of cocaine on place preference. Females showed CPP at doses of 5 and 10mg/kg cocaine ($t(4) = 5.45$, $p < 0.05$, and $t(4) = 4.92$, $p < 0.05$, respectively), but not at 20mg/kg ($p > 0.05$). On the other hand, males showed a significant CPP for the cocaine-paired chamber at a dose of 20mg/kg ($t(5) = 9.22$, $p < 0.05$), but not at 5 or 10mg/kg ($p > 0.05$). Furthermore, there was a significant interaction between sex and cocaine dose on CPP scores [$F(3,61) = 16.09$, $p < 0.05$], where males conditioned with 20mg/kg had higher CPP scores than all other groups ($p < 0.05$).

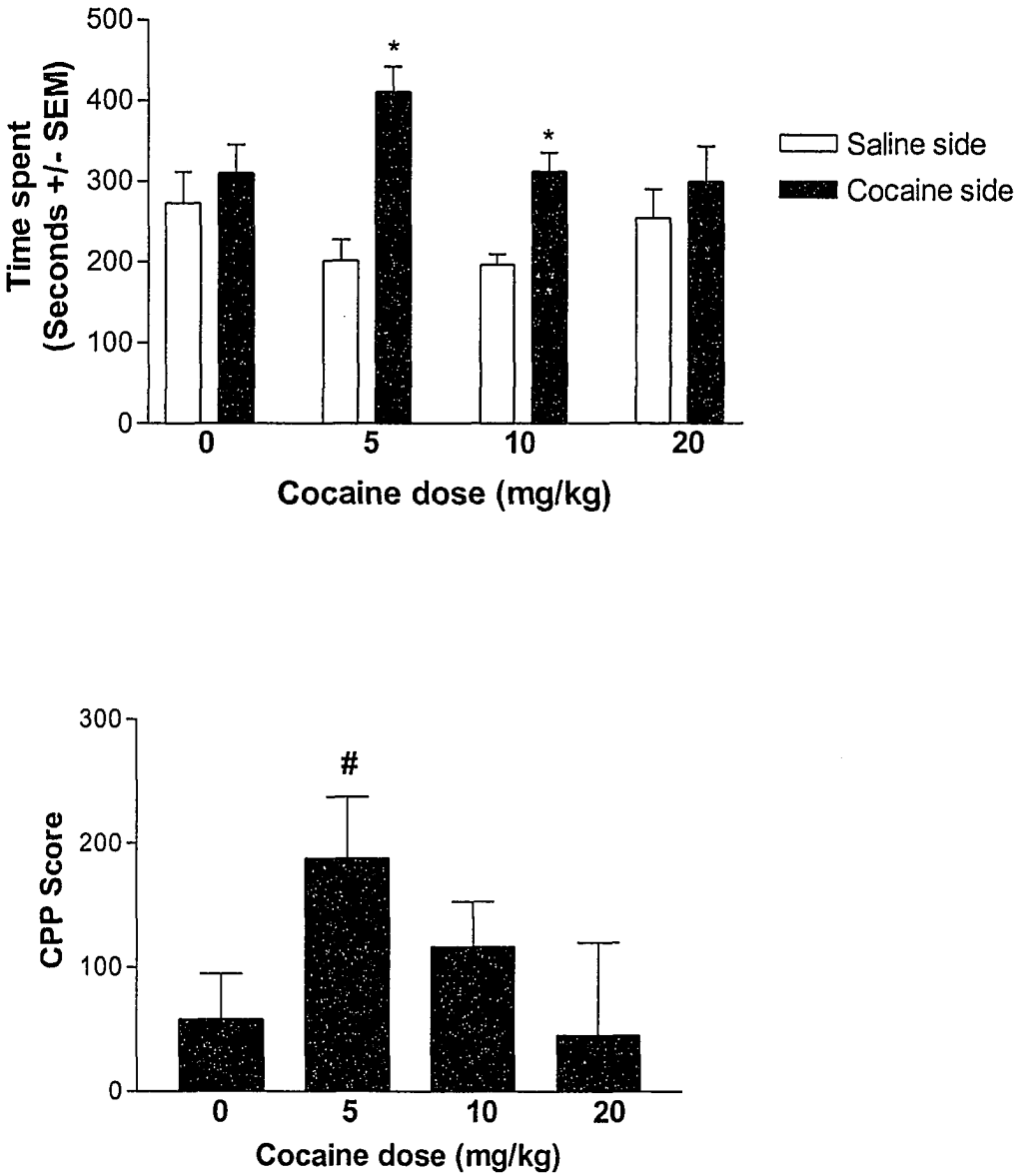

Figure 5. Dose response of cocaine-induced CPP using a 4-day conditioning paradigm in female rats. Time spent in the saline-paired (white bars) and cocaine-paired (black bars) chambers **(A)** and CPP Scores **(B)** on the test day, represented as mean (± SEM). * Indicates statistically significant differences at $p < 0.05$ ($n = 5$-10).

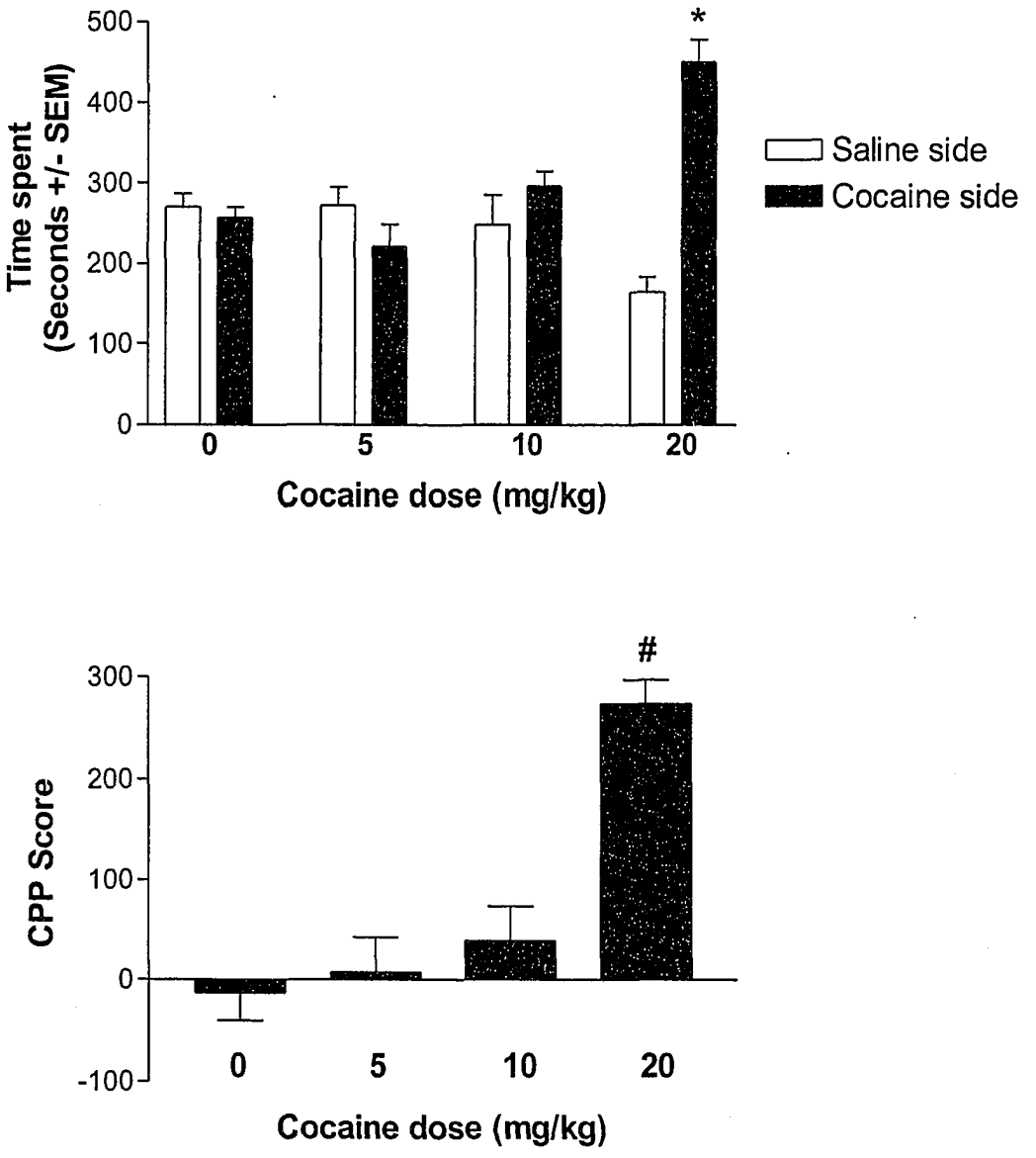

Figure 6. Dose response of cocaine-induced CPP using a 4-day conditioning paradigm in male rats. Time spent in the saline-paired (white bars) and cocaine-paired (black bars) chambers **(A)** and CPP Scores **(B)** on the test day, represented as mean (± SEM). * Indicates statistically significant differences at $p < 0.05$ ($n = 5$-10).

As shown in Table III below, there was a dose-dependent effect of cocaine on entrances and explorations in both male and female rats. Female rats conditioned with 5mg/kg cocaine entered the cocaine-paired chamber more often than the saline-paired chamber ($t(4) = 2.57$, $p < 0.05$) though no significant differences in the number of explorations were observed at any dose ($p > 0.05$). On the other hand, after conditioning with 20 mg/kg (but not 5 and 10 mg/kg cocaine), male rats entered and explored the cocaine-paired chamber more often than the saline-paired chamber [$t(5) = 2.57$, $p < 0.05$, and $t(5) = 2.57$, $p < 0.05$, respectively]. Although the total locomotor counts of females were higher than those of males [$F(1,37) = 15.37$, $p < 0.05$], cocaine conditioning (at all doses) did not significantly alter locomotor behavior in either sex ($p > 0.05$). Furthermore, there was a significant effect of cocaine dose on serum levels of corticosterone ($F(3,32) = 3.98$, $p < 0.02$; Table II). Females conditioned with 20mg/kg cocaine had higher levels of serum corticosterone than did females conditioned with 5mg/kg cocaine ($p < 0.05$). On the other hand, none of these cocaine doses significantly altered serum levels of corticosterone in male rats ($p > 0.05$).

Role of adrenal hormones in cocaine CPP:

To determine if endogenous adrenal hormones affect place preference for cocaine, SHAM and ADX male and female rats were conditioned with 20mg/kg and 5mg/kg cocaine, respectively (doses which produce similar CPP scores in males and females). As shown in Figure 7, all experimental groups (SHAM and ADX male and female rats) developed a significant CPP for cocaine [SHAM males: $t(9) = 5.44$, $p < 0.05$; ADX males: $t(5) = 2.62$, $p < 0.05$; SHAM females: $t(9) = 2.33$, $p < 0.05$; ADX females $t(8) = 3.87$, $p < 0.05$], but no significant differences were observed in the magnitude of CPP scores ($p > 0.05$). As shown in table I, adrenalectomy did not significantly alter the number of entrances or explorations in either chamber, nor did it alter locomotor behavior in males or females ($p > 0.05$). As seen in Table II, serum levels of corticosterone in ADX male and female rats were drastically reduced compared to SHAM animals.

Table III. The effects of cocaine conditioning on other CPP-related behaviors.

				ENTRANCES		EXPLORATIONS		LOCOMOTOR
EXPERIMENT	GROUP		DRUG	UNPAIRED	PAIRED	UNPAIRED	PAIRED	COUNTS
LENGTH OF CONDITIONING	Female	4 days	Saline	55.2 ± 4.2	56.2 ± 6.0	86.5 ± 6.0	87.5 ± 11.0	1598.5 ± 388.2
		4 days	Cocaine	52.4 ± 9.0	***77.2 ± 11.4**	72.8 ± 13.8	***103.4 ± 11.2**	2111.8 ± 454.2
		8 days	Saline	56.7 ± 8.3	59.2 ± 8.5	80.8 ± 12.7	98.0 ± 22.2	1762.7 ± 270.5
		8 days	Cocaine	49.2 ± 8.1	65.2 ± 7.3	90.7 ± 11.5	90.0 ± 8.0	1844.0 ± 313.6
	Male	4 days	Saline	65.5 ± 6.9	77.8 ± 6.7	70.5 ± 9.1	80.8 ± 9.3	1588.2 ± 199.4
		4 days	Cocaine	52.0 ± 3.1	62.7 ± 8.0	68.2 ± 10.3	70.5 ± 5.6	1702.2 ± 209.6
		8 days	Saline	64.6 ± 4.8	55.8 ± 4.7	87.8 ± 11.8	74.2 ± 6.1	1561.8 ± 162.9
		8 days	Cocaine	66.5 ± 4.7	77.0 ± 10.9	90.3 ± 8.6	94.8 ± 21.8	1834.8 ± 313.3
DOSE RESPONSE	Female	INT	Saline	62.8 ± 6.8	65.0 ± 7.8	91.0 ± 9.4	81.7 ± 13.4	1640.8 ± 343.0
			5mg Cocaine	57.3 ± 7.2	***73.8 ± 5.5**	89.8 ± 12.7	102.7 ± 18.3	1622.8 ± 248.8
			10mg Cocaine	53.0 ± 6.6	55.3 ± 9.5	96.2 ± 13.7	97.5 ± 14.9	1644.8 ± 128.7
			20mg Cocaine	63.3 ± 9.0	57.5 ± 8.5	97.5 ± 14.7	89.0 ± 15.8	1523.8 ± 320.5
	Male	INT	Saline	77.0 ± 8.8	57.2 ± 6.3	94.3 ± 8.0	79.8 ± 8.7	1305.8 ± 153.7
			5mg Cocaine	56.6 ± 7.2	54.6 ± 7.2	50.6 ± 12.8	58.6 ± 11.5	1249.4 ± 231.1
			10mg Cocaine	63.8 ± 6.2	59.3 ± 8.3	73.5 ± 7.6	80.0 ± 4.7	1372.4 ± 72.2
			20mg Cocaine	46.3 ± 4.0	***75.2 ± 6.5**	57.0 ± 5.6	***98.5 ± 9.7**	1299.8 ± 194.2
ADX vs SHAM	Female	SHAM	Saline	53.7 ± 8.8	57.6 ± 5.4	79.3 ± 14.8	77.1 ± 10.0	1053.3 ± 188.3
			Cocaine	45.2 ± 4.5	49.2 ± 5.9	77.4 ± 8.7	82.4 ± 12.4	1160.6 ± 84.3
		ADX	Saline	40.5 ± 4.8	37.1 ± 5.8	54.8 ± 5.2	45.7 ± 8.1	982.4 ± 68.7
			Cocaine	44.9 ± 4.8	48.1 ± 6.9	57.3 ± 9.9	61.3 ± 45.0	1199.4 ± 85.0
	Male	SHAM	Saline	62.1 ± 3.6	55.7 ± 6.8	76.0 ± 7.0	78.6 ± 9.3	1004.1 ± 81.0
			Cocaine	53.8 ± 4.0	67.7 ± 7.4	71.7 ± 3.7	76.4 ± 10.0	1157.2 ± 66.4
		ADX	Saline	44.5 ± 7.7	39.0 ± 5.7	57.5 ± 6.1	45.7 ± 6.0	841.9 ± 111.3
			Cocaine	35.5 ± 6.0	43.0 ± 6.5	47.2 ± 6.4	62.8 ± 9.4	759.5 ± 102.8

Data is represented as mean (± SEM) number of entrances and explorations in the saline-paired and cocaine-paired chambers on the test day. Locomotor activity is represented as the mean (± SEM) sum of all locomotor counts in all chambers. * In bold indicates statistically significant differences from saline-paired chamber at $p < 0.05$. [ADX=Adrenalectomy; SHAM=SHAM surgery].

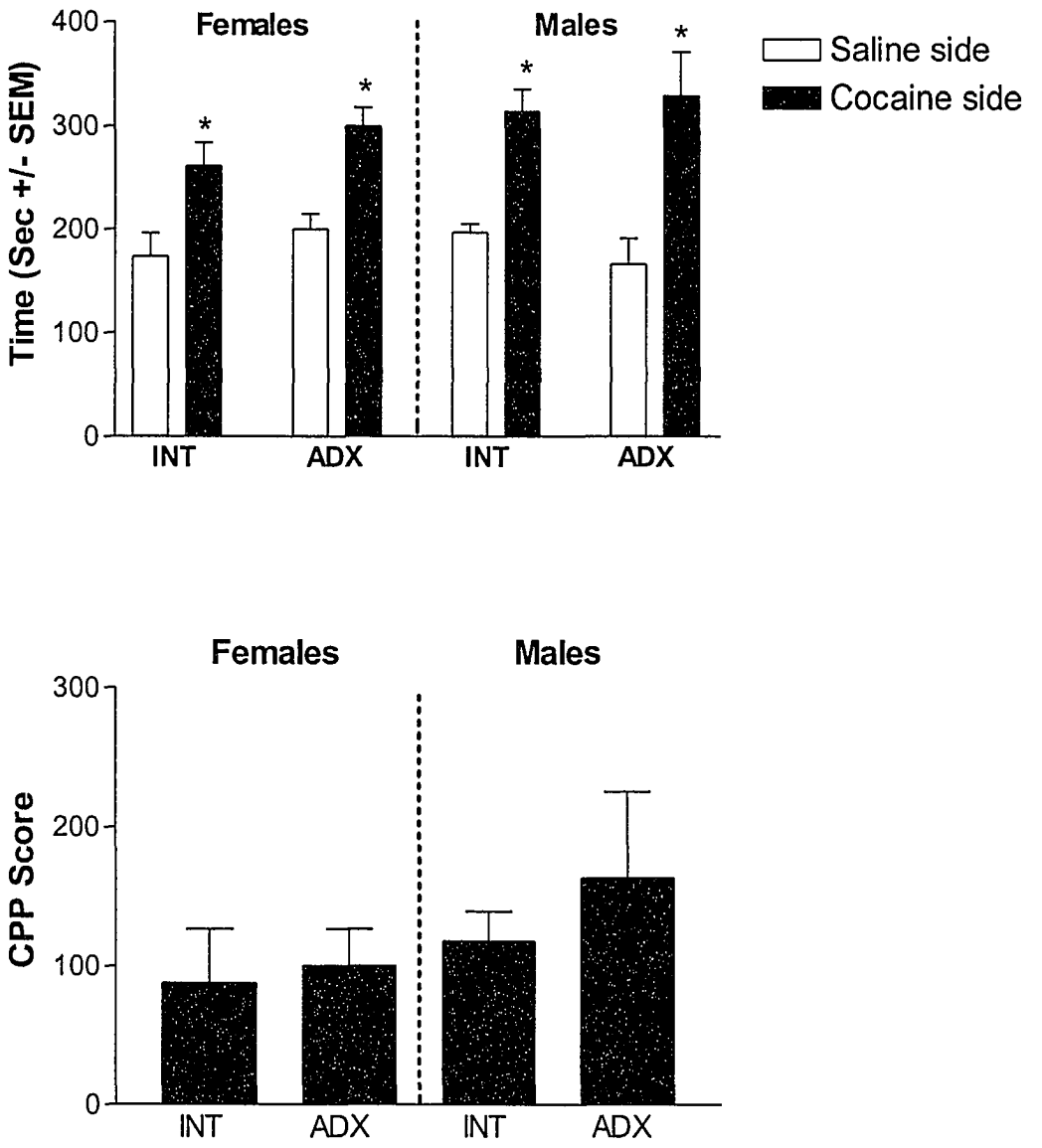

Figure 7. The effects of adrenalectomy on cocaine-induced CPP in female and male rats. Time spent in the saline-paired (white bars) and cocaine-paired (black bars) chambers **(A)** and CPP scores **(B)** on the test day, represented as mean (± SEM). * Indicates statistically significant differences at $p < 0.05$ ($n = 5$-10). [Abbreviations: SAL=Saline; COC=Cocaine; ADX=Adrenalectomy; SHAM=SHAM surgery].

IV. Discussion

Our results show that female rats acquire CPP after fewer pairing sessions and at lower doses of cocaine than those of male rats. This suggests that female rats are more responsive than males to contiguous associations between drug administration and environmental stimuli, which implies that females are more sensitive to the rewarding properties of cocaine. It has been previously shown that female rats acquire both cocaine and heroin self-administration more quickly than do males, and on average, mean drug intake is slightly higher in females [147]. Furthermore, reinstatement of self-administration following a cocaine priming injection is quicker in female than in male rats [48]. Our findings extend on those of Lynch and Carroll [147] by demonstrating that like previously reported sex differences in the acquisition of intravenously self-administered cocaine, there are also sex differences in reward and/or the ability to develop contiguous associations between environmental stimuli and cocaine's rewarding properties. In both humans and animals, there are drastic differences in the response to conditioned stimuli and patterns of cocaine intake [5;92;168]. It is possible that a female's increased sensitivity to drug-conditioned stimuli affects not only behavioral and subjective responses to cocaine but also relapse after exposure to environmental cues. These important clinical issues need further investigation.

There are various mechanisms by which these behavioral differences might occur. First, it has been postulated that HPA axis activation plays a role in the psychomotor response to cocaine as well as cocaine reinforcement [176]. It has been suggested that sex differences in HPA axis activation may influence cocaine-induced behavioral responses [176-178]. Consistent with a previous report, we observed that female rats had

higher serum levels of corticosterone than did male rats [46]. Furthermore, in a drug-free state, females showed an enhanced corticosterone response to cocaine-associated environmental cues when conditioned with higher doses of cocaine. However, as previously shown in males [179], the removal of the adrenals did not affect the overall expression of cocaine CPP in either sex. Marinelli & Piazza [180], have suggested that corticosterone regulates other aspects of the behavioral response to cocaine but has little or no effect on the acquisition and/or expression of cocaine CPP. Consistent with these theories, our observations suggest that adrenal hormones are playing a minor role, if any, in the observed sex differences in the acquisition and/or expression of cocaine CPP. Alternatively, since the HPG axis has been previously shown to play a role in cocaine-induced behavioral activation [71;152;159;181], gonadal hormones may mediate the observed sex differences in cocaine CPP. This hypothesis needs further investigation.

It has been suggested that learning and memory serve as a basis for the development of CPP [182-185]. Interestingly, there are a number of reports demonstrating intrinsic sex differences in the neuronal circuitry that controls learning and memory [186-188]. Nestler [26] has suggested that cocaine addiction and behavioral sensitization are to a certain extent controlled by these complex processes. The observed sex differences in cocaine-induced CPP may be based in part on intrinsic sex differences in the ability to associate and recall rewarding events. The extent to which sex differences in the ability to store and remember rewarding and subjective events are based on cognitive differences between the sexes remains to be elucidated. However, there may be interactions between the HPG and CNS mechanisms controlling learning and memory that in turn may have an impact upon the rewarding effects of cocaine.

Chapter 3: Gonadal hormones modulate conditioned place preference for cocaine.

I. Introduction

Estimates over the past 10 years by the National Household Survey on Drug Abuse indicate that women make up approximately 30% of the cocaine-using population. Recent studies in humans and animals have demonstrated that there are sex differences in the behavioral, hormonal, and subjective responses to cocaine [5;6;46;189]. For example, female rats more readily self-administer cocaine and have both exaggerated locomotor responses to cocaine and increased corticosterone release after acute and chronic cocaine administration when compared to male rats [46;148;189]. Female rats also sensitize to cocaine's behavioral effects faster than do male rats and maintain this sensitization for a longer period of time after cocaine withdrawal [92].

It has been postulated that differences in the subjective and behavioral effects of drugs may be an important factor in sex differences in drug-taking behaviors [190]. Although it is not clear whether sex differences in cocaine's rewarding effects contribute to these differences in drug-taking behaviors, it has been demonstrated that there are few sex differences in the discriminative stimulus effects of cocaine [47]. Compared to male rats, female rats acquire both cocaine and heroin self-administration faster and at lower doses, and also respond at higher levels during reinstatement testing [48;147;148]. Using a CPP paradigm, a test to determine the rewarding effects of cocaine, we have recently demonstrated that female rats develop associations between environmental stimuli and cocaine's rewarding effects more quickly and at lower doses than do males [191]. This implies that in female rats cocaine may have a greater reward potential than in male rats. Thus, the evidence suggests that female rats may be more vulnerable than male rats to

developing learned associations between environmental cues and cocaine-induced reward effects.

Cocaine produces its psychomotor and rewarding effects by blocking the reuptake of monoaminergic neurotransmitters. In male rats monoamines in the ventral tegmental area (VTA) and the nucleus accumbens (NAc) are considered to play an important role in the rewarding properties of cocaine [26;27;192;193], which may contribute to the acquisition and/or expression of cocaine-induced place preference. For example, parallel microdialysis and CPP studies suggest that increased dopamine activity in the NAc is important in the expression of CPP [194]. Recently, the role of serotonin and dopamine in regulating the rewarding properties of cocaine has received strong support with the characterization of serotonin and dopamine transporter knockout mice. Unlike single mutations where cocaine-induced CPP is preserved [42], a double serotonin and dopamine transporter knockout completely abolishes cocaine-induced CPP [43], strongly indicating that both monoamine systems are important regulators of cocaine's rewarding properties. The extent to which sex differences in the regulation of these monoamine systems alter the rewarding properties of cocaine is not well understood.

Gonadal hormones have been postulated to be important determinants of cocaine's effects by influencing neuronal activity and plasticity in the brain [10;88;195]. For example, after acute and chronic cocaine administration, estrogen and progesterone have been shown to regulate different aspects of the behavioral response [71;159;196;197]. In turn, cocaine modulates plasma levels of progesterone in intact and pregnant females as well as in male rats [55;57;198]. On the other hand, in male rats,

plasma levels of testosterone are significantly lower after chronic and acute cocaine administration [58;63;92]. This suggests that endogenous hormones may be involved in the cascade of events, which occur following cocaine administration. However, little is known about the contributions of endogenous gonadal hormones to cocaine reward. Therefore in this study, we conducted experiments to address the questions of how endogenous gonadal hormones as well as estrogen and progesterone replacement affect cocaine CPP. Neurochemical measurements were taken to determine if dopamine and 5HT activity in the VTA and NAc underlie the hormonal effects on the acquisition and/or expression of cocaine CPP.

II. Methods

Animals and cocaine administration paradigm:

Eight-week-old intact (INT) or gonadectomized (GDX) female and male Fischer rats (Charles River, NY) were single-housed in a standard cage with free access to food and water and maintained on a 12-hour light/dark cycle (lights on at 9:00 A.M.). Thirty minutes after lights were on, rats (n=9-15/group) received either i.p. injections of saline (0.9%) or cocaine (5mg/kg for females and 20mg/kg for males dissolved in 0.9% saline) in the conditioning chambers. On the testing day (Phase III), rats were decapitated following a brief (20 seconds) exposure to CO_2. Brains were removed and trunk blood was collected and centrifuged at 3,000 rpm for 15 minutes at 4 °C. Serum was collected and stored at -80 °C until used. Animal care was in accordance with the *Guide for the Care and Use of Laboratory Animals* (NIH publication 865-23, Bethesda, MD) and approved by the Institutional Animal Care and Use Committee. All chemicals were purchased from Sigma Scientific (Saint Louis, MI).

Hormone replacement paradigm:

After being anesthetized with isoflourane, ovariectomized (OVX) rats were implanted with SILASTIC capsules containing cholesterol (1mm, 100%), estrogen (1 mm, 10% estrogen: 90% cholesterol), progesterone (3 mm, 100%), or estrogen + progesterone (1 mm, 10% estrogen: 90% cholesterol; 3 mm, 100% progesterone) into the nape of the animal's neck. These steroid concentrations have been previously shown to regulate behavioral responses to cocaine and endocrinological alterations in females [71;199]. One week after surgery, rats were conditioned with cocaine or saline treatments.

Conditioned place preference boxes:

Place preference cages, purchased from Med Associates (Georgia, VT), consisted of a rectangular box with three distinct chambers. The center neutral gray chamber was 12 cm long, with a smooth PVC floor. The two conditioning chambers were 28 cm long and had different visual and tactile cues [one was black with a stainless steel grid rod floor and the other was white with a stainless steel mesh floor]. The chambers were separated by computer-automated guillotine doors to allow access to all three chambers.

Conditioned place preference procedure:

Conditioning was conducted as described previously [191]. For pre-conditioning, rats were placed into the neutral gray area (for a 5-minute acclimation period) and then allowed to freely explore all three chambers for 15 minutes. Rats were then randomly assigned to cocaine/saline conditioning groups or saline/saline control groups. The next phase consisted of 4 days (2 cocaine/saline pairings) of conditioning, with male and

female rats receiving 20mg/kg and 5mg/kg cocaine, respectively. These doses have previously been shown to represent the optimal dose needed to produce conditioned place preference in both sexes [191]. On conditioning days 1 and 3, rats were injected with cocaine and immediately confined to one chamber for 30 minutes. On alternate days, rats were injected with saline and immediately confined to the opposite chamber for 30 minutes. Control rats received saline in both chambers on alternate days. On the testing day, rats were placed into the neutral gray chamber (5-minute acclimation period) and then allowed 15 minutes with free access to all three chambers in a drug-free state. The time spent, entrances and explorations in each chamber, and total locomotor activity were recorded.

Corticosterone radioimmunoassay:

Serum levels of corticosterone were analyzed using Coat-A-Count Radioimmunoassay kits (Diagnostic Product Corporation, CA). The intra-assay coefficient of variance averaged less than 10%. Results were determined using a log-logit analysis within Graph Pad Prism (Graph Pad Software, Inc., CA).

High performance liquid chromatography (HPLC) analysis:

Bilateral tissue punches (0.5mm x 0.3mm) were taken from 300 μm sections [8 punches in the NAc shell (ranging from 1.70mm to1.00mm anterior to Bregma) and 4 punches in the VTA (ranging from -5.20mm to -5.80mm posterior to Bregma)]. Tissue punches were preserved overnight with 60 μl of sodium acetate buffer + internal standard (3,4-dihydroxybenzylamine) + ascorbate oxidase. On the following day samples were freeze-thawed and centrifuged, and supernatant was collected. Tissue pellets were re-

suspended in 100 µl of 1.0 N NaOH and total protein levels were measured using a Bradford assay from Bio-Rad (Bio-Rad Laboratories, CA).

As previously described [88], monoamine levels and their metabolites were measured in 30 μl of supernatant with a Waters Associates chromatographic system, containing a refrigerated 717 plus auto sampler and 1525 binary pump (Waters Associates, MA). In line was an MD-150/RP C_{18} column with MD-TM mobile phase at pH 2.3 (ESA, MA) pumped through the system. For electrochemical detection, we used an ESA Coulchem II EC detector with the screening electrode set at –150 mv, the detecting electrode at +325 mv, and the guard cell at +350mv. Concentrations of dopamine and its metabolites [3,4 dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA)] and serotonin and its metabolite [5-hydroxyindole acetic acid (5HIAA)] were calculated with reference to standards using peak integration with Breeze software (Waters Associates, MA).

Data analysis:

Dependent measure t-test analyses were used to determine statistically significant differences between time spent, entrances, and explorations in each chamber. Within each sex, one-way ANOVAs were used to determine the effect of gonadectomy on CPP scores (time spent in cocaine-paired chamber minus time spent in saline-paired chamber). One-way ANOVAs were also run to examine the effect of hormone replacement (cholesterol, estrogen, progesterone, or estrogen + progesterone) on CPP scores. Two-way ANOVAs were used to analyze locomotor activity, serum levels of corticosterone, and monoamine/metabolite levels [drug (cocaine or saline) x experimental group (intact

vs. GDX, or steroid replacement)]. All ANOVAs were followed by Newman-Kheuls post hoc analysis. $P < 0.05$ was considered statistically significant.

III. Results

Throughout the study, control rats receiving saline treatment in both conditioning compartments did not exhibit significant differences in the time spent in each chamber, confirming the unbiased nature of the procedure. Average time spent (seconds ± S.E.M.) in each chamber was 251.5 ± 11.7 and 241.0 ± 9.3, respectively. Moreover, no differences in the number of explorations or entrances were observed in control rats [85.6 ± 3.4 and 91.0 ± 3.4 or 59.5 ± 2.7 and 60.2 ± 2.3, respectively].

Role of endogenous gonadal hormones in cocaine-induced conditioned place preference:

To determine the role of endogenous gonadal hormones in the rewarding aspects of cocaine, we examined the ability of intact and gonadectomized male and female rats to acquire cocaine-induced CPP. As shown in Figure 8A, all experimental groups acquire place preference for the cocaine-paired chamber [intact males: $t(11) = 3.52$, $p < 0.05$; castrated males: $t(11) = 2.90$, $p < 0.05$; intact females: $t(13) = 6.40$, $p < 0.05$; OVX females: $t(14) = 2.20$, $p < 0.05$]. After ovariectomy, the magnitudes of cocaine-induced CPP scores were significantly decreased when compared to those of intact female rats [$F(1,26) = 6.07$, $p < 0.05$; Figure 8B].

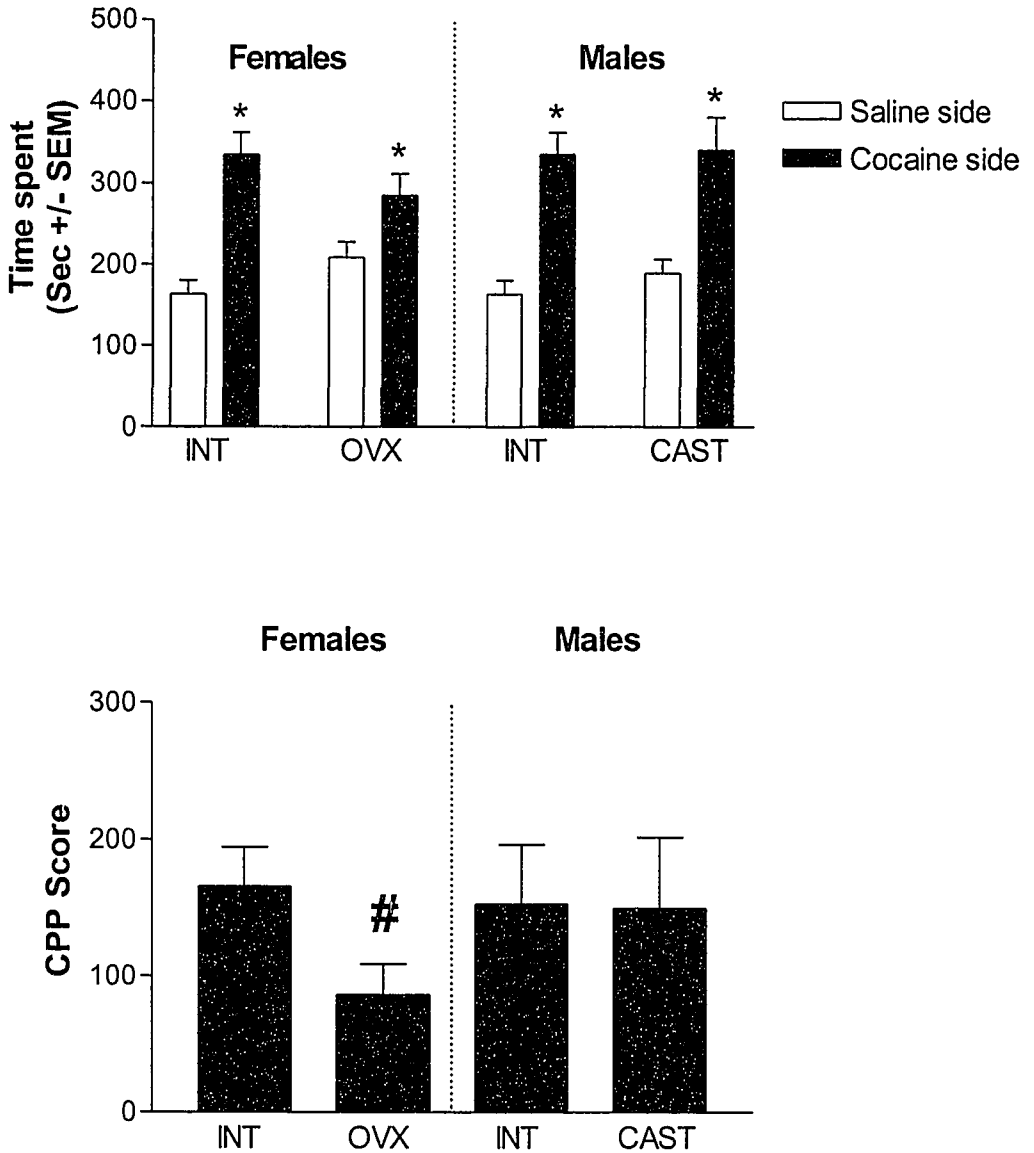

Figure 8: The effects of gonadectomy on cocaine CPP. Data are represented as the mean (± S.E.M.) time spent in each chamber **(A)** and CPP scores **(B)**. * Indicates statistically significant differences at $p < 0.05$ (n=9-15). [Abbreviations: SAL=Saline; COC=Cocaine; OVX=Ovariectomy; CAST=Castrated INT=Intact].

As shown in Figure 9, intact and OVX female rats had a greater number of entrances into the cocaine-paired chamber than the saline-paired chamber [$t(8) = 2.62$, $p < 0.05$, and $t(10) = 2.32$, $p < 0.05$, respectively]. However, no significant differences in the number of explorations in the saline- and cocaine-paired chambers were observed between intact or OVX females. On the other hand, regardless of the conditioning treatment, intact females have higher total locomotor counts [$F(3, 67) = 14.29$, $p < 0.05$] than do OVX females, intact males, and castrated males [$p < 0.05$ for all groups]. In both intact and castrated male rats, there were no statistically significant differences observed in either the number of entrances or explorations in the chambers after cocaine conditioning. However, in contrast to ovariectomy, castration did not affect total locomotor activity (see Figure 9C).

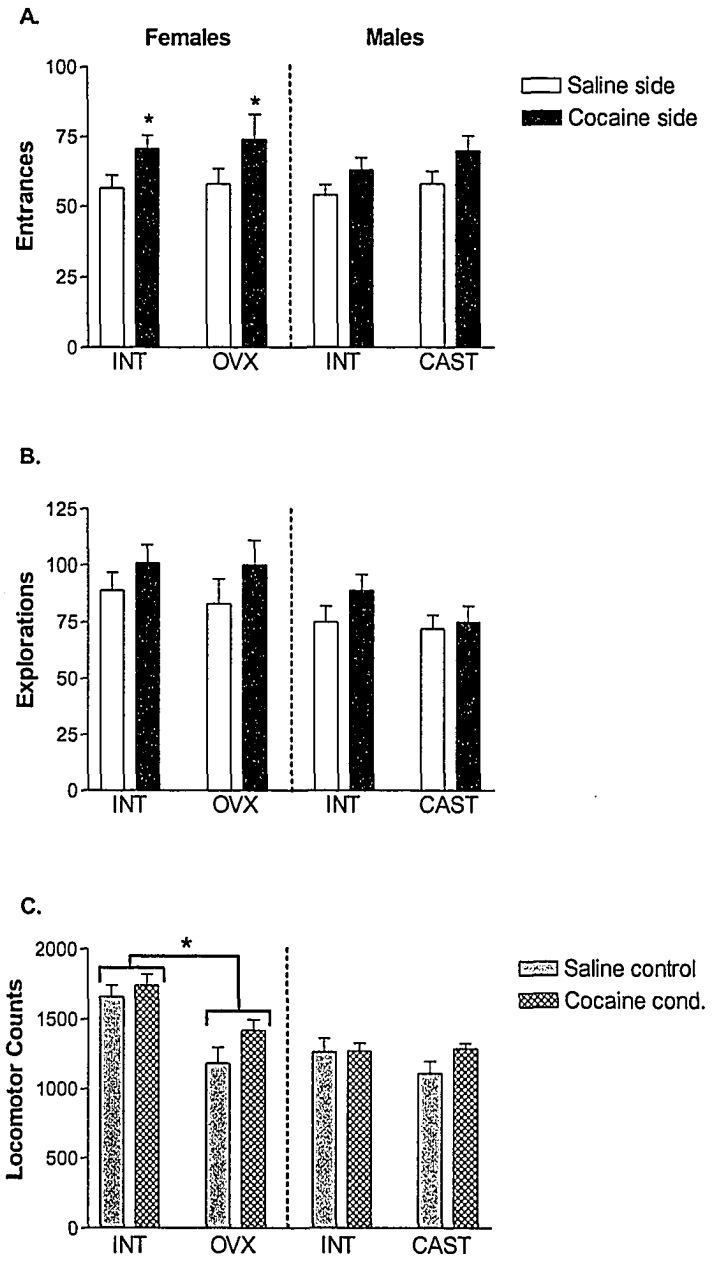

Figure 9: The effects of gonadectomy on other CPP behaviors. Total number of entrances and explorations to the saline- (white bars) and cocaine-paired (black bars) chambers in cocaine-conditioned animals, are shown in Figures **(A and B)** respectively. Locomotor activity **(C)** is represented as the sum of all locomotor counts in all chambers (gray bars represent saline-control animals and hatched bars represent cocaine-conditioned animals). All Data are represented as mean ± S.E.M. * Indicates statistically significant differences at $p < 0.05$. [Abbreviations: COC=Cocaine; OVX=Ovariectomy; CAST=Castrated INT=Intact].

Role of estrogen and progesterone replacement in cocaine-induced place preference:

To determine if ovarian hormones affect conditioned place preference for cocaine, OVX female rats received cholesterol, estrogen, progesterone, or estrogen + progesterone replacement (Figure 10). After conditioning, cholesterol-, estrogen-, and estrogen + progesterone-treated rats showed preference for the cocaine-paired chamber [$t(10) = 2.96$, $p < 0.05$; $t(10) = 3.083$, $p < 0.05$; $t(8) = 5.156$, $p < 0.001$; respectively, Figure 10A]. However, rats receiving progesterone replacement did not exhibit preference for either the saline- or cocaine-paired chambers [$t(9) = 0.72$, $p > 0.05$]. Furthermore, as shown in Figure 10B, a one-way ANOVA reveals a significant effect of hormone on the magnitude of CPP scores in cocaine-conditioned rats [$F(3,37) = 3.89$, $p < 0.05$] where estrogen + progesterone-treated rats had significantly higher CPP scores than rats receiving other steroid replacement paradigms [$p < 0.05$].

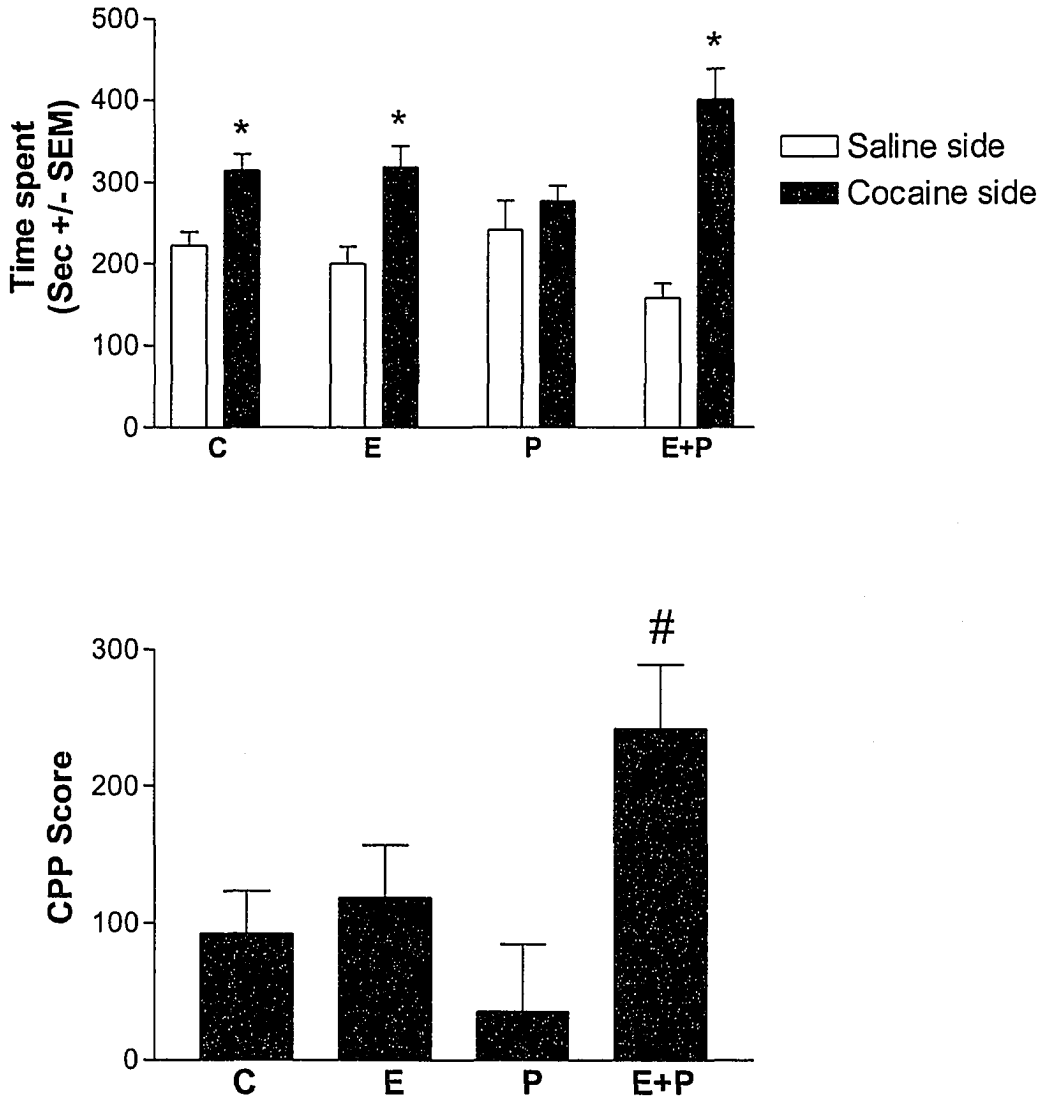

Figure 10. The effects of ovarian hormone replacement on cocaine-induced CPP in ovariectomized female rats. Data are represented as the as mean (± S.E.M) time spent in each chamber **(A)** and CPP scores **(B)**. * Indicates statistically significant differences at $p < 0.05$ (n=9-15). [Abbreviations: C=Cholesterol; E=Estrogen; P=Progesterone; E+P=Estrogen plus Progesterone].

Rats given estrogen + progesterone replacement also had a greater number of entrances and explorations into the cocaine-paired chamber than into the saline-paired chamber [$t(9) = 2.81$, $p < 0.05$, $t(10) = 2.26$, $p < 0.05$, Figures 11A and 11B respectively]. A main effect of hormone on total locomotor behavior was observed [$F(3,73) = 9.70$, $p < 0.0001$; Figure 11C] where estrogen and estrogen + progesterone replacement decreased total locomotor activity when compared to cholesterol or progesterone-treated rats [$p < 0.05$].

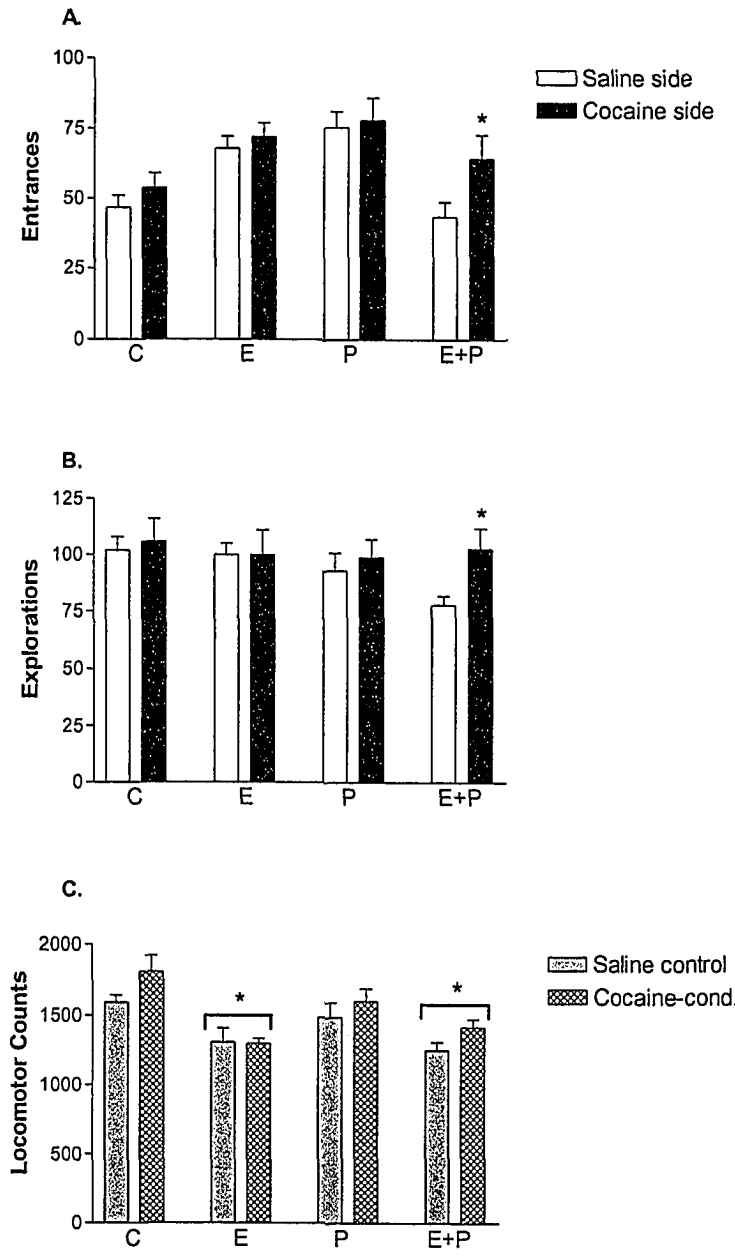

Figure 11: The effects of ovarian hormone replacement on other CPP behaviors in ovariectomized female rats. Total number of entrances and explorations to the saline- (white bars) and cocaine-paired (black bars) chambers in cocaine-conditioned animals, are shown in Figures **(A and B)** respectively. Locomotor activity **(C)** is represented as the sum of all locomotor counts in all chambers (gray bars represent saline-control animals and hatched bars represent cocaine-conditioned animals). All Data are represented as mean ± S.E.M. * Indicates statistically significant differences at $p < 0.05$. [Abbreviations: C=Cholesterol; E=Estrogen; P=Progesterone; E+P=Estrogen plus Progesterone].

Serum levels of corticosterone:

As shown in Table IV, no significant differences in serum levels of corticosterone were observed between cocaine-conditioned and saline-control groups. However, females had overall higher serum levels of corticosterone than male rats [$F(3,70) = 34.43$, $p < 0.05$] where both OVX and intact females had significantly higher levels of serum corticosterone than intact and castrated males [$p < 0.05$, for all comparisons]. Furthermore, OVX female rats had lower serum levels of corticosterone when compared to intact female rats [$F(1,34) = 7.93$, $p < 0.05$]. No significant differences in serum levels of corticosterone were observed between castrated or intact male rats or between the different steroid replacement groups in OVX female rats [$p > 0.05$].

Table IV. Serum levels of corticosterone in saline- and cocaine-conditioned animals.

Group	Saline-control	Cocaine-conditioned
INT-Female	316±43.7	283±35.8
OVX-Female	**215±17.4***	**212±20.8***
INT-Male	92±8.6	119±14.7
CAST-Male	67±13.3	96±15.7
OVX-C	282±23.7	280±29.3
OVX-E	315±28.9	368±26.8
OVX-P	290±39.7	304±45.9
OVX-E+P	327±39.2	321±72.4

Data is presented as the mean (± S.E.M.) serum levels of corticosterone (ng/ml). * Indicates differences from INT group at $p < 0.05$. [Abbreviations: INT=Intact; OVX=Ovariectomized; CAST=Castrated; C=Cholesterol; E=Estrogen; P=Progesterone; E+P=Estrogen plus Progesterone].

Monoamine and metabolite levels after CPP test:

Gonadectomy effects: Levels of serotonin, dopamine, metabolites, and turnover ratios after cocaine/saline conditioning in intact and GDX male and female rats are summarized in Table V. Overall, there were no differences in either serotonin or dopamine levels between cocaine-conditioned and saline-control groups. However, in the VTA of female rats, total levels of dopamine and serotonin were affected by ovariectomy [$F(1,18) = 4.97$, $p < 0.05$, and $F(1,19) = 19.31$, $p < 0.001$, respectively]; dopamine and serotonin levels were significantly lower in OVX female rats when compared to intact female rats [$p < 0.05$]. In addition, serotonin turnover ratios in this brain region were decreased after ovariectomy [$F(1,19) = 7.19$, $p < 0.05$]. In the VTA of male rats no significant effects of castration were observed. Furthermore, in the NAc shell no statistically significant changes in dopamine, serotonin, metabolite levels, or turnover ratios after gonadectomy were observed in either sex.

Table V. Effects of gonadectomy on total levels of monoamines, metabolites, and turnover ratios in cocaine- and saline-conditioned animals.

		Treatment	DA	DOPAC	HVA	DOPA/DA	HVA/DA	5HT	5HIAA	5HIAA/5HT
VTA										
Females	**OVX**	Saline control	**41.5±6.2***	**7.2±1.2**	**N/D**	0.17±0.02	**N/D**	**6.0±1.0***	9.9±0.7	**2.0±0.4***
		Cocaine-cond.	**38.2±7.0**	**6.9±1.4**		0.19±0.03		**6.0±0.7**	11.6±0.7	**2.1±0.2**
	INT	Saline control	70.4±15.3	8.9±0.8	**N/D**	0.13±0.01	**N/D**	11.4±1.2	10.0±1.1	0.9±0.2
		Cocaine-cond.	45.3±8.2	7.2±1.7		0.14±0.03		9.1±1.0	12.4±2.1	1.5±0.3
Males	**CAST**	Saline control	55.9±9.5	9.4±1.2	**N/D**	0.19±0.04	**N/D**	7.2±1.1	9.4±1.2	1.5±0.3
		Cocaine-cond.	47.1±2.9	6.5±1.1		0.13±0.02		7.9±0.9	10.7±1.2	1.5±0.3
	INT	Saline control	44.8±16.2	7.0±2.0	**N/D**	0.20±0.04	**N/D**	7.2±2.1	9.5±0.8	1.2±0.2
		Cocaine-cond.	56.4±11.3	8.0±1.8		0.15±0.01		6.0±1.0	9.8±1.0	1.9±0.4
NAc Shell										
Females	**OVX**	Saline control	48.4±5.8	93.6±12.8	22.8±8.4	1.9±0.6	2.9±0.5	12.9±3.0	30.6±4.6	3.2±0.8
		Cocaine-cond.	22.6±3.0	80.5±16.6	24.4±7.0	3.6±0.5	4.0±0.5	9.2±1.8	22.6±2.8	3.6±0.5
	INT	Saline control	46.8±12.2	91.5±17.4	18.1±5.8	2.0±0.8	3.6±1.0	9.4±2.4	26.1±2.7	3.7±0.9
		Cocaine-cond.	47.7±4.5	94.2±15.8	39.7±14.7	2.0±0.3	4.0±0.9	9.3±1.7	23.5±3.4	3.1±0.8
Males	**CAST**	Saline control	49.4±10.6	86.8±7.5	35.4±16.8	1.8±0.6	5.0±1.1	11.0±1.8	29.1±7.5	2.6±0.6
		Cocaine-cond.	48.7±6.3	79.1±13.8	32.7±24.8	1.6±0.2	2.8±0.3	10.6±1.2	29.5±10.8	2.8±0.9
	INT	Saline control	38.8±8.7	85.5±23.6	36.2±19.2	2.2±0.4	6.9±4.1	10.5±1.6	27.6±2.5	2.9±1.0
		Cocaine-cond.	43.4±8.5	72.6±11.4	31.9±13.2	1.7±0.8	3.8±0.9	11.7±2.1	35.4±7.5	4.3±1.3

Data is presented as the mean (± S.E.M.) levels of monoamines in pg/ug protein. * Indicates differences from other hormone treatment group regardless of cocaine or saline condition. [Abbreviations: INT=Intact; OVX=Ovariectomized; CAST=Castrated; for monoamine abbreviations see methods section].

Hormone replacement effects: serotonin and dopamine content, metabolites, and turnover ratios after cocaine/saline conditioning in steroid replaced OVX females are summarized in Table VI. Overall, there were no differences in either serotonin or dopamine levels between cocaine-conditioned and saline-control groups. However, in the VTA, a main effect of hormone replacement was observed on levels of serotonin [$F(3,46) = 3.84$, $p < 0.05$] and dopamine [$F(1,44) = 4.61$, $p < 0.005$] as well as on some metabolites [HVA: $F(3,43) = 4.03$, $p < 0.05$ and 5HIAA: $F(3,38) = 3.44$, $p < 0.05$] and turnover ratios [5HIAA/5-HT: $F(3,38) = 3.44$, $p < 0.05$ and HVA/ DA: $F(3,38) = 4.15$, $p < 0.05$]. Specifically, progesterone-treated rats had higher levels of serotonin and dopamine than control- and estrogen-treated rats, lower 5HIAA/5-HT turnover ratios than cholesterol-treated rats, and lower HVA/DA turnover ratios than estrogen + progesterone-treated rats [$p < 0.05$ for all comparisons]. On the other hand, estrogen treatment decreased HVA levels when compared to all other groups [$p < 0.05$] while estrogen + progesterone-treated rats had higher levels of 5HIAA than estrogen-treated rats [$p < 0.05$]. In the NAc shell, co-administration of estrogen and progesterone increased dopamine levels [$F(3,40) = 3.25$, $p < 0.05$] when compared to estrogen- and progesterone- [$p < 0.05$, for both comparisons] but not cholesterol-treated rats [$p = 0.07$].

Table VI. Effects of ovarian hormone replacement on total levels of monoamines, metabolites, and turnover ratios

	Treatment		DA	DOPA	HVA	DOPA/DA	HVA/DA	5-HT	5HIAA	5-HT/5HIAA
VTA										
	C	Saline control	12.4±1.1	7.3±0.9	3.6±0.2	0.7±0.06	0.3±0.01	4.3±0.1	15.0±1.1	4.0±0.2
		Cocaine-cond.	18.4±3.5	9.5±1.0	3.8±0.4	0.6±0.05	0.3±0.03	6.0±1.0	17.8±3.5	2.8±0.3
	E	Saline control	12.0±3.5	4.7±0.7	**2.8±0.2***	0.6±0.04	0.3±0.05	4.7±1.4	12.7±3.5	3.5±0.6
		Cocaine-cond.	13.6±2.2	7.6±1.1	**2.9±0.2**	0.6±0.07	0.2±0.02	6.1±1.3	15.0±2.2	2.8±0.5
	P	Saline control	**30.0±4.3***	9.0±1.6	4.0±0.3	0.5±0.12	**0.2±0.03***	**9.0±2.0***	17.7±4.3	**2.5±0.5***
		Cocaine-cond.	**21.4±3.9**	8.1±1.4	3.6±0.3	0.4±0.08	**0.2±0.03**	**9.3±2.0**	15.8±4.0	**1.9±0.2**
	E+P	Saline control	23.0±7.7	10.0±2.9	4.5±0.6	0.5±0.07	0.3±0.06	6.7±1.3	**18.0±7.7***	2.8±0.3
		Cocaine-cond.	12.3±2.7	7.0±2.6	4.1±0.5	0.3±0.09	0.4±0.09	7.0±1.1	**15.8±2.7**	3.3±0.4
NAc Shell										
	C	Saline control	46±4.4	68±8.6	24±2	1.5±0.3	0.5±0.1	12.9±3.7	34.4±8.7	5.8±2.2
		Cocaine-cond.	47±7.4	42±6.9	23±2.7	1.0±0.1	0.5±0.1	12.1±3.8	25.7±4.9	3.6±1.2
	E	Saline control	49±6.1	37±6.3	18±2.9	0.8±0.2	0.4±0.1	16.7±6.6	21.5±2.4	2.2±0.5
		Cocaine-cond.	46±3.4	59±10.2	23±1.9	1.2±0.2	0.5±0.0	24.2±9.2	26.6±2.6	3.1±1.0
	P	Saline control	42±2.2	77±21.1	27±2.4	2.2±0.5	0.6±0.1	12.5±4.0	26.6±3.0	3.4±1.0
		Cocaine-cond.	39±5.1	54±20.1	19±2.7	1.4±0.6	0.5±0.1	13.5±4.6	24.8±2.7	3.5±0.9
	E+P	Saline control	**55±6.9***	57±8.5	22±1.9	1.1±0.3	0.5±0.1	12.9±3.6	31.0±4.3	3.2±1.0
		Cocaine-cond.	**65±9.3**	51±14.6	33±8.1	0.8±0.2	0.4±0.1	4.4±0.6	27.6±5.3	6.2±0.8

Data is presented as the mean (± S.E.M.) levels of monoamines in pg/ug protein. * Indicates differences from other hormone treatment group regardless of cocaine or saline conditioning. [C=Cholesterol; E=Estrogen; P=Progesterone; E+P=Estrogen plus Progesterone; for monoamine abbreviations see method section]

IV. Discussion

This study demonstrates that endogenous ovarian hormones in female rats in part, mediate cocaine's rewarding effects, where estrogen and progesterone may have direct effects on the magnitude of cocaine CPP. Contrary to females, endogenous gonadal hormones in males seem to play a limited role in the expression of cocaine-induced reward responses. Hormone replacement experiments demonstrated a complex relationship between ovarian steroids in mediating the acquisition and/or expression of cocaine-induced CPP. Depending upon the steroid replacement paradigm, cocaine CPP was either inhibited or potentiated. These effects were not completely based upon steroid modulation of locomotor activity since decreases in locomotor activity were not correlated with cocaine CPP responses. For example, total locomotor activity was decreased in ovariectomized rats as well as estrogen and estrogen + progesterone replaced rats. However, estrogen + progesterone replacement increased the magnitude of cocaine CPP, estrogen alone had no effect, and ovariectomy decreased it. Based on neurochemical assays, our results also suggest that ovarian hormones may effect the expression of cocaine-induced CPP in part through alterations of the mesocorticolimbic monoamine systems. Although castration in males did not affect the development of cocaine CPP in male rats, further studies are needed to address the role of testosterone. It is possible that testing rats after longer periods after castration may affect cocaine CPP.

These findings are consistent with the growing body of literature, which suggests that both endogenous and exogenous gonadal hormones can influence cocaine-induced behavioral alterations [59;71;159;168;200]. For example, we have previously demonstrated that there are sex differences in the development of cocaine-induced

behavioral sensitization, which are in part controlled by endogenous gonadal hormones [i.e., ovariectomy but not castration delayed the development of sensitization after a challenge dose of cocaine] [46]. Similarly, in this report we observed that gonadectomy produced more profound effects on the expression of cocaine CPP in female rats. Taken together, these studies suggest that to a certain extent, female rats are more susceptible to gonadal hormone regulation of cocaine-induced locomotor activity and reward than male rats.

Lynch and Carroll [147] and our group [191] have demonstrated sex differences in cocaine's rewarding/reinforcing effects. Female rats acquire cocaine self-administration [147] as well as cocaine-induced CPP [191] faster and with lower doses of cocaine than do male rats. Our results extend upon those previously reported by Lynch and Carroll [147] as well as Russo et al. [191] by demonstrating that sex difference in the acquisition of cocaine self-administration and cocaine-induced CPP may in part be mediated by sex differences in the effects of endogenous gonadal hormones and estrogen + progesterone replacement on cocaine-reward learning.

It has previously been demonstrated that estrogen and progesterone administration differentially affect cocaine-induced behavioral responses. Progesterone has been shown to attenuate cocaine-induced locomotor activity in rats [159] and the subjective effects of cocaine in humans [201]. On the other hand, co-administration of estrogen and progesterone potentiated brain stimulation reward in OVX rats [119]. Gonadal hormone interactions have been postulated to be critical in the modulation of reproductive and cocaine-induced behaviors as well as of neuronal activity and plasticity [10]. Moreover,

Roberts et al. [64] reported that females in diestrous (when progesterone levels are high and estrogen levels are low) self-administer cocaine with lower frequency than during other stages of the estrous cycle while females during estrus (when both estrogen and progesterone levels are high) administer cocaine with greater frequency. Consistent with these reports, our findings showed that while progesterone alone inhibited cocaine-induced CPP, co-administration of both gonadal steroids potentiated the magnitude of cocaine-induced CPP. Taken together, our results support the idea that fluctuations of estrogen and progesterone during the estrous cycle may render female rats more or less vulnerable than male rats to developing learned associations between environmental cues and cocaine-induced reward effects. Therefore, the stage of a female's cycle may change the experience of cocaine-induced reward, which, in turn, may influence the level of cocaine use in females. Although these important clinical issues remain to be elucidated, an appealing explanation is that cyclical changes in the sensitivity to cocaine reward may contribute to sex disparities in the percentage of male and female cocaine users.

Activation of the HPA has been postulated to be essential for the control of cocaine reinforcement [97;176]. Consistent with Kuhn and Francis [202] and Chin et al. [46], we observed sex differences in serum levels of corticosterone. Although we observed that gonadectomy decreased corticosterone levels as well as the magnitude of CPP responses in female rats, we have previously shown that adrenalectomy does not affect cocaine CPP in either sex [191]. Furthermore, since intact and castrated male rats as well as female rats treated with gonadal hormones did not show any differences in serum levels of corticosterone. This suggests that the HPA axis may play only a limited role, if any, in the expression of cocaine CPP. It is possible that the HPG axis in female

rats may be more critical in modulating the cascade of events that regulate the acquisition and/or expression of cocaine CPP.

The dopaminergic and serotonergic systems, which project from the VTA of the midbrain to the NAc, have been postulated to serve as a final common neural pathway for mediating reinforcement processes [26;27;193]. In our study, ovariectomy decreased overall levels of dopamine and serotonin as well as increased serotonin turnover ratios compared to intact females in the VTA. After progesterone pre-treatment in OVX rats, both dopamine and serotonin levels were elevated while their respective turnover ratios were significantly decreased in the VTA. This, in turn, suggests that in progesterone-treated rats dopamine and serotonin metabolism were lower than in other steroid replacement groups. On the other hand, after estrogen and progesterone co-administration, elevated levels of dopamine in the NAc were observed, suggesting that increases in the magnitude of cocaine CPP may, in part, be mediated by a higher availability of dopamine in the NAc. However, it is possible that gonadal hormones may produce alterations in other neuronal systems, such as the regulation of GABAergic and glutamatergic activity by progesterone [203;204]. For example, an induction of the GABAergic system by progesterone may down-regulate the overall dopamine /serotonergic tone within the VTA and NAc circuit, thereby inhibiting cocaine-induced CPP. Taken together, our results suggest that the hormone-induced alterations in the acquisition and expression of cocaine CPP may be mediated by direct or indirect alterations of serotonin and dopamine activity within the reward circuit.

It has been suggested that activation of learning and memory processes are the basis for the development of cocaine-induced CPP [182-185]. Since gonadal hormones affect learning and memory processes [160;161;205;206], it is possible that gonadectomy and/or gonadal hormone replacement in female rats may ultimately affect the development of cocaine-induced CPP via learning and memory processes. For example, allopregnanolone, an active progesterone metabolite, inhibits learning in the Morris water maze [160] and progesterone inhibits avoidance conditioning [161]. On the other hand, co-administration of estrogen and progesterone enhanced the acquisition of a spatial memory task in OVX rats [206]. Thus, the observed effects on cocaine-induced CPP may directly reflect an inhibition or potentiation by gonadal hormones on learning and memory processes involved in the development of long-term memories necessary to form associations between cocaine reward and environmental cues. There are a number of reports demonstrating that hormone-dependent changes in dopamine and serotonin activity are critical in learning and memory processes [75-77] [78]. Based on the current studies, it is possible that estrogen and progesterone administration (alone or in combination) may affect the ability to store and recall cocaine-induced rewarding events through alterations of serotonergic/dopaminergic systems. To date, the direct relationship between cocaine's rewarding properties and experience-dependent memory as well as the extent to which these processes are affected by gonadal hormones remain an important challenge. Elucidating these mechanisms will have a profound effect on our understanding of the sex differences in cocaine abuse currently being studied.

Chapter 4: Progesterone inhibits the acquisition of conditioned place preference for cocaine in intact female rats.

I. Introduction:

Sex differences in the behavioral and subjective response to cocaine are well established. Several recent reports have indicated that gonadal hormones are a key factor in these sexually dimorphic responses. Lynch and Carroll [147] and our group [191] have demonstrated sex differences in cocaine's rewarding/reinforcing effects. Female rats acquire cocaine self-administration [147] as well as cocaine-induced CPP [191] faster and with lower doses of cocaine than do male rats. We have also demonstrated that sex difference in the acquisition of cocaine self-administration and cocaine-induced CPP may in part be mediated by sex differences in the effects of endogenous gonadal hormones [191].

It has been previously demonstrated that gonadal hormones modulate reward-related behaviors including brain stimulation reward, cocaine self-administration, sexual receptivity in females (Table I), and more recently cocaine CPP in OVX rats [201]. Interestingly, progesterone attenuates cocaine-induced locomotor activity [159], as well as the subjective effects of cocaine in humans [201]. Moreover, Roberts et al. [64] reported that females in diestrous (when progesterone levels are high and estrogen levels are low) self-administer cocaine with lower frequency than during other stages of the estrous cycle while females during estrus (when both estrogen and progesterone levels are high) administer cocaine with greater frequency. Taken together, this suggests that progesterone changes an animal's sensitivity to reward-related behaviors and may

contribute to sex disparities in cocaine addiction. The purpose of the current experiment is to determine whether progesterone administration block cocaine-induced CPP in intact male and female rats.

II. Methods

Animals:

Eight-week-old intact, female and male Fischer rats (Charles River, Kingston, NY) were single-housed in a standard cage with free access to food and water and maintained on a 12-hour light/dark cycle (lights on at 9:00 A.M.). One week after arrival, animals were randomly assigned to either saline- or cocaine-treatment groups and conditioned as described below. After testing, rats were decapitated following a brief (20 seconds) exposure to CO_2. Animal care was in accordance with the *Guide for the Care and Use of Laboratory Animals* (NIH publication 85-23, Bethesda, MD) and approved by the Institutional Animal Care and Use Committee at Hunter College. All chemicals were purchased from Sigma Chemical Co. (St. Louis, MI).

CPP apparatus:

Place preference cages, purchased from Med Associates (Georgia, VT), consisted of a rectangular box with three distinct chambers. The center neutral gray chamber was 12 cm long, with a smooth PVC floor. The two conditioning chambers were 28 cm long and had different visual and tactile cues [one was black with a stainless steel grid rod floor and the other was white with a stainless steel mesh floor]. Computer-automated guillotine doors to allow access to all three chambers separated the chambers.

CPP procedure:

The conditioning procedure was conducted as described previously [191]. For pre-conditioning, rats were placed into the neutral gray area (for a 5-minute acclimation period) and then allowed free access to all three chambers for 15 minutes. Rats were then randomly assigned to either cocaine/saline conditioning groups or saline/saline control groups. On conditioning days 1 and 3 (for 4-day paradigm) male and female rats were injected with cocaine (20mg/kg and 5mg/kg; respectively) and immediately confined to one chamber for 30 minutes. On alternate days, rats were injected with saline and immediately confined to the opposite chamber for 30 minutes. Control rats received saline in both chambers on alternate days. On the testing day, rats were placed into the neutral gray chamber (for a 5-minute acclimation period) and then allowed 15 minutes with free access to all three chambers in a drug-free state. Time-spent, total locomotor activity (sum of all horizontal counts), and number of entrances (multiple beams broken as animal enters the chamber) and explorations (single beam broken in adjacent chamber without entry into the chamber) were recorded using a computerized photo-beam system run with MED-PC software.

Hormone replacement paradigm:

Acute paradigm: Animals were injected with either progesterone (500 μg dissolved in sesame oil; s.c.) or vehicle (sesame oil; s.c.) 4 hours before each conditioning session (for acquisition). This dose has been previously shown to inhibit the behavioral response to cocaine [207].

Chronic paradigm: Animals were surgically implanted with silastic capsules containing progesterone (100%) or empty capsules into the nape of the animal's neck. This dose has been previously shown to inhibit the behavioral response to cocaine [208].

Data analysis:

Dependent measure t-test analyses within each group were used to determine statistically significant differences between time spent, entrances, and explorations in each chamber. Two-way ANOVAs were used to examine the magnitude of CPP scores (time in drug minus time in saline) and locomotor behavior [DRUG (cocaine vs. saline) x HORMONE (vehicle vs. progesterone).

III. Results

Throughout the study, control rats receiving saline treatment in both conditioning compartments did not exhibit significant differences in the time spent in each chamber, confirming the unbiased nature of the procedure. Average time spent (seconds ± S.E.M.) in each chamber was 234.9±9.7 and 236.6±9.5; respectively. Moreover, no differences in the number of explorations or entrances were observed in control rats [86.3±3.9 and 81.6±3.1 or 61.6±2.6 and 59.5±2.4 respectively].

Effects chronic progesterone on cocaine CPP:

As shown in Figure 12 all groups showed a significant CPP for cocaine [vehicle-males; $t(8) = 3.621$, $p < 0.05$; progesterone-males $t(9) = 5.924$, $p < 0.05$; vehicle-females $t(11) = 2.908$, $p < 0.05$; progesterone-females $t(11) = 3.695$, $p < 0.05$]. Chronic progesterone did not affect the magnitude of cocaine CPP in either sex ($p > 0.05$). Table

VII shows mean entrances and explorations in all groups. Although none of the groups significantly explored the cocaine-paired chamber more often than the saline-paired chamber, vehicle-treated females significantly entered the cocaine-paired chamber more often than the saline-paired [$t(10) = 2.17$, $p < 0.05$]. Moreover, female had overall higher locomotor counts, neither hormone or cocaine treatment affected locomotor behavior in either sex ($p > 0.05$).

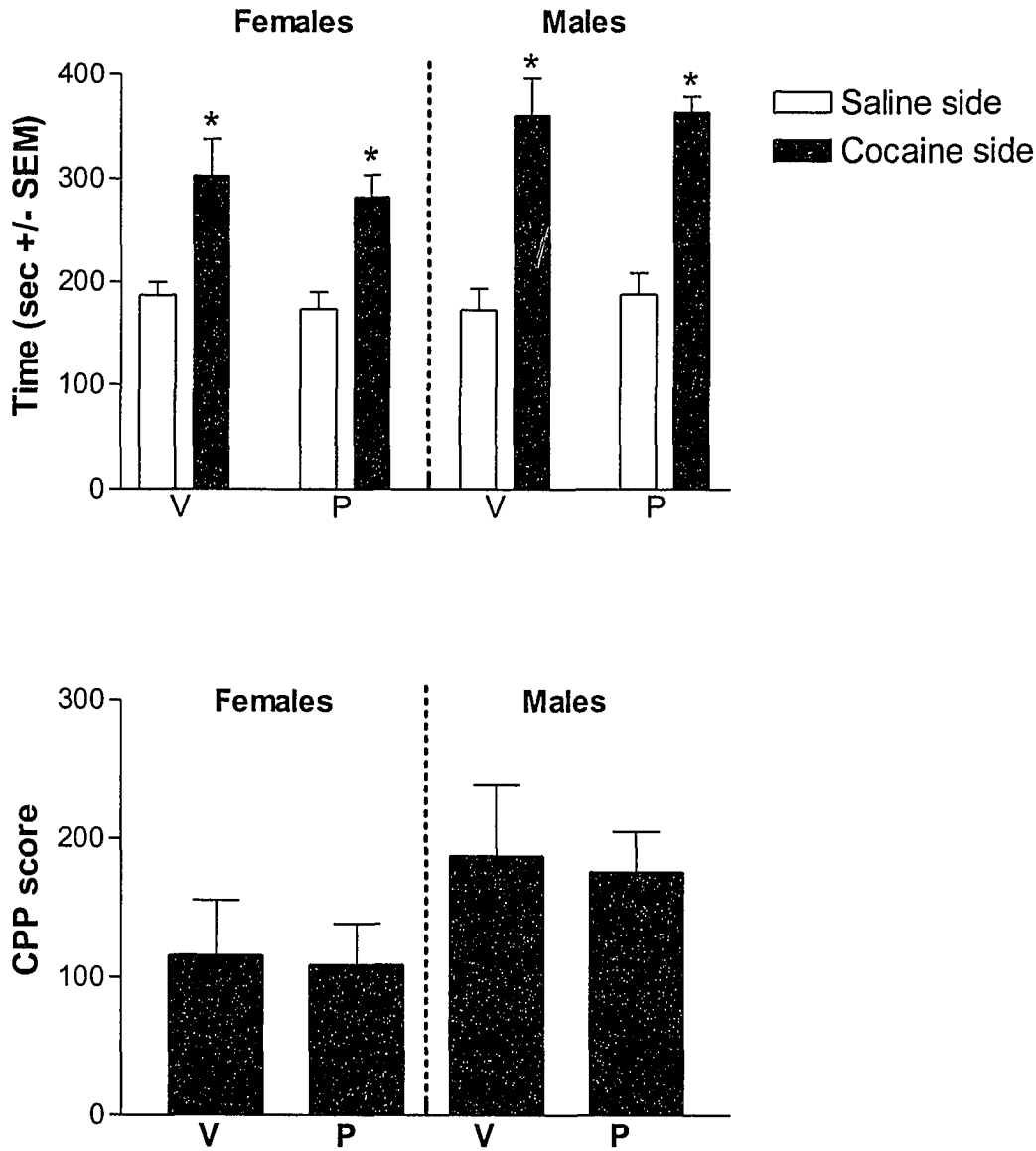

Figure 12: The effects of chronic progesterone on conditioned place preference for cocaine in female and male rats. Time spent in the saline-paired (white bars) and cocaine-paired (black bars) chambers **(A)** and CPP scores **(B)** on the test day, represented as mean seconds (± SEM). * Indicates statistically significant differences at $p < 0.05$ (n=8-15 animals/ group). [Abbreviations: V=Vehicle; P=Progesterone].

Table VII: The effects of chronic progesterone on other CPP behaviors in male and female rats.

			Explorations		Entrances		Locomotor
Sex	**Hormone**	**Drug**	**Unpaired**	**Paired**	**Unpaired**	**Paired**	**Counts**
Females	Vehicle	Saline	101.2±10.1	83.5±9.7	57.2±11.3	52.0±7.6	1567.5±114.9
		Cocaine	80.3±7.2	100.8±8.9	50.5±3.7	***63.6±5.5**	1476.8±80.9
	Progesterone	Saline	104.1±14.3	90.7±7.6	61.6±5.6	53.3±5.9	1461.3±74.6
		Cocaine	103.4±10.5	98.7±7.1	52.5±5.6	61.7±5.7	1524.4±67.5
Males	Vehicle	Saline	92.3±11.5	71.4±4.3	77.9±7.3	59.0±4.5	1170.1±52.6
		Cocaine	83.9±6.4	84.1±6.1	66.0±4.0	65.7±5.0	1279.8±64.5
	Progesterone	Saline	64.8±4.9	84.6±9.7	62.9±8.7	72.9±5.7	1301.5±67.4
		Cocaine	86.1±8.4	90.3±8.3	59.3±6.7	71.5±5.7	1358.1±85.8

Total number of entrances and explorations to the saline- and cocaine-paired chambers and locomotor activity is represented as the sum of all locomotor counts in all chambers are represented as mean ± S.E.M. (*indicates statistically significant differences at $p < 0.05$).

The effects of acute progesterone administration during the acquisition phase of cocaine CPP:

Figure 13 shows the effects of progesterone administration on the acquisition of cocaine-induced CPP in intact male and female rats. Vehicle-treated females and males as well as males treated with progesterone developed a significant CPP for cocaine [vehicle-males; $t(13) = 2.450$, $p < 0.05$; progesterone-males $t(14) = 3.221$, $p < 0.05$; vehicle-females $t(10) = 3.119$, $p < 0.05$]. However, in intact females rats, acute progesterone administration inhibited cocaine CPP ($p > 0.05$).

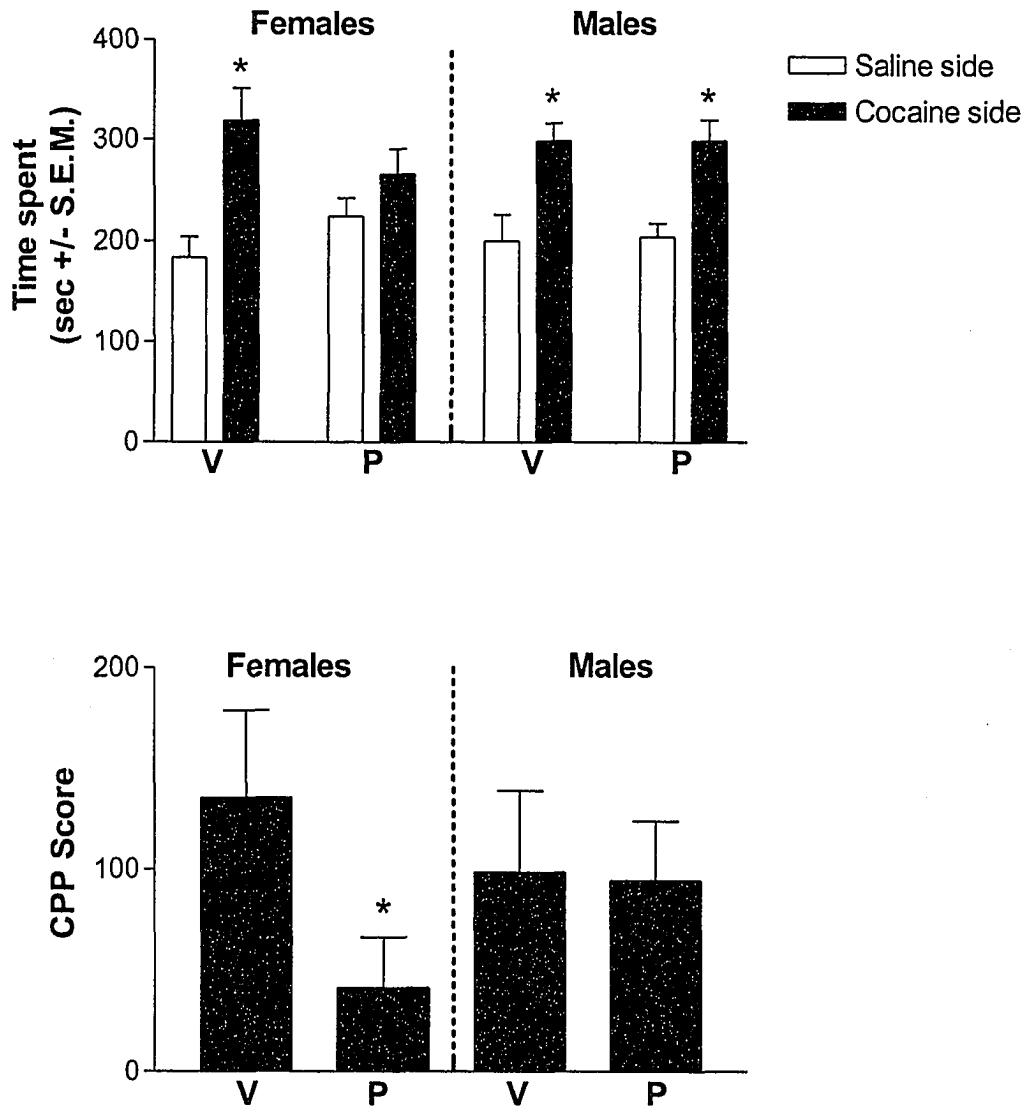

Figure 13: The effects of acute progesterone injections on conditioned place preference for cocaine in female and male rats. Time spent in the saline-paired (white bars) and cocaine-paired (black bars) chambers **(A)** and CPP scores **(B)** on the test day, represented as mean seconds (± SEM). * Indicates statistically significant differences at $p < 0.05$ (n=8-15 animals/ group). [Abbreviations: V=Vehicle; P=Progesterone].

As shown in Table VIII, vehicle-treated females and males as well as progesterone-treated males entered the cocaine-paired chamber more often than the saline-paired chamber [vehicle-males; $t(13) = 2.947$, $p < 0.05$; progesterone-males $t(14) = 2.074$, $p < 0.05$; vehicle-females $t(10) = 2.094$, $p < 0.05$]. However, no statistically significant differences in the number of entrances or exploration in the saline and cocaine-paired chambers were observed ($p > 0.05$). Although females had significantly higher locomotor counts than males, we did not observe any effects of hormone replacement or cocaine treatment in either sex. ($p > 0.05$).

Table VIII: The effects of acute progesterone on other CPP behaviors in ovariectomized female rats.

			Explorations		Entrances		Locomotion
Sex	**Hormone**	**Drug**	**Unpaired**	**Paired**	**Unpaired**	**Paired**	**Counts**
Females	Vehicle	Saline	99.0±14.8	88.5±11.1	54.0±7.8	53.1±7.1	1304.1±152.0
		Cocaine	86.8±10.1	***107.4±7.6**	61.8±7.5	***80.5±7.3**	1478.8±76.3
	Progesterone	Saline	89.7±6.3	99.2±7.5	67.3±6.8	73.7±7.0	1500.4±97.9
		Cocaine	97.3±6.9	95.6±8.9	70.7±6.4	61.4±6.2	1513.0±115.8
Males	Vehicle	Saline	80.1±6.6	67.1±11.0	60.6±5.6	51.4±9.4	1089.7±75.8
		Cocaine	68.6±7.1	80.3±7.8	48.8±4.9	***67.0±5.9**	1083.4±63.3
	Progesterone	Saline	58.6±5.9	64.5±6.7	47.4±4.8	56.8±5.1	991.4±42.6
		Cocaine	59.7±5.4	***70.1±7.6**	52.1±5.3	***65.6±6.1**	1226.9±89.1

Total number of entrances and explorations to the saline- and cocaine-paired chambers and locomotor activity is represented as the sum of all locomotor counts in all chambers are represented as mean ± S.E.M. (*indicates statistically significant differences at $p < 0.05$).

IV. Discussion

Contrary to previous observations in ovariectomized females, constant delivery (via silastic capsule) during all phases of the conditioning paradigm, did not block cocaine-induced CPP. It's possible that the presence of estrogen blunts the inhibitory properties of progesterone, rendering this route of administration less affective than subcutaneous injections during the conditioning phase. When progesterone is injected during the acquisition phase of the conditioning paradigm, female rats do not exhibit a significant cocaine CPP. These findings are consistent with previous reports that indicate progesterone inhibits the expression of other reward-related behaviors [64;157;201]. Specifically, progesterone decreases lordosis behavior [157] and the subjective effects of cocaine in humans [201]. Furthermore, Roberts et al. [64] reported that females in diestrous (when progesterone levels are high and estrogen levels are low) self-administer cocaine with less frequency than during other stages of the estrous cycle while females during estrus (when both estrogen and progesterone levels are high) administer cocaine with greater frequency.

In other areas of research it has been reported that progesterone inhibits performance on learning and memory tasks [160;161]. Since CPP is largely controlled by circuitry involved in learning and memory [182;183], we have previously hypothesized that progesterone's affects on CPP may reflect an inhibition of either learning and memory processes or the rewarding properties of cocaine [208]. However, in these studies, progesterone was administered during all phases of the conditioning paradigm and thus it is unclear whether progesterone affects the rewarding properties of cocaine or the expression of the behavior on the test day. The current data shows that

progesterone blocks the association of the rewarding event during the conditioning session. Taken together, it's possible that exogenous progesterone in female rats, and fluctuations of progesterone during the female cycle may have inhibitory effects on cocaine-induced reward behaviors by blocking the association between environmental cues and cocaine's rewarding properties.

In the current experiment females were more susceptible to hormonal regulation of cocaine-induced CPP than males. Changes in CPP after ovarian hormone replacement may have clinical implications when considering oral contraceptive use and as a potential treatment for cocaine addiction in females. A previous report has shown that estrogen + progesterone co-administration enhance, while progesterone alone inhibits CPP in OVX females [208]. Extending upon these results, progesterone blocks the acquisition of cocaine CPP in intact females and has clinical implications in the treatment of cocaine addiction. Since estrogen + progesterone have been shown previously to enhance cocaine CPP in OVX rats [208], further studies are needed to determine whether estrogen and progesterone co-administration also enhance cocaine CPP in intact females and to determine whether endogenous fluctuations of estrogen and progesterone affect cocaine CPP. Understanding the hormonal mechanisms that modulate cocaine reward, may explain sex differences in these responses.

Chapter 5: General Discussion

Estimates over the past 10 years by the National Household Survey on Drug Abuse indicate that women make up approximately 30% of the cocaine-using population. Furthermore, sex differences in the behavioral and subjective response to cocaine have been reported in both humans and animals. For example, Lukas et al. [5] reported that male subjects achieve a faster and higher peak of plasma cocaine levels with more episodes of euphoria or "good feeling" than woman. Although there are no gender differences in drug taking behaviors such as the time spent using, total amount used, and money spent, a study has shown that men remain abstinent longer than woman [7]. Therefore, shorter abstinence periods in females may be related to an increased sensitivity to drug-conditioned stimuli and increased craving [6]. Furthermore, women in proestrous (when progesterone is high and estrogen is low) or woman taking progesterone-based oral contraceptives may be less sensitive than males to the rewarding properties of cocaine. Therefore, It is possible that difference in the subjective and rewarding effects of cocaine in humans influences the desire to engage in drug taking behaviors, partially explaining the current sex disparities in overall cocaine use and rates of relapse. These clinical issues need further investigation.

Sex differences have also been reported in a variety of reward-related behaviors in animals. It has been shown that females consume greater amounts of alcohol than males [145]. Further studies revealed significant sex differences in the acquisition of cocaine and heroin self-administration, showing that females acquire cocaine self-administration quicker and show higher rates of responding [147]. Here we observed that female rats develop cocaine CPP at lower doses and after fewer pairing sessions than male rats,

which suggests that females are more sensitive to the conditioned rewarding properties of cocaine. Therefore, sex differences in the hormonal regulatory mechanisms may, in part, explain sex differences in these behaviors.

Ovariectomy and ovarian hormone replacement has a dramatic effect on a female's response to the rewarding properties of cocaine and monoamines within the brain's reward circuit. In contrast to ovariectomy, castration did not affect cocaine CPP, nor does it alter monoaminergic function in the VTA or NAc. This may be due to the length of castration in males, and further studies are needed using acute and chronic paradigms to assess the role of testosterone in cocaine CPP. Although there are sex differences in serum levels of corticosterone and females show an increased corticosterone response when conditioned with higher doses, adrenalectomy does not alter CPP in either sex. These findings support the idea that gonadal but not adrenal hormones are playing a major role in sex differences in cocaine CPP.

Changes in the rewarding properties of cocaine after ovarian hormone replacement may have clinical implications when considering oral contraceptive use and as a potential treatment for cocaine addiction in females. Here, we have shown that estrogen + progesterone co-administration enhance, while progesterone alone inhibits CPP in OVX females [208]. The inhibitory effects of progesterone on cocaine CPP were replicated in intact females, indicating a possible role for progesterone in the treatment of cocaine addiction in females. Clinical studies have indicated that progesterone attenuates the subjective effects of cocaine in woman. Furthermore, estrogen + progesterone co-administration have been shown previously to enhance

cocaine CPP in OVX rats [208], and further studies using intact female rat. It may be that estrogen + progesterone based contraceptives and endogenous fluctuations of estrogen and progesterone enhance a female's sensitivity to the addictive properties of cocaine. Understanding the hormonal mechanisms that modulate cocaine reward will aide in the development of sex specific treatments designed to compensate for differences in the behavioral effects of cocaine.

Sex differences in the hormonal mechanisms that control the mesolimbic dopamine system, and in particular the VTA-NAc circuit, may be a key factor influencing sex differences in reward-related behaviors. Monoamine depletion induced by acute gonadectomy was only observed in females, where serotonin and dopamine levels in the VTA of OVX females were significantly lower than intact females. Cocaine, which binds with the greatest affinity to the serotonin transporter [28], relies heavily on serotonin/dopamine interactions in the VTA to control reward behaviors [209], and at lower concentrations cocaine's effects *in vitro* are mediated mainly by alterations of the serotonin system [210]. Serotonin acts by disinhibiting $GABA_B$ inhibition of A10 dopamine neurons altering dopamine release in the NAc *in vivo* [211]. Interestingly, serotonin agonists injected directly into the VTA enhance accumbal dopamine release [212;213] and cocaine reinforcement [214], through serotonin $_{1B/1D}$ receptor stimulation. Though no effects of ovariectomy were observed on total levels of accumbal monoamines, it's possible that decreases in serotonin in the VTA alter the response output of dopamine projection neurons.

According to Creutz & Kritzer [215], intracellular estrogen receptors are co-localized with A8-A10 dopamine projection neurons. Therefore estrogenic effects in the VTA may act through genomic mechanisms to control dopamine activity in the NAc. However, the current results suggest that estradiol replacement at this concentration was not sufficient to alter the degree of place preference, nor were any effects on central monoamines observed. Interestingly, progesterone, which blocks place preference for cocaine, enhances both serotonin and dopamine in the VTA. This paradoxical effect of progesterone may act via GABAergic and/or glutamatergic mechanisms [203;204]. In the VTA progesterone increases $GABA_A$ receptor subunit expression [216] and binds directly to $GABA_A$ receptors [217]. Further studies revealed that estrogen and progesterone receptors are colocalized with glutamatergic cells [218;219], and ovarian hormone replacement affects the release of both GABA and glutamate [220;221]. Taken together, our results suggest that the hormone-induced alterations in the acquisition and expression of cocaine CPP may be mediated by direct or indirect alterations of serotonin and dopamine activity within the reward circuit.

Indirect regulation of monoamines by glutamate and GABA has been shown previously [211;222-225]. Increases in dopamine levels in the VTA oppose serotonin inhibition of $GABA_B$ through a D1 receptor mechanism [211] and oppose A10 dopamine neurons via D2 inhibitory receptors [223-225]. Furthermore, in vitro, progesterone affects N-methyl-D-aspartate (NMDA)-evoked dopamine release from the striatum [222]. Thus, direct and indirect effects of progesterone on dopamine projection neurons in the mesolimbic dopamine system may partially explain cocaine's failure to produce place preference. Though estrogen + progesterone treatment did not alter dopamine or

serotonin in the VTA, an increased availability of NAc dopamine, could account for increases in the magnitude of place preference.

It has been suggested that activation of learning and memory processes are the basis for the development of cocaine-induced CPP [182-185]. Since gonadal hormones affect learning and memory processes [160;161;206], it is possible that gonadal hormone replacement in female rats may ultimately affect the development of cocaine-induced CPP via learning and memory processes. Therefore, the current results may reflect sex differences in the hormonal mechanisms that control learning and memory processes involved in the development of long-term memories necessary to form associations between cocaine reward and environmental cues. Furthermore, it is possible that estrogen- and progesterone-based oral contraception may affect the ability to store and recall cocaine-induced rewarding events through alterations of serotonergic and dopaminergic systems. Although current theories have begun to address issues of learning and memory and addiction [184;185], the direct relationship between cocaine's rewarding properties and experience-dependent memory as well as the extent to which these processes are affected by gonadal hormones remain an important challenge. Elucidating these mechanisms will have a profound effect on our understanding of the sex differences in cocaine abuse currently being studied.

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