The Role of the Mesocorticolimbic Dopamine System in the Sexually Dimorphic Motor Response to Acute Cocaine

by

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

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THE CITY UNIVERSITY OF NEW YORK

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Abstract

Cocaine has been shown to increase locomotor behaviors in rats by altering monoaminergic tranmission. It has been previously demonstrated that female rats have a more robust behavioral response to acute cocaine administration. The neurobiological mechanisms underlying these differences remain unclear. The aim of this proposal was to determine potential mechanisms underlying sexual disparities in motor behavior following acute cocaine administration. We found that cocaine injection frequency, dose, and metabolism may contribute to sex differences in cocaine-induced motor behavior. It was observed that cocaine was more potent in female rats and that the motor behavior of female rats persisted for a longer time frame as compared to male rats. Cocaine is also differentially metabolized in female rats; norcocaine levels in female rats were higher in the brain and serum, indicating that this bioactive metabolite may influence sex differences in cocaine-induced behaviors. These results may help in explaining the increased sensitivity to cocaine's addictive properties observed in human females.

We have also demonstrated that the dopaminergic response to acute cocaine administration is sexually dimorphic in the caudate putamen and the nucleus accumbens. Our neurochemical results suggest that autoregulatory feedback pathways modulating monoamine function in the striatum could be the basis for these disparities. In the dopamine system, we found that D1 receptors modulate cocaine-induced motor activity in a sex-dependent manner. Cocaine-induced motor activity in female rats was more sensitive to D1 receptor blockade. However, cocaine-induced alterations in D1 mRNA and binding levels were only observed in male rats. These results suggest that sex-dependent modulation of the D1 receptor system contributes to the differences observed in cocaine-induced motor activity, and to the initial cellular adaptations that occur following cocaine administration. Since pharamcological agents that target the dopamine system have long been the subject of treatment options for cocaine addiction, conceivably, sex should be an important variable in the development of therapeutics for drug addiction.

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I dedicate this thesis to my parents, Eugene and Grace Festa. Their unwavering support throughout the years has made it possible for me to attain my goals and has shown me I truly can do anything I set my mind to. I am also grateful to all the members of my family for supporting and encouraging me to chase my dreams.

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CHAPTER 1

I. History of cocaine

Cocaine is an active alkaloid found in the leaves of *Erythroxylon coca*, a tree indigenous to Peru and Bolivia. The drug was used as a psychostimulant among the Indians of Colombia dating back 2,000 to 5,000 years (Platt, 1997). After the Incas conquered Colombia during the 10th century, coca's usage was limited to priests and nobility for special ceremonies (McKim, 1996). Coca was completely banned by the Spanish when they first conquered the Inca's empire because they viewed its use as idolatrous and pagan. Unfortunately for the Incas, the Spanish later discovered that if the Indians were given coca they would work harder, longer hours, and require less food. Consequently, coca leaves were distributed to workers three to four times a day during resting periods (McKim, 1996; Platt, 1997).

In the United States, however, its use remained relatively limited until the late 1800s when the famous Austrian physician, Sigmund Freud, advocated its use as a treatment for a multitude of personality disorders and even morphine addiction (McKim, 1996; Platt, 1997). Cocaine gained heightened popularity and it was not long before there were reports of cocaine addiction, toxicity, and death by 1888. During this time, an associate of Freud demonstrated the only real medical use of cocaine, as a potent local anesthetic (McKim, 1996; Platt, 1997). By 1894, the American Medical Association was beginning to question its use and made a statement expressing its concern (Platt, 1997).

Finally, in 1914, the use of cocaine was banned by the Harrison Narcotic Act and cocaine use was reduced for decades (Platt, 1997).

By the 1960s, a wave of cocaine use amongst the upper and middle class was reported. In the 1970s and into the early 1980s, cocaine became popular among the inner city poor (Platt, 1997). By 1985, cocaine use was reported by over six million Americans (SAMHSA, 1998). Since this peak, cocaine use has declined to around 2 million users (SAMHSA, 2002). However, cocaine abuse remains a public health problem.

II. Cocaine's effects in the CNS at the behavioral and cellular level

Cocaine's effects on the DArgic system

Cocaine binds directly to monoamine transporters and prevents their re-uptake, increasing the concentration of these neurochemicals in the synapse (Heikkila et al., 1975; Figure 1). However, DA has been the most studied monoamine and is highly involved in cocaine's actions. Cocaine's effects on DA re-uptake occur within the mesocorticolimbic pathway, which has been postulated as having a primary role in mediating the reinforcing properties of many drugs of abuse including cocaine, amphetamine, and opioids (Koob, 1992; see Figure 2). The circuitry that mediates the behavioral effects following acute psychostimulant administration include DArgic cell bodies which project from the ventral tegmental area (VTA) to the frontal cortex, NAc, and CPu as well as nigrostriatal dopaminergic

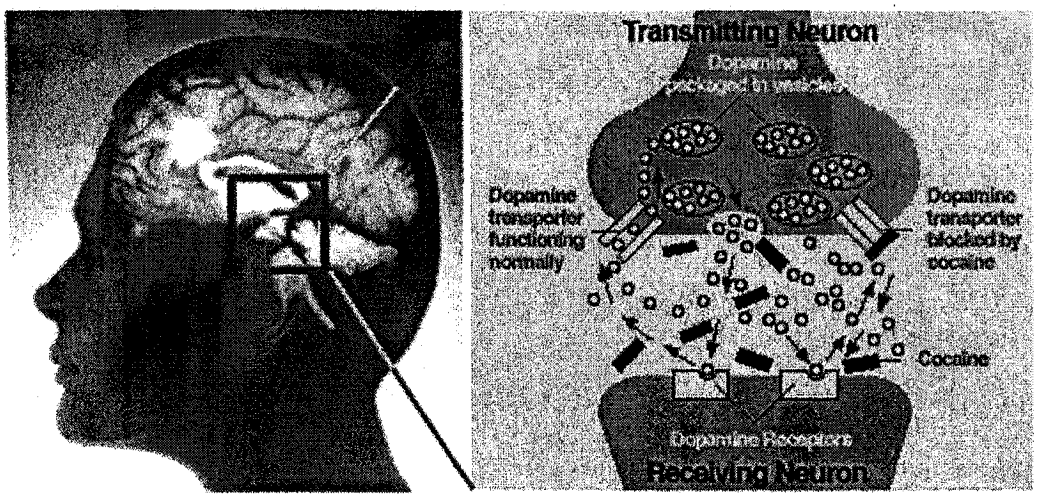
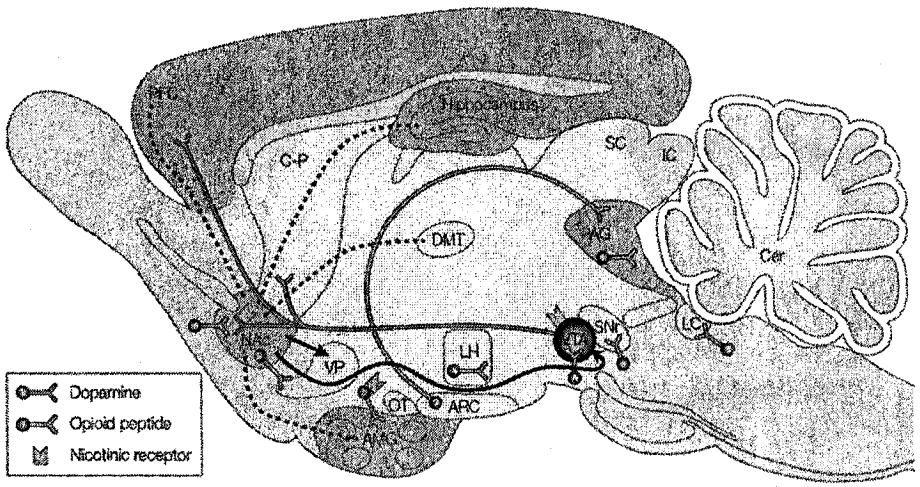


Figure 1: Cocaine's effects at the synaptic level. Under normal conditions, the DAT removes DA from the synapse. Cocaine binds to the transporter and increases DA in the synapse by preventing its re-uptake (N.I.D.A.).

projections to the dorsal striatum (Hyman and Malenka, 2001; Koob et al., 1993; Robbins and Everitt, 1996). In particular, VTA projections to the NAc have been postulated to play a pivotal role in the rewarding effects of cocaine. It has been shown that cocaine administered i.p. increases extracellular DA concentrations in the VTA and the NAc of freely moving rats, but with time course differences in DA release (Reith et al., 1997). In the NAc, DA activates DA receptors located on GABAergic medium spiny neurons projecting to the VTA to exert inhibitory control on DA firing (Nestler, 2001). It has been shown that chronic cocaine alters the normal physiological state by attenuating the GABA mediated inhibition, which in turn potentiates neuronal activity (Bonci and Williams, 1996), thus making GABA less effective at inhibiting midbrain DA neurons. It is generally assumed that changes within this neural circuit following chronic self-administration of cocaine may underlie



(Nestler et al., 2001)

Figure 2: The mesocorticolimbic DA system. DArgic cell bodies originate in the substantia nigra pars compacta and ventral tegmental area. These projections have targets in the forebrain including the pre-frontal cortex, striatum, amygdala, and hippocampus.

addiction (McFarland and Kalivas, 2001). There is a plethora of data indicating that there are semi-permanent cellular adaptations in these limbic nuclei after repeated administration of drugs of abuse (Nestler, 2001). It is still not well delineated as to how alterations in the mesolimbic circuit contribute to the craving and relapse seen in chronic drug users.

Cocaine's behavioral effects are mediated by the monoamines

Cocaine can stimulate a wide range of behaviors in rodents. For example, it has been demonstrated that an acute injection of cocaine induces hyperactivity and behavioral stereotypy in rodents. Cocaine also possesses discriminative stimulus properties and produces a robust place preference (McKenna and Ho, 1980; Tzschentke, 1998). Moreover, cocaine acts as a powerful reinforcer. Rats will readily self-administer cocaine on various schedules of reinforcement (Koob, 1992). Chronic intermittent administration of cocaine leads to a phenomenon known as behavioral sensitization. Behavioral sensitization is characterized as an increased locomotor response following chronic drug administration as compared to the first day of drug administration (Robinson and Berridge, 1993). Sensitization is of particular interest because the neuroadaptations that occur during the development of sensitization are thought to be similar to those which lead to an addictive state in humans (Robinson and Berridge, 1993).

Early evidence indicated that the mesocorticolimbic DA system was essential for cocaine's rewarding and psychomotor effects. For example, when DArgic neurons in the NAc are lesioned, cocaine-induced locomotor activity is abolished (Kelly and Iversen, 1976). The two classes of DA receptors, D1 and D2, are differentially distributed in the striatum. D1 receptors are located post-synaptically on striatonigral projection neurons (Gerfen et al., 1990). D2 receptors are located post-synaptically on striatopallidal

projection neurons (Gerfen et al., 1990) and also function as autoreceptors pre-snynaptically on DA terminals to modulate DA release and synthesis (Surmeier et al., 1996). Both D1 and D2 receptors have been shown to modulate cocaine-induced motor activity (for review, see Hummel and Unterwald, 2002). For example, D2 activation replaces cocaine-induced motor activity with stereotyped behaviors (Ushijima et al., 1995). It has been demonstrated that selective knockouts of D1 and D2 receptor, but not the D3 receptor, effectively reduce cocaine-induced hyperactivity (Glickstein and Schmauss, 2001). D1 receptor deletion also attenuates cocaine self-administration (Waddington et al., 2001). However, the D2 receptor is involved, but not required, for cocaine self-administration (Caine et al., 2002). D1 receptor, but not D2 receptor, antagonists are potent in blocking place preference for cocaine (Baker et al., 1998; Cervo and Samanin, 1995). D1 and D2 receptor antagonists have been shown to reduce cocaine self-administration in some studies, while D2 antagonists at low doses reduce feeding but not self-administration of cocaine (Caine and Koob, 1994; Hubner and Moreton, 1991). The role of the D4 and D5 DA receptors are less clear. Although, it has been shown that mice lacking the D4 receptor are supersensitive to the locomotor activating effects of cocaine and other drugs of abuse (Rubinstein et al., 1997). Recently, Uhl et al. (Uhl et al., 2002) demonstrated that selective knockout of the DA transporter also reduces cocaine-induced hyperactivity. This suggests that blockade of DA re-uptake at the DA transporter is essential for psychomotor activation by cocaine. The DA transporter, though necessary for cocaine-induced locomotion, is not required for self-administration

or induction of a place preference for cocaine (Uhl et al., 2002). Thus suggesting that other neurotransmitter pathways are involved in cocaine's reinforcing effects.

Serotonin has also been implicated in cocaine's behavioral effects. When both the DA transporter and the serotonin transporter are deleted in mice, place preference is abolished (Uhl et al., 2002). In a recent report it was demonstrated that antagonists of the serotonin (2a) receptor attenuate cocaine-induced locomotor behavior and discrimination (McMahon and Cunningham, 2001). Further studies implicated other serotonin receptor subtypes in cocaine's motor effects (Filip and Cunningham, 2000). Serotonin's role in cocaine-induced motor activity is not surprising, as it has been shown that serotonin cells innervate the mesocorticolimbic system at the levels of the VTA and NAc (Broderick and Phelix, 1997). Bubar et al. (Bubar et al., 2003) have recently demonstrated that serotonin re-uptake inhibitors injected into the NAc enhance cocaine's behavioral effects. Moreover, studies by Parsons and Justice (Parsons and Justice, 1993) and Parsons et al. (Parson et al., 1999) have demonstrated that accumbal elevation of serotonin modulates cocaine-induced DA efflux. However, it has also been demonstrated that 5-HT exerts both inhibitory and excitatory effects, depending on the 5-HT concentration, on DA cell bodies in the VTA (Liu et al., 2003). Taken together, these studies suggest a complex interaction of 5-HT and DA at the level of the striatum to regulate cocaine-induced activity.

III. Gonadal hormones provide the biological basis for sex differences in cocaine

abuse

Recent clinical and preclinical studies have invalidated the long-standing view of cocaine abuse as a problem predominately limited to males. The newest evidence suggests that females are more sensitive to the addictive properties of cocaine and could be more vulnerable to its powerful psychostimulating effects. Both clinical and preclinical studies demonstrate a sexually dimorphic pattern in the behavioral responses to cocaine in all phases of the addiction process, including initiation, maintenance, and relapse. A clearer picture is emerging which suggests that the biological basis of sex differences in cocaine addiction resides in a disparate regulation of the central nervous system (CNS) by male and female gonadal hormones. This chapter aims to discuss work from our laboratory and others demonstrating the biological basis of sex differences in the behavioral responses to cocaine.

Sex differences in cocaine use

Clinical studies show sex differences in cocaine's behavioral effects

Approximately 600,000 of the estimated 2 million Americans who use cocaine are woman (National Survey on Drug Use and Health, 2002). Although males are more likely than females to have opportunities to try cocaine, both males and females progress to actual use equally once exposure to cocaine occurs (Van Etten et al., 1999). Moreover, although no gender differences in rates of cocaine use in adulthood have been found, Chen and Kandel (2002) have recently demonstrated that cocaine use is higher in

adolescent females than in adolescent males. Adolescent females are also more likely than adolescent males to use cocaine at an earlier age with a greater frequency and to report more symptoms, such as "inability to cut down" and "need for larger quantities," at lower doses (Chen and Kandel, 2002). Interestingly, adult women report shorter abstinence periods between cocaine use than adult men (Kosten et al., 1996). Women experience more nervousness after internasal administration of cocaine (Kosten et al., 1996), take longer to feel the subjective effects of cocaine, report less euphoria and dysphoria as compared to men (Lukas et al., 1996), and have more severe drug use at intake (Kosten et al., 1993). Women also reported stronger craving in response to cocaine cues as compared to men (Robbins et al., 1999). These effects in women are most likely not due to differences in cocaine metabolism as it has been demonstrated that there is no sex difference in plasma cocaine levels after an acute intravenous dose (Mendelson et al., 1999). However, it is possible that other routes of administration could result in differential cocaine levels in males and females. Taken together, these studies suggest that the pattern of cocaine use and onset of addiction is more rapid in women than in men. Thus, these drastic gender differences in cocaine use patterns and behavioral responses indicate that the biological basis of addiction is sexually dimorphic.

Rodent studies addressing sex differences in cocaine-induced behavioral activity

As reviewed in Table I, sex differences in response to acute cocaine administration have been extensively studied. Overall, female rats have greater

locomotor and stererotypic behavior than male rats following acute cocaine administration (Chin et al., 2002; Sell et al., 2000; Van Haaren and Meyer, 1991; Walker et al., 2001a). Leret et al. (1994) and Tropp and Markus (2001) have suggested that higher baseline motor activity in female rats may contribute to the enhanced motor responsivity to acute cocaine administration. However, when closely examined, numerous reports have found no sex differences in behavior in saline-treated male and female rats (Glick et al., 1983; Sell et al., 2000; Walker et al., 2001a), suggesting that female hyperactivity after cocaine administration is a direct effect of cocaine rather than sex differences in overall baseline activity.

Walker et al. (2001a) demonstrated that ambulatory, rearing, and stereotypic behaviors following an acute cocaine injection are dose- and sex-dependent. It was demonstrated that higher doses of cocaine are required in male rats to achieve responses similar to those of female rats. This suggests that cocaine is less effective in producing hyperactivity in male rats. This in agreement with reported sex differences in cocaine's rewarding effects. Russo et al. (2003b) reported that female rats were more sensitive to the rewarding effects of cocaine through acquisition of conditioned place preference (CPP) at lower doses and at shorter conditioning lengths. Similarly, female rats acquired cocaine self-administration faster than males and at lower cocaine doses (Lynch and Carroll, 1999). Thus, cocaine is more potent in female rats at producing motor activity and establishing contiguous associations between environmental context and cocaine's

Table I. Sex differences in cocaine-induced behavior following acute or chronic cocaine administration

State of Animal		Male vs. Female Rats	Cocaine Dose	References
Acute Cocaine				
	↑	Females display greater cocaine-induced locomotor and/or stereotypic activity	15 mg/kg i.p.	*Chin et al., 2002*
			10, 20, 40 mg/kg i.p.	*Walker et al., 2001*
			5, 15 mg/kg i.p.	*Sell et al., 2000*
			1, 10 mg/kg s.c.	*van Haaren and Meyer, 1991*
			5,15,20,30 mg/kg i.p.	*Festa et al., 2003a, 2003b*
Chronic Cocaine				
	↑	Female rats have greater sensitizatized response to cocaine than male rats	15 mg/kg i.p.	*Chin et al., 2002*
			1, 10 mg/kg s.c.	*van Haaren and Meyer, 1991*
	Δ	Rotational behavior in female rats sensitizes to single cocaine injection	20 mg/kg i.p.	*Glick and Hinds, 1984*
		GDX vs. Intact		
Acute Cocaine				
Males	-	Did not affect cocaine-induced activity	15 mg/kg i.p.	*Chin et al., 2002*
			5, 10, 20 mg/kg i.p.	*Hu and Becker, 2003*
	↑	Cocaine-induced ambulatory activity	10, 20, 40 mg/kg i.p.	*Walker et al., 2001*
	↓	Cocaine-induced activity	1, 10 mg/kg s.c.	*van Haaren and Meyer, 1991*
Females	↓	Cocaine-induced ambulatory activity	10-40 mg/kg i.p.	*Chin et al., 2002*
			10, 20, 40 mg/kg i.p.	*Walker et al., 2001*
			1, 10 mg/kg, s.c.	*van Haaren and Meyer, 1991*
Chronic Cocaine				
Males	↓	Cocaine-induced activity as	15 mg/kg i.p.	*Chin et al., 2002*
	-	Rotational behavior in male rats does not sensitize	5, 10, 20 mg/kg i.p.	*Hu and Becker, 2003*
	-	Locomotor behavior in male rats did not sensitize to cocaine	1, 10 mg/kg, s.c.	*van Haaren and Meyer, 1991*
Females	↓	Cocaine-induced activity as compared to intact animals	15 mg/kg i.p.	*Chin et al., 2002*
			1, 10 mg/kg s.c.	*van Haaren and Meyer, 1991*

↑ Represents an increase in behavioral activity. ↓ Represents a decrease in behavioral activity.

Δ Represents an effect observed only in female rats.

rewarding properties.

Rodent studies addressing sex differences in behavior after chronic cocaine administration

Behavioral sensitization, defined as a progressive increase in motor behavior following repeated cocaine injections, produces long-lasting neural adaptations which are thought to be relevant to drug craving and addiction (Robinson and Berridge, 1993). As seen in Table I, unlike acute cocaine administration paradigms, studies addressing sex differences in the development and expression of behavioral sensitization have been limited. However, regardless of dose, injection frequency, or length of cocaine administration, female rats consistently demonstrated behavioral sensitization to cocaine (Chin et al., 2002; Glick et al., 1983; Van Haaren and Meyer, 1991). Chin et al. (2002) showed that both male and female rats express cocaine sensitized motor behavior following 14 days of chronic cocaine. However, other studies assessing behavioral sensitization to cocaine in male and female rats show that male rats do not develop sensitization. This effect can be attributed to the sensitization paradigm used. For example, Van Harren and Meyer (1991) showed that only female rats displayed behavioral sensitization following 10 days of cocaine administration (2 mg/kg, s.c.). Similarly, Glick and Hinds (1984) showed that 7 days following a single cocaine-injection (20 mg/kg, i.p.), female rats elicit higher cocaine-induced rotational behavior after a challenge dose while male rats do not display sensitization. After 7 days of

withdrawal from chronic cocaine treatment, female rats that received a cocaine challenge injection (15 mg/kg, i.p.) retained the sensitized motor response, while male rats showed a diminished response (Chin et al., 2002). However, no sex differences in behavioral stereotypy were observed following chronic cocaine administration, suggesting that locomotion and motor stereotypy respond differentially to chronic cocaine (Chin et al., 2002). Thus, not all behavioral responses to chronic cocaine are sexually dimorphic.

All studies taken together, observed sex differences in behavioral responses following acute and chronic cocaine administration demonstrate females as having augmented behavioral responses regardless of the dose or schedule of administration. This suggests that sex differences exist in all stages of the cocaine abuse process, including induction, maintenance, and relapse to cocaine use. Since this is consistent with the human literature, these results may reflect overall differences in the pattern of cocaine use between sexes.

Menstrual/Estrous cycle affects cocaine-induced behavior

Clinical studies demonstrate that menstrual cycle affects subjective responses to cocaine

The dynamic endocrinological profile of females has been shown to affect cocaine-induced responses in both clinical and pre-clinical studies. In humans, female cocaine users during the luteal phase had an attenuated subjective response to smoked cocaine and less desire to smoke cocaine as compared to those in the follicular phase

(Sofuoglu et al., 1999). Additionally, women also reported a greater "high or good feeling" during the follicular phase (Sofuoglu, et al., 1999). Since the luteal phase of the cycle is characterized by high levels of progesterone, these studies suggest that progesterone attenuates the subjective effects of cocaine.

Differences in cocaine pharmacokinetics during the menstrual cycle may account for some of the reported differences. For example, Lukas et al. (1996) reported that cocaine levels during the follicular phase were higher as compared to the luteal phase. However, Mendelsen et al. (1999) reported no differences in subjective effects across the luteal or follicular phases and found no differences in blood cocaine levels. Since the route of administration and dose of cocaine differed in each study (Lukas et al. (1996) administered cocaine intranasally, Mendelsen et al. (1999) administered cocaine i.v., while Sofuoglu et al. (2002) administered smoked cocaine), a side-by-side comparison of administration route and pharacokinetics is necessary to clarify these important clinical issues.

Rodent studies show cocaine-induced motor activity is dependent on stage of the estrous cycle.

As summarized in Table II, the estrous cycle influenced the behavioral response to acute cocaine administration in rats. Overall, most studies show that cocaine-induced

Table II. Effects of estrous cycle on cocaine-iduced activity following acute or chronic cocaine administration

	Result	Cocaine Dose	References
Acute Cocaine			
↑	Cocaine-induced locomotor and stereotypic activity in estrous vs. other stages	15 mg/kg i.p.(x 3)	*Quinones-Jenab et al., 1999*
↑	Cocaine-induced locomotor activity in estrous/proestrous vs. diestrous	5 mg/kg i.p.	*Sell et al., 2000*
-	(non-lavaged rats)	10 mg/kg i.p.	*Walker et al., 2002*
↓	Attenuates estrous cycle effects (lavaged rats)	10 mg/kg i.p.	*Walker et al., 2002*
Chronic Cocaine			
Δ	Cocaine disrupts estrous cyclicity dose-dependently	1-20 mg/kg s.c.	*King et al., 1993*
-	Cocaine does not disrupt estrous cyclicity	3 m/kg i.v.	*Booze et al., 1999*
↑	Sensitized in diestrous only compared to OVX females	15 mg/kg i.p.	*Sell et al., 2002*

↑ Represents an increase in behavioral activity. ↓ Represents a decrease in behavioral activity.

Δ Represents a disruption in estrous cycle cyclicity.

activity was lowest during diestrus after acute cocaine treatment (Quinones-Jenab et al., 1999; Sell et al., 2000; Walker et al., 2002). However, Quinones-Jenab et al. (1999) found higher locomotor activity in estrus than in proestrus, while Sell et al. (2000) and Walker et al. (2002) observed similar levels of behavioral activity during both estrus and proestrus after acute cocaine administration. Discrepancies between these studies may be attributed in part to either the frequency of cocaine injections (single vs. "binge") and/or the timing of cocaine administration during the proestrus phase since progesterone sharply increases at the end of proestrus.

Walker et al. (2002) showed that while the estrous cycle effects on behavioral responses after cocaine administration are attenuated by vaginal lavage, a single lavage prior to acute cocaine maintained estrous cycle effects on cocaine-stimulated behavioral responses. Moreover, as shown by Merlemstein and Becker (1995), repetitive vaginal lavage increases DA in the NAc. As a result of these findings, Walker et al. (2002) suggests that caution must be used when interpreting results from estrous cycle studies, since the methodology used to determine the stage of the cycle may affect the behavioral outcome produced by cocaine.

Estrous cycle affects cocaine-induced motor behavior after chronic cocaine administration

Unlike studies of acute cocaine administration, results from studies examining

estrous cycle effects during chronic cocaine administration have not yielded consistent results. For example, as summarized in Table II, chronic cocaine administration has been shown to either interrupt (King et al., 1990) or to have no effect on estrous cyclicity (Booze et al., 1999). However, Sell et al. (2000) and Booze et al. (1999) demonstrated that interruption of estrous cyclicity was dependent on the cocaine treatment regimen. Estrous cyclicity was maintained after a shorter schedule of cocaine administration (Sell et al., 2002). Sell et al. (2002) also demonstrated that sensitization to cocaine was only evident in females during diestrous.

Taken together, clinical and rodent studies indicate that hormonal fluctuations during the menstrual/estrous cycle modulate acute responses to cocaine. Furthermore, rodent studies illustrate that estrous cycle affects the expression of cocaine sensitization.

Effects of cocaine administration on the endocrinological profile of humans and rodents

In men and women, acute cocaine administration increases luteinizing hormone (LH) levels (Mendelson et al., 2001) and increases follicle stimulating hormone (FSH) in men (Heesh et al., 1996). However, acute cocaine treatment does not alter serum levels of testosterone in male subjects (Mendelson et al., 2003). Little is known about cocaine's effects on the endocrinological profile of females.

In the rodent model, cocaine has been shown to decrease testosterone levels in male rats after acute and chronic treatment (Chin et al., 2002; Quinones-Jenab et al., 2000d) while others have shown no change in testosterone levels following an acute cocaine injection (Walker et al., 2001b). Cocaine transiently increases LH, but not FSH, in female rats (King et al., 2001). Furthermore, cocaine increases plasma levels of progesterone following acute and chronic administration in male and female rats (Chin et al., 2002; Quinones-Jenab et al., 2000b; Walker et al., 2001b). In female rats, levels of cocaine-induced progesterone fluctuate with the estrous cycle (Quinones-Jenab et al., 2000c; Walker et al., 2001b). Although acute cocaine administration does not affect plasma levels of estrogen, it has been shown that when estrogen levels are at their highest, cocaine-stimulated progesterone release is greatest (Walker et al., 2001b).

Cocaine's direct effects on gonadal hormones in both clinical and rodent studies, as well as cocaine's interaction with the menstrual/estrous cycles, strongly suggest gonadal hormones provide the basis for sex differences in the behavioral responses to cocaine. To determine the role of gonadal hormones in cocaine-induced activity, two approaches have been utilized in rodents. First, studies have examined the role of endogenous hormones through gonadectomy (GDX) and side-by-side comparisons with intact rats, and secondly the individual contributions of testosterone, estrogen, and progesterone, have been determined by hormone replacement in GDX rats.

The role of endogenous gonadal hormones in cocaine-induced motor behaviors

The effects of endogenous gonadal hormones in acute and chronic cocaine-induced responses are sexually dimorphic

As shown in Table III, GDX of female rats either decreased or had no effect on behavioral responses to acute cocaine (Chin et al., 2002; Walker et al., 2001a) respectively. On the other hand, GDX of male rats either increased or had no effect on behavioral responses to acute cocaine (Chin et al., 2002; van Luijtelaar et al., 1996; Walker et al., 2001a). Discrepancies in the results of these studies may be due to the cocaine dose. For example, Chin et al. (2002) used 15 mg/kg, while Walker et al. (2001) used 10, 20, or 40 mg/kg of cocaine, the major effects of GDX on horizontal and stereotypic activity were seen at 10 mg/kg. Furthermore, it has been shown that female Fischer rats (Chin et al. study) have greater hyperactivity in response to acute cocaine as compared to Sprague-Dawley rats (Walker et al. study) (Sircar and Kim, 1999).

Similar to the observed results in behavioral activity after acute cocaine administration, chronic administration in GDX male and female rats did not alter cocaine-induced responses in a consistent manner (Table III). For example, Chin et al. (2002) showed that 14 days of cocaine injections reduced ambulatory behavior in male and female GDX rats as compared to intact controls, while castration reduced cocaine-induced stereotypic behavior (Chin et al., 2002). However, a challenge injection of cocaine caused an induction of stereotypy in castrated male rats suggesting that

Table III. Effects of gonadal hormones on cocaine-induced activity following acute or chronic cocaine administration

Acute Cocaine		GDX vs. Hormone Replacement	Cocaine Dose	Hormone Dose	References
Female Rats					
Estrogen	↑	Cocaine-induced activity as compared	5 mg/kg i.p.	Silastic Implant	*Sell et al., 2000*
			15 mg/kg i.p.	Silastic Implant	*Perrotti et al., 2000*
	-	Does not affect cocaine-induced activity	10 mg/kg i.p.	Silastic Implant	*Peris et al., 1991*
			15 mg/kg i.p.	2ug E s.c.	*Sicar and Kim, 1999*
			15 mg/kg i.p.(x 3)	50ug E s.c.	*Quinones-Jenab et al., 2000*
			5-20 mg/kg i.p.	5ug E s.c.	*Hu and Becker, 2003*
Progesterone	↓	Cocaine-induced activity	5 mg/kg i.p.	Silastic Implant	*Sell et al., 2000;*
	-	Does not affect cocaine-induced activity	10 mg/kg i.p.	Silastic Implant	*Peris et al., 1991*
			15 mg/kg i.p.	500ug P s.c.	*Sicar and Kim, 1999*
			15 mg/kg i.p.	500ug P s.c.	*Perrotti et al., 2000*
			15 mg/kg i.p.(x 3)	500ug P s.c.	*Quinones-Jenab et al., 2000*
E + P	↑	Cocaine-induced activity as	5 mg/kg i.p.	Silastic Implant	*Sell et al., 2000;*
			15 mg/kg i.p.	2ug E/500ug P s.c.	*Sicar and Kim, 1999*
	-	Does not affect cocaine-induced activity	10 mg/kg i.p.	Silastic Implant	*Peris et al., 1991*
	↑↓	Inhibited cocaine-induced activity following 1st injection, increased activity following multiple injections	15 mg/kg i.p.(x 3)	50ug E/500ug P s.c.	*Quinones-Jenab et al., 2000*
Male Rats					
Testosterone	↓	Cocaine-induced activity as compared to intact GDX rats	20-80 mg/kg i.p.	100 mg Implant	*Long et al., 1994*

Chronic Cocaine		GDX vs. Hormone Replacement			
Female Rats					
Estrogen	↑	Sensitization to cocaine as compared to OVX females	15 mg/kg i.p.	Silastic Implant	*Perrotti et al., 2000*
			10 mg/kg i.p.	Silastic Implant	*Peris et al., 1991*
			15 mg/kg i.p.	Silastic Implant	*Sell et al., 2002*
			5-20 mg/kg i.p.	5ug E s.c.	*Hu and Becker, 2003*
			15 mg/kg i.p.	2ug E s.c.	*Sircar and Kim, 1999*
Progesterone	↓	Cocaine-induced sensitization	15 mg/kg i.p.	500ug P s.c.	*Sircar and Kim, 1999*
E + P	↑	Cocaine-induced sensitization compared to OVX females	15 mg/kg i.p.	2ug E/500ug P s.c.	*Sircar and Kim, 1999*
			15 mg/kg i.p.	E Implant/500ug P s.c.	*Perrotti et al., 2000*
Male Rats					
Testosterone	↓	Cocaine-induced sensitzation as compared to GDX males	24 mg/kg i.p.	2 mg/kg s.c.	*Chen et al., 2003*

↑ Represents an increase in behavioral activity. ↓ Represents a decrease in behavioral activity.
↑↓ Represents changes in behavioral activity across "binge" injections of cocaine.

behavioral stereotypy can sensitize to cocaine independently of testosterone (Chin et al., 2002). In female rats, OVX did not affect vertical activity (rearing and stereotypy) (Chin et al., 2002). These results show that endogenous gonadal hormones regulate certain components of the behavioral response to chronic cocaine in both sexes, while other behaviors, particularly vertical behavior, may be mediated by different neuronal substrates. Hu and Becker (2003) report that sham and castrated males do not develop sensitized rotational behavior in response to a cocaine challenge injection. Other reports find that intact and castrated males sensitize to cocaine, but castration reduces (Chin et al., 2002) or increases the response (Sorg et al., 2002). These discrepancies could be based on differences in sensitization paradigms or the length of time the animal is castrated (Pfaff and Schwartz-Giblin, 1995).

GDX of male and female rats affects the endocrinological response to cocaine

GDX of male rats has been shown to attenuate cocaine-induced increases of testosterone after both acute and chronic administration (Chin et al., 2002; Quinones-Jenab, 2000d). GDX in female rats is also associated with a significant decrease in progesterone levels following acute administration (Walker et al., 2001b). However, there is still a significant induction of progesterone following cocaine administration in GDX females. Following removal of the adrenal glands, cocaine-stimulated progesterone was abolished, suggesting that the adrenal gland may contribute to cocaine-stimulated progesterone (Walker et al., 2001b).

Taken together, after both acute and chronic cocaine administration, GDX of female rats, decreases behavioral stimulation of cocaine overall, while in male rats these effects are not consistent (no changes, increases, or decreases have been observed). Thus, adaptations following GDX in male rats results in inconsistent behavioral responses, while removal of estrogen and progesterone in female rats seems to be more directly involved in potentiating cocaine's behavioral effects.

Gonadal hormone replacement modulates cocaine-induced behaviors

Clinical studies show that exogenous hormone administration affects subjective responses to cocaine

A recent clinical study demonstrated that oral contraceptives which contain both estrogen and progesterone do not change the response to intranasally administered cocaine (Kouri et al., 2002). However, Sofuoglu et al. (2002) reported that an acute dose of progesterone administered prior to smoked cocaine administration results in an attenuation of cocaine's subjective effects as compared to placebo-treated females. More recently, Sofuoglu et al. (2003) has shown that progesterone administration attenuates the subjective effects of cocaine in male subjects as well. Progesterone treatment did not reduce cocaine self-administration in male or female subjects (Sofuoglu et al., 2003). Moreover, progesterone treatment did not alter other parameters, such as blood pressure and heart rate (Sofuoglu et al., 2002; Sofuoglu et al., 2003). This suggests that while exogenous administration of both estrogen and progesterone may not produce changes in

the subjective response to cocaine, progesterone administered alone may reduce cocaine's rewarding effects to a certain extent.

Rodent studies show that gonadal hormone replacement affects cocaine-induced motor activity after acute cocaine administration

As shown in Table II, much of the current research to determine the role of ovarian hormones has focused on estrogen's effects on cocaine-induced motor behavior. For example, estrogen replacement via silastic capsules has consistently been shown to potentiate cocaine's behavioral effects after an acute injection (Perrotti et al., 2001; Sell et al., 2000). On the other hand, pulsatory estrogen administration via subcutaneous injections does not affect the overall response to acute cocaine (Hu and Becker, 2003; Mori et al., 1994; Sircar and Kim, 1999). These differential effects of estrogen may be attributed to the method of estrogen replacement. While estrogen replacement via silastic capsules provide relatively steady levels of estrogen, subcutaneous (s.c.) injections produce more variability in levels of estrogen. This may induce differential modifications of neuronal activity (genomic vs. non-genomic effects). Therefore, the method of estrogen replacement may underlie the variability in the observed effects across these studies.

Fewer reports have considered the role of progesterone as a modulator of cocaine-induced behaviors. However, similar to estrogen, the method of progesterone

replacement determines its effect on cocaine-induced responses. For example, Quinones-Jenab et al. (2000), Perrotti et al. (2001), and Sicar and Kim (1999) have demonstrated that when progesterone is acutely injected four hours prior to acute cocaine administration, there is no effect on cocaine-induced locomotor and stereotypic behavior. However, when progesterone is administered via silastic capsules, a significant reduction in cocaine-induced activity was observed (Sell et al., 2000). Interestingly, Russo et al. (Russo et al., 2003a) have recently demonstrated that progesterone and estrogen administered via silastic capsules inhibited and potentiated, respectively, cocaine-induced CPP in female rats. Preliminary results from our lab suggest that in intact animals, progesterone inhibits the acquisition and expression of cocaine-induced CPP in female, but not male, rats (unpublished findings).

Co-administration of estrogen and progesterone have produced consistent results irrespective of administration route, across all studies, estrogen and progesterone replacement produced an observed augmentation of cocaine-induced activity as compared to OVX females (Quinones-Jenab et al., 2000c; Sell et al., 2000; Sircar and Kim, 1999). Moreover, co-administration of estrogen and progesterone potentiate cocaine CPP (Russo et al., 2003a).

Testosterone replacement in male rats attenuates cocaine-induced behavioral stereotypy and locomotion (Chen et al., 2003; Long et al., 1994). Furthermore, Chen et

al. (2003) suggest that testosterone's effects may be due to an interaction with cocaine-induced alterations in the mesocortical DA system.

Rodent studies suggest that ovarian hormone replacement affects cocaine-stimulated responses following chronic cocaine administration

The role of estrogen and progesterone replacement in behavioral sensitization to cocaine has been extensively studied (see Table III). Using different concentrations and replacement paradigms, estrogen has been reported to enhance the behavioral effects of cocaine (see Fig. 4) (Hu and Becker, 2003; Perrotti et al., 2001; Sell et al., 2002; Sircar and Kim, 1999). For example, Sircar and Kim (1999) show that estrogen (given 48 and 24 hours prior to cocaine administration) increases behavioral activity in female rats. Hu and Becker (2003) also show that estrogen enhances rotational behavior following a cocaine challenge injection. Sell et al. (2002) found that sensitization to cocaine occurred in both OVX and estrogen-treated rats at 13 and 34 days following withdrawal, but not at 3 days post-withdrawal (Sell et al., 2002). Moreover, sensitization to challenge injections of cocaine on days 13 and 34 was higher in estrogen treated female rats that received either cocaine or saline (Sell et al., 2002). However, the magnitude of sensitization was the same in both OVX and OVX+estrogen treated animals as compared to their respective control groups. Thus, as suggested by Sell et al. (2002) estrogen may merely enhance sensitization to cocaine causing an additive effect, possibly accounting for their higher activity as compared to male rats.

The effects of progesterone and estrogen+progesterone replacement during chronic cocaine administration have not been sufficiently studied. Using the same progesterone dose and administration route, Perrotti et al. (2001) and Sircar and Kim (1999) demonstrated that progesterone did not affect behavioral responses following chronic cocaine administration. Studies that co-administer estrogen and progesterone have shown consistently augmented behavioral sensitization to cocaine as compared to rats that receive either estrogen or progesterone alone (Sircar and Kim, 1999, Perrotti et al., 2001).

Overall, acute and chronic cocaine administration studies demonstrate that the method of hormone replacement affects cocaine-induced responses and estrogen increases, testosterone reduces, and progesterone reduces or has no effect on cocaine-induced activity in rodents. Although clinical studies have been limited, the inhibitory effects of progesterone are consistent in both clinical and rodent studies.

Current state of the hormone replacement literature

Despite advances in the research of sex differences in cocaine abuse, many questions remain. For example, it is unclear how differences in hormone concentrations, administration paradigm and the time length following GDX affect cocaine-induced responses. Moreover, since both hormones circulate simultaneously at different levels across the reproductive cycle, we do not fully understand the extent to which hormone

concentrations and combinations modulate behavioral responses in humans and rats. Studies delineating how these dynamic hormonal interactions affect cocaine-induced responses are therefore necessary. The effects of estrogen on cocaine-induced behavioral responses remain to be elucidated since there is a possible interaction between exogenous estrogen and adrenally released progesterone. Finally, there have been reports of neural adaptations at different time points following GDX (Pfaff and Schwartz-Giblin, 1995). This suggests that exogenously administered hormones may produce differential behavioral outcomes depending on when the animal is tested following GDX. This important variable has not been studied extensively.

Clinical implications and conclusions

Important clinical implications can be drawn from the studies discussed in this overview. Recently, female adolescents have emerged as an important group considered to be at increased risk for cocaine abuse. Following alcohol, cocaine is the second most common drug found in the system of persons treated in the emergency room (ER). From 1999 to 2000, total drug ER visits increased 20 percent for patients age 12 to 17 (from 52,783 to 63,448) and 13 percent for patients age 18 to 25 (from 109,580 to 123,438). Total drug-related ER visits involving females increased 9 percent (from 258,079 to 281,994) between 1999 and 2000, but were statistically unchanged for males (SAMHSA, 2000). These statistics demonstrate the importance of studying the biological basis of sex differences in substance abuse. Moreover, the use of estrogen- or progesterone-based

contraceptives could affect the way a woman experiences the subjective effects of cocaine. Further investigations delineating the regulation of cocaine-induced responses by gonadal hormones are needed to develop sex-specific treatments for cocaine addiction.

IV. Mechanisms modulating sex differences to acute and chronic cocaine administration

Sex differences in monoaminergic systems

There are extensive sex differences in the organization of the CNS. Sex differences in monoaminergic pathways have been postulated to underlie sex differences in drug-induced behaviors. Sex differences in both DA and 5-HT systems exist (reviewed in Becker, 1999; Klink et al., 2002). For example, in the NAc, female rats have lower D1 DA receptor levels while in the CPu, female rats have greater striatal DA release and re-uptake than male rats (Andersen et al., 1997; Walker et al., 2000). It has recently been shown that there are differences in firing rates of 5–HT neurons in the dorsal raphe nucleus where male rats have higher spontaneous firing rates and lower GABAergic tonic inhibition of 5-HT firing than those of female rats (Klink et al., 2002). Additionally, female rats also have lower levels of 5-HT_{2A} receptor mRNA in the hypothalamus and greater levels in the hippocampus (Zhang et al., 1999). Becker (1999) has postulated that enhanced DA release in the CPu of female rats could account for the observed sex differences in cocaine-induced activity. It is possible, as suggested by Becker (1999), that the endocrinological profile of female rats contributes to the

downregulation of D_2 autoreceptors in the VTA which results in enhanced DA release in the CPu.

It has also been shown that there are sex differences in basal levels of 5-HT (Carlsson and Carlsson, 1988). Carlsson and Carlsson (1987) demonstrated that sex differences in baseline levels of 5-HT were more prevalent than in the DA system suggesting the likelihood that sex differences in basal 5-HT levels have functional consequences (i.e., a greater potential to modulate monoaminergic function), a suggestion made by other groups as well (Broderick and Phelix, 1997). Cocaine-stimulated activity in female rats may be in part influenced by higher basal serotonin levels, thus, contributing to a more robust DA efflux in the mesoaccumbens pathway. Indeed, Bubar et al. (2003) have recently demonstrated that serotonin re-uptake inhibitors injected into the NAc enhance cocaine's behavioral effects in male rats. Sex differences in serotonin's modulation of DA activity remains to be determined.

Ovarian hormones regulate monoaminergic pathways

Estrogen has been shown to modulate several aspects of mesocorticolimbic DA pathways. Estradiol increases stimulated DA release in the CPu and NAc (Becker, 1999), and decreases DA clearance (Thompson et al., 2000) and uptake (Thompson, 1999) in the NAc. Estrogen priming has been shown to increase or decrease DA transporter levels (Attali et al., 1997; Morissette and Di Paolo, 1993b), increases D1 DA receptors

(Levesque et al., 1989), increase striatal and accumbal D2 DA receptors (Levesque and Di Paolo, 1993; Shimizu and Bray, 1993), and affects receptor affinity for D2 DA receptors (Levesque and Di Paolo, 1988). Furthermore, DA release in the NAc peaks during diestrous and release and re-uptake of DA are highest during proestrous (Thompson, 1999), and DA receptors fluctuate across the estrous cycle (Levesque et al., 1989). These results suggest that regulation of the mesocorticolimbic DA system by estrogen is likely to regulate, in part, cocaine-induced behavioral activity in females.

There is evidence that progesterone has opposite effects on DA systems. Progesterone has been shown to attenuate estrogen-induced increases in DA activity in striatal and limbic DA projections (Fernandez-Ruiz et al., 1990). Motor behaviors induced by cocaine can also be blocked by progesterone antagonists and anti-sense microinjected into the third ventricle (Apostolakis et al., 1996). Acute progesterone treatment does not change DA transporter density (Morissette and Di Paolo, 1993a) but dose decrease DA metabolism in the striatum (Di Paolo et al., 1986). Moreover, DA release in the NAc is attenuated by progesterone administration, but restored by concurrent estrogen and progesterone administration (Saigusa et al., 1997). These results suggest that although progesterone may have inhibitory effects on DA pathways when administered alone, estrogen and progesterone replacement togther can potentiate DA activity.

Serotonergic systems are also modulated by estrous cycle and through estrogen and progesterone replacement. For example, in the NAc, 5-HIAA/5-HT turnover was decreased during proestrus, however, progesterone replacement in female rats did not affect 5-HT levels (Shimizu and Bray, 1993). 5-HT binding to cortical membranes also varied with stage of the estrous cycle where the lowest binding found during proestrus and the highest binding during estrus (Uphouse et al., 1986). Estrogen replacement in female rats has been shown to affect mRNA levels of many 5-HT receptor subtypes. For example, estrogen decreases 5-HT1A, 1B, 2A, and 2C receptor mRNA levels in the amygdala, and increases 5-HT1B amd 5-HT2C receptor mRNA levels in the midbrain (Zhou et al., 2002). However, Zhou et al. (2002) suggests that these estrogen effects on 5-HT receptors are temporally dependent. OVX causes a decrease in striatal DA uptake sites and this decreases is restored by estrogen, estrogen + progesterone treatment, but not progesterone treatment alone (Attali et al., 1997).

Taken together, these studies show that ovarian hormones in the female rat regulate monoaminergic pathways. Thus, the hormonal profile of the female rat may underlie sexually dimorphic responses following acute and chronic cocaine administration.

Hypothesis and specific aims of the thesis

The mesocorticolimbic DA system, particularly DArgic projections to the CPu

and NAc, has been postulated to mediate cocaine's motor effects. The aims of this thesis postulate that sexual dimorphisms in the mesocorticolimbic DA pathway underlie sex differences in the motor response to acute cocaine administration. These sex differences may occur at the neurochemical level, the receptor activation level, or at the level of cellular mRNA and protein expression. Sex differences in the DA system may be present at the organizational level or may occur following acute cocaine administration. Thus, the goal of this research is to elucidate sex differences within this pathway. The specific aims of the research presented here are:

Specific Aim One: To determine if sex differences in cocaine-induced motor behavior are dependent on injection frequency, cocaine dose, and cocaine metabolism. Moreover, we will determine if there are sex differences in DA levels by examining baseline and cocaine-induced alterations in the CPu and NAc.

Specific Aim Two: To determine if sex differences in the locomotor response to cocaine are mediated by D1 and/or D2 receptors. To this end, we will use D1- and D2-like receptor antagonists to investigate this hypothesis.

Specific Aim Three: To determine if there are sex differences in baseline and/or cocaine-induced levels of D1 and D2 receptor mRNA and binding.

CHAPTER 2: FREQUENCY OF COCAINE ADMINISTRATION AFFECTS BEHAVIORAL AND ENDOCRINOLOGICAL RESOPNSES IN MALE AND FEMALE FISCHER RATS.

Accumulating evidence suggests that cocaine affects men and women differently. In the clinical literature, sexual disparities in cocaine's physiological and subjective effects as well as rates of addiction have been reported (Lukas et al., 1996). Recent research has also indicated sex differences in cocaine's behavioral and endocrine effects in rodents. Acute cocaine administration stimulates greater locomotor activity in female rats as compared to male rats (Chin et al., 2002; Van Haaren and Meyer, 1991; Walker et al., 2001a). Female rats acquire cocaine self-administration more quickly, have higher rates of self-administration, and are more sensitive in the reinstatement of self-administration (Lynch and Carroll, 2000). Moreover, female rats acquire a place preference for cocaine faster and at lower doses than male rats. At the endocrinological level, cocaine-induced release of progesterone and corticosterone is higher in female than in male rats, indicating differences in the endocrine response to cocaine (Chin et al., 2002; Kuhn and Francis, 1997; Walker et al., 2001b).

In male rats, the frequency of cocaine administration has been previously demonstrated to affect cocaine-induced behavioral and molecular alterations (Post, 1980; Unterwald et al., 1994b; Unterwald et al., 1994a; Unterwald, 1995; Unterwald et al., 2001). For example, Segal and Kucsensky (1997) reported that "binge" cocaine

administration produces burst-like behavioral activity while single administration produces a steadier behavioral activation. However, it is not clear if sex differences in behavioral responses to cocaine are also affected by the frequency of cocaine administration. The aim of the current study is to determine if the frequency of cocaine administration affects sex differences in the behavioral and endocrinological responses to cocaine. Since adolescent females have reported more frequent use of cocaine than males (Chen and Kandel, 2002), a better understanding of sex differences in behavioral and endocrinological responses following different cocaine administration frequencies in rodents may advance our understanding of mechanisms underlying sex differences in rates of addiction.

Materials and Methods

Animals

Eight-week-old male and female Fischer rats purchased from Charles River (Kingston, NY) were individually housed in standard cages for one week prior to the experiment with free access to food and water and maintained on a 12-hour light/dark cycle with lights on at 9:30 a.m. Animal care was in accordance with the *Guide for the Care and Use of Laboratory Animals* (NIH publication 865-112, Bethesda, MD) and approved by the Institutional Animal Care and Use Committee.

Drugs

Male and female rats (n=6-8/ group) were randomly assigned to single-saline, single-cocaine, binge-saline, or binge-cocaine treatment groups. Thirty minutes after the lights were turned on, rats received a single injection of cocaine (i.p, 15 mg/kg) or saline, or three injections (one hour apart) of either cocaine (i.p, 15 mg/kg) or saline. This binge administration pattern was chosen to parallel previously published results in male rats (Unterwald et al., 1994a; Yuferov et al., 1999; Zhou et al., 1998). The dose used for the single injection of cocaine was the equivalent to one injection of the "binge" administration paradigm. For acute administration, animals were sacrificed 30 minutes or three hours after a single cocaine or saline injection. For "binge" administration, animals were sacrificed 30 minutes following the last injection.

Locomotor activity

Locomotor activity was monitored for three hours following treatment in each rat's home cage with a Photobeam Activity System from San Diego Instruments (San Diego, CA) which records vertical and horizontal activities. Ambulatory activity represents the number of counts produced by two consecutive photobeam interruptions in the lower frame. Rearing activity represents total counts of vertical motion as recorded by the upper frame.

Testosterone and progesterone radioimmunoassys

Thirty minutes after cocaine/saline administration, rats were sacrificed by

decapitation, following a brief exposure (30 seconds) to CO_2, and trunk blood was collected. Blood was allowed to clot and centrifuged at 3,000 RPM for 15 minutes at 4 °C. Plasma was collected and stored at -80° C until analyzed by radioimmunoassay (RIA) using Coat-A-Count kits for progesterone and testosterone (National Diagnostic, San Diego, CA). Intra-assay coefficients of variation averaged 10.0± 1.0%. Results for these assays were determined using a log-logit computer program. Progesterone and testosterone plasma levels are expressed as ng/ml.

Data Analysis

For locomotor activity, three-way ANOVAs were used to determine the effects of drug treatment (saline vs. cocaine) x sex (male vs. female) x time after drug administration. One-way ANOVAs were used to determine the effect of time on ambulatory and rearing activities within cocaine-treated groups in both single and binge conditions. ANOVAs were followed by Newman-Keuls *post-hoc* tests if appropriate. Each behavioral measurement was analyzed separately. For hormone measurements, dependent measure t-tests were used to assess cocaine's effects on plasma levels of testosterone in male rats and progesterone in male and female rats. Significance in all cases was considered to be $p<0.05$.

Results

Sex differences in locomotor activity after single and "binge" pattern administration

As seen in Figures 3 and 4, both single and "binge" pattern cocaine administration increased ambulatory activity as compared to their respective saline-treated groups [$F(1, 20)=8.23$, $p=0.0095$; $F(1, 27)=49.38$, $p=0.0001$, respectively]. Overall, cocaine-treated females had the highest ambulatory counts after single or "binge" cocaine administration when compared their respective saline controls and male rats that received either single or "binge" cocaine/saline administration ($p<0.05$ for all comparisons). Furthermore, a significant interaction between sex, drug, and time on ambulatory activity after single cocaine administration was observed [$F(2, 40)=7.95$, $p=0.0012$; Figure 3] where male rats had a time-dependent decrease in ambulatory behavior [$F(1, 5)=11.77$, $p=0.0020$; Figure 3B].

As seen in Figures 5 and 6, single and "binge" pattern cocaine administration increased rearing activity as compared to their respective saline-treated controls [$F(1, 20)=32.85$, $p=0.0001$; $F(1, 27)=319.42$, $p=0.0001$, respectively]. A significant interaction between sex, drug, and time after cocaine administration was observed for both single and binge-pattern administration [$F(2, 40)=3.82$, $p=0.0302$; $F(2, 54)=10.85$, $p=0.0001$, respectively]. Overall, after both administration paradigms, females had higher cocaine-induced rearing behavior when compared to their respective saline controls and male rats that received either single or "binge" cocaine/saline treatment ($p<0.05$) for all comparisons). Furthermore, there was a significant decrease in rearing activity across time after single cocaine administration in both male and female rats [$F(1, 5)=8.01$,

p=0.0080; F(1, 5)=21.35, p=0.0001, respectively; Figure 5]. Alternatively, after "binge" cocaine administration, female rats had a time-dependent increase in rearing activity across injections [F(1, 7)=9.38, p=0.0030, Figure 6A] while in males there were no significant changes across injections (p>0.05).

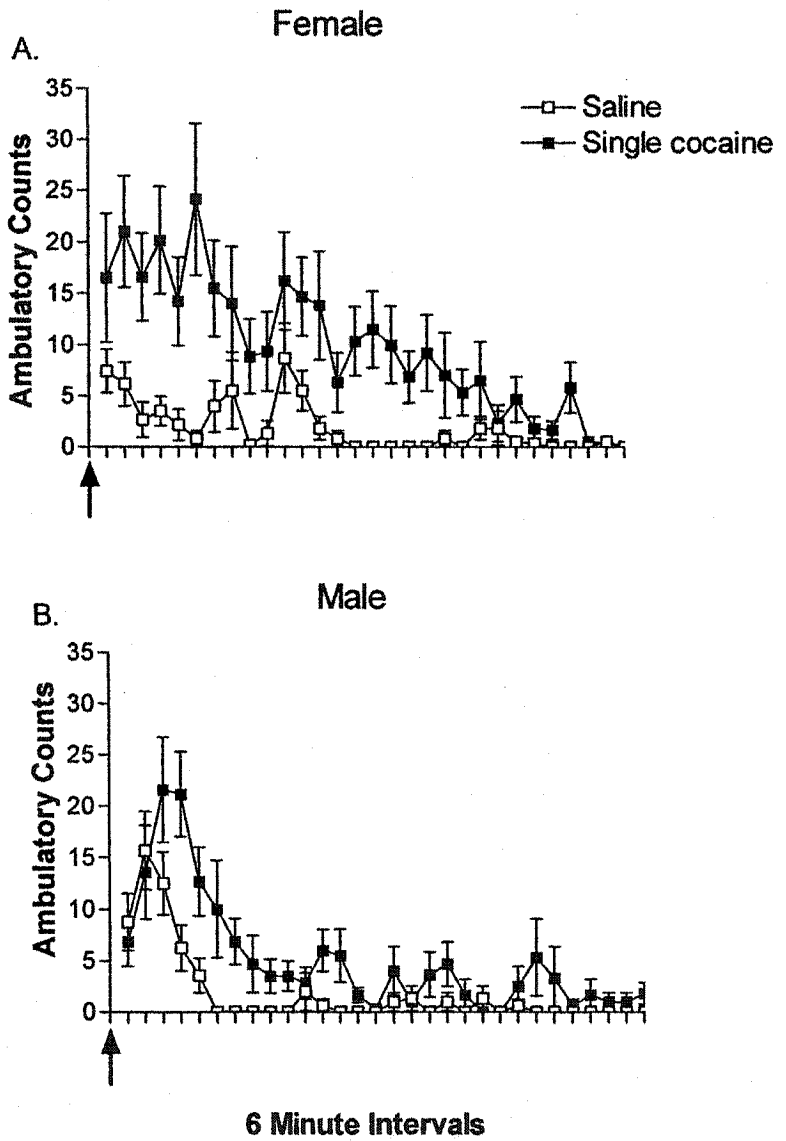


Figure 3 Ambulatory counts of female (A) and male (B) Fischer rats after a single cocaine (i.p., 15 mg/kg) or saline injection. Behavior was measured for three hours and is represented in 6-minute intervals. The values represent mean ± SEM. Arrows indicate time of injections.

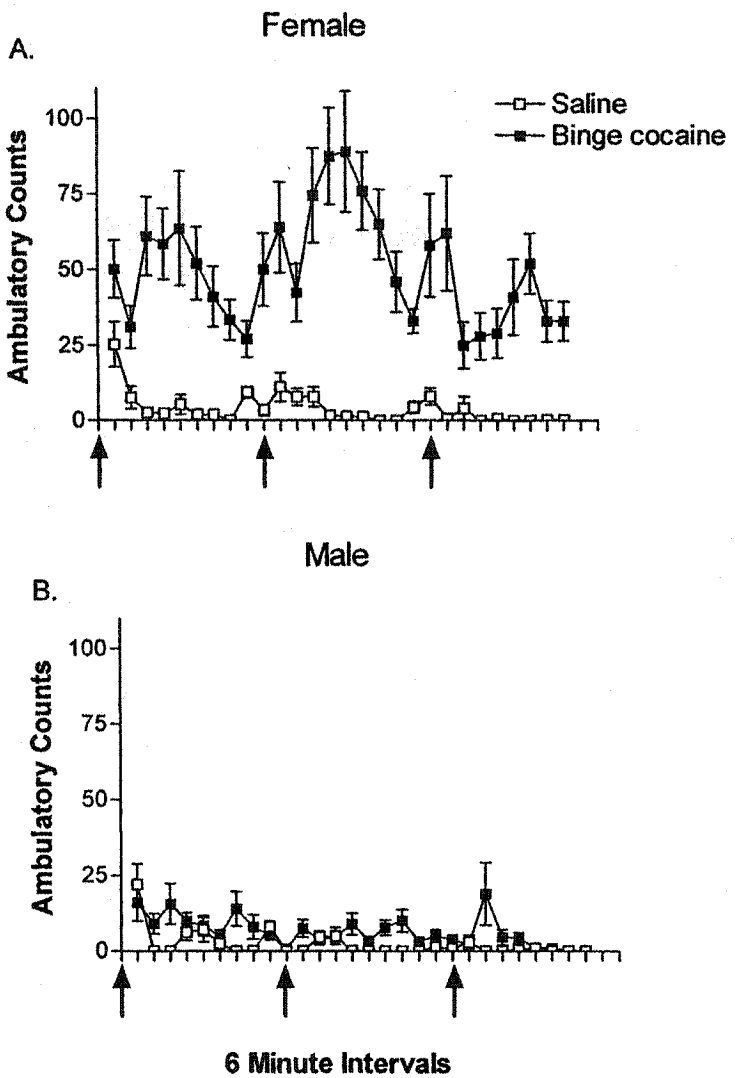


Figure 4 Ambulatory counts of female (A) and male (B) Fischer rats after binge-pattern cocaine (3 x 15 mg/kg/, i.p., 1 hour apart) or binge-pattern saline administration. Behavior was measured for three hours and is represented in 6-minute intervals. The values represent mean ± SEM. Arrows indicate time of injections.

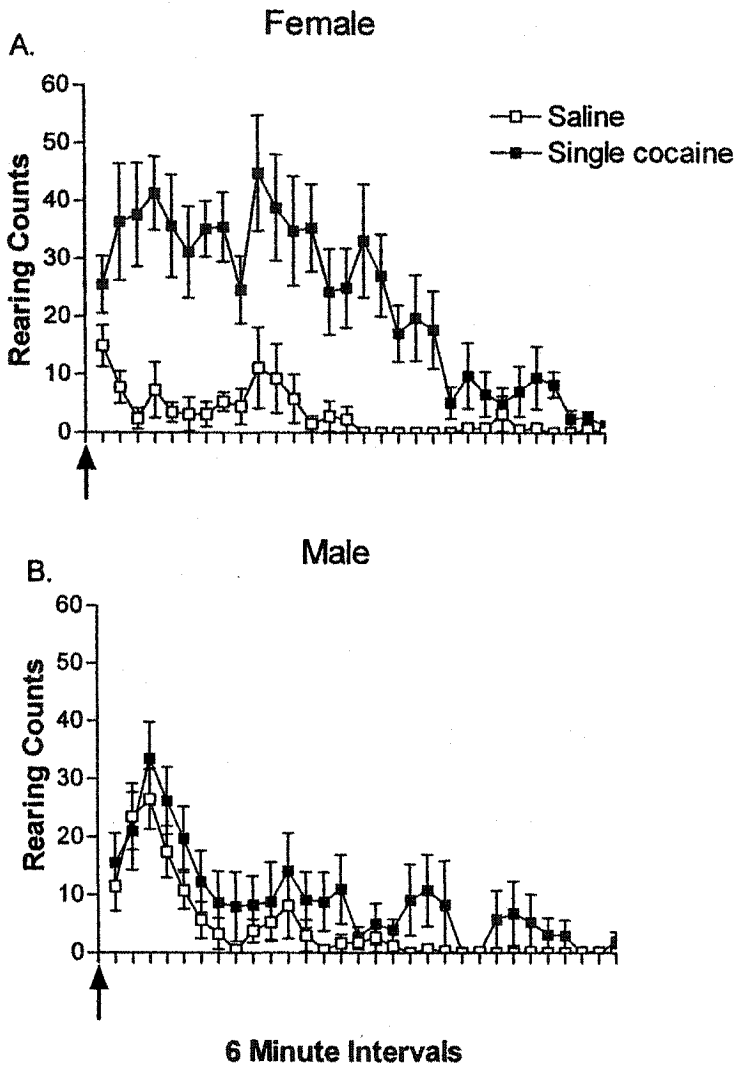


Figure 5 Rearing counts of female (A) and male (B) Fischer rats after a single cocaine (i.p., 15 mg/kg) or saline injection. Behavior was measured as described in Figure 1. The values represent mean ± SEM. Arrows indicate time of injections.

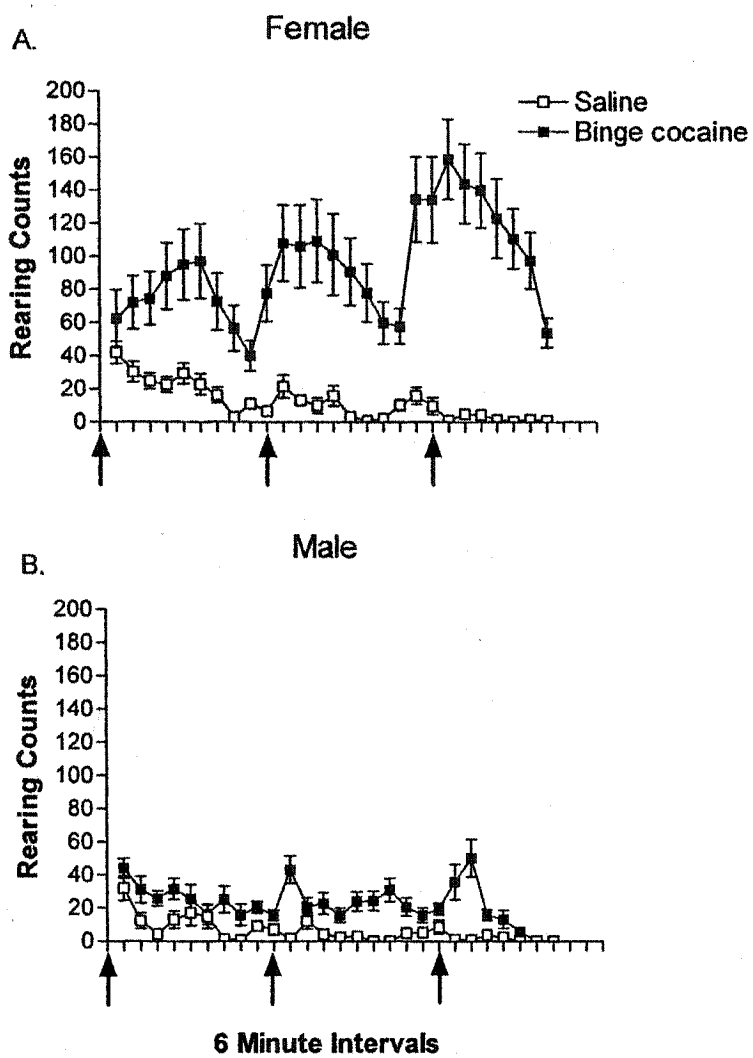


Figure 6 Rearing counts of female (A) and male (B) Fischer rats after binge-pattern cocaine (3 x 15 mg/kg/, i.p., 1 hour apart) or binge-pattern saline administration. Behavior was measured as described in Figure 2. The values represent mean ± SEM. Arrows indicate time of injections.

Single and "binge" cocaine affect testosterone and progesterone levels

As shown in Figure 7, testosterone plasma levels were significantly decreased in male rats after "binge" pattern cocaine administration when compared to saline-treated controls (p=0.005, Figure 7A). Testosterone levels also were significantly decreased in cocaine-treated animals 30 minutes after an acute single dose of cocaine when compared to controls (p=0.002, Figure 7B). However, 3 hours after a single cocaine administration, testosterone plasma levels had returned to levels comparable to those of the saline-treated group (p=0.082, Figure 7B). In saline- and cocaine-treated female rats, testosterone levels were not detectable (data not shown).

Progesterone plasma levels were significantly higher in male Fischer rats after "binge" pattern cocaine administration than in saline-treated animals (p=0.003, Figure 7C). Unlike testosterone plasma levels, progesterone plasma levels were not significantly increased following a single dose of cocaine either at 30 minutes or 3 hours after cocaine injection (Figure 7D). However, in female rats, cocaine increased progesterone levels following "binge" cocaine administration (p=0.033, Figure 8A). Following a single injection of cocaine, progesterone levels were increased 30 minutes (p=0.001, Figure 8B) but not 3 hours (p=0.702, Figure 8B) following treatment.

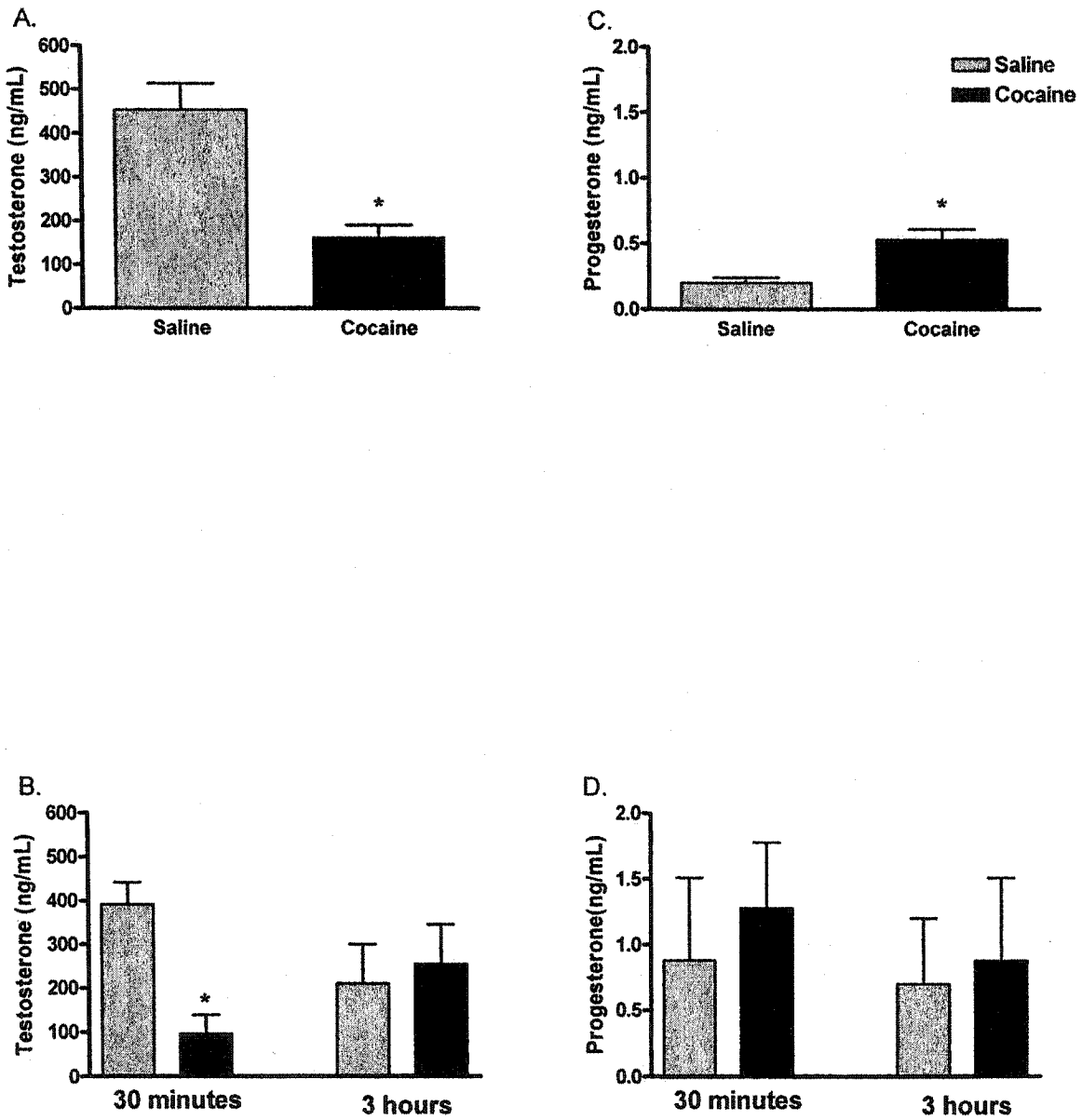


Figure 7 Plasma levels of (A) testosterone and (C) progesterone in male rats after "binge" pattern cocaine or saline administration (3 x 15 mg/kg/, i.p., 1 hour apart). Plasma levels of (B) testosterone and (D) progesterone in male rats after an acute administration of 15 mg/kg cocaine or saline. Animals received a single i.p. injection and were sacrificed 30 minutes or three hours later. The values represent mean + S.E.M.

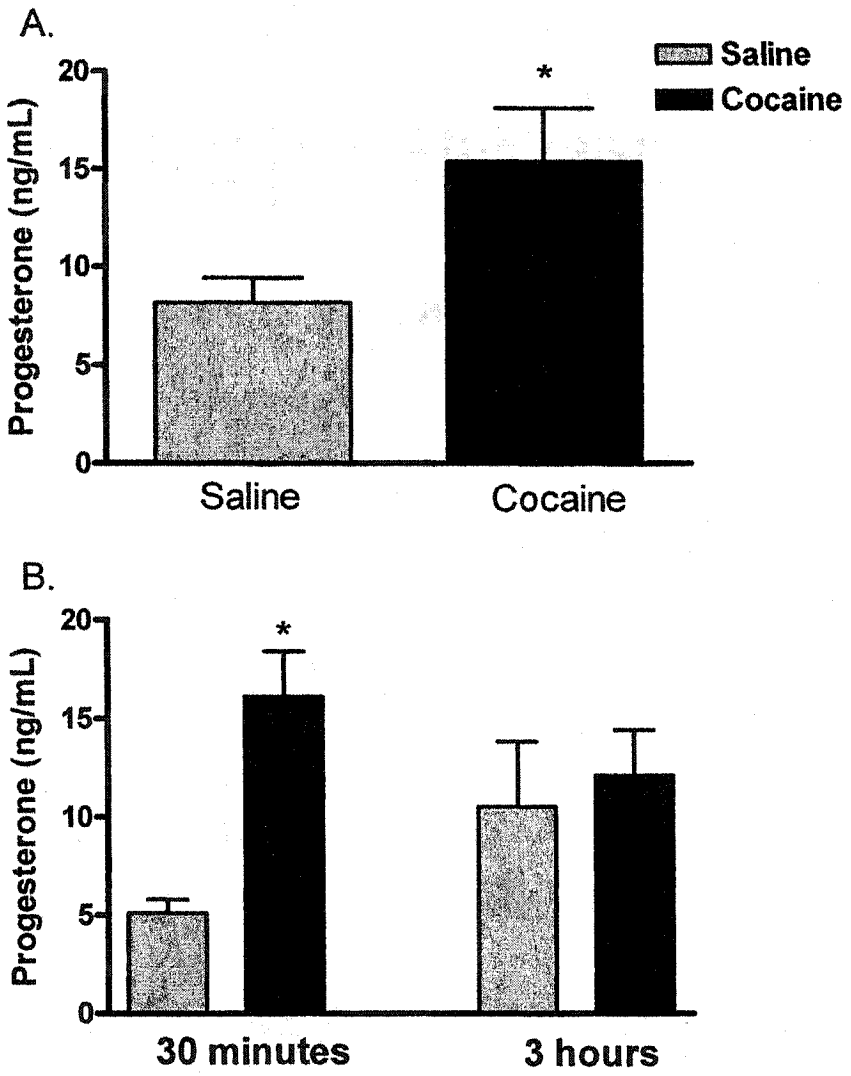


Figure 8 (A) Progesterone levels in female rats after saline or "binge" pattern cocaine administration (3 x 15 mg/kg/ i.p.,1 hour apart). (B) Plasma levels of progesterone in female rats after an acute administration of 15 mg/kg cocaine or saline. Animals received a single i.p. injection and were sacrificed 30 minutes or three hours later. The values represent mean + S.E.M.

Discussion

In the current study we demonstrated that sex differences in the behavioral and endocrine effects induced by cocaine were impacted by the frequency of cocaine administration. In agreement with prior studies, after single cocaine administration, female rats have greater locomotor activity than do male rats (Chin et al., 2002; Sell et al., 2000; Walker et al., 2001a). However, our results extend these observations by demonstrating that in female rats, cocaine-induced behavioral activation is more prolonged and robust than in males. For example, less than an hour after single cocaine administration, male behavioral activity was not statistically significant when compared to that of control groups. In contrast, female behavioral activity was sustained two and a half hours after a single injection of cocaine. This, in turn, may provide female rats with a longer temporal period to experience cocaine's psychomotor and/or rewarding effects. Russo et al. (2003b) reported sex differences in cocaine-induced conditioned place preference where female rats were more sensitive to the rewarding effects of cocaine through acquisition of conditioned place preference at lower doses and shorter conditioning lengths. Thus, the prolonged cocaine-stimulated responses in female rats at lower doses may facilitate a more rapid establishment of contiguous associations between environmental context and cocaine's rewarding properties.

Similar to single cocaine administration, "binge" pattern cocaine administration in female rats led to significantly higher ambulatory and rearing activity when compared to

male rats. Upon further inspection of the cocaine-induced behavioral patterns, we observed ambulatory and rearing counts returned to control levels in male rats after each "binge" injection, whereas in female rats, these activities did not return to that of controls. Interestingly, cocaine-induced behavioral activity in male rats remained stable across injections (period of behavioral activation followed by a return to baseline). In contrast, female rats had a more complex behavioral profile where after the third injection of cocaine, rearing counts were significantly increased while ambulatory activity significantly decreased. This differential behavioral pattern in female rats may represent the rapid development of sensitization or tolerance to specific behavioral components following repeated "binge" cocaine injections.

Consistent with previous reports in intact and pregnant female rats (Quinones-Jenab et al., 2000a; Quinones-Jenab et al., 2000b), "binge" pattern cocaine administration significantly increased plasma progesterone levels in male rats. This is consistent with other reports of male rats in which plasma progesterone levels were increased after amphetamine treatment (Budziszewska et al., 1996) and other drugs of abuse (Walker et al., 2001b). Although in female rats it has been shown that an acute injection of cocaine is sufficient to increase plasma progesterone (Quinones-Jenab et al., 2000b), this was not observed in male rats. Thus, male Fischer rats may need higher doses of cocaine to affect progesterone secretion, and/or the modulation of progesterone may follow a different time-course of activation than in female rats. Interestingly, Walker et al. (Walker et al.,

2001b), using the same dose as this study, reported an increase in progesterone levels in male Sprague-Dawley rats using the same dose administered here. Discrepancies between these two studies suggest that there may be strain differences in cocaine's modulation of progesterone plasma levels.

In male rats, testosterone levels were decreased after "binge" and single-dose cocaine administration. Testosterone levels returned to control levels 3 hours after cocaine administration, suggesting that cocaine's effect on testicular hormones is transient. Interestingly, Berul and Harclerode (Berul and Harclerode, 1989) hypothesized that cocaine's effect on testosterone levels is a direct effect of testosterone synthesis and/or secretion since the levels of lutenizing hormone never increase above the level of control animals. The fact that testosterone plasma levels were decreased after cocaine administration is consistent with previous reports that DA plays an inhibitory role in the control of testosterone secretion (Edwards et al., 1980). The current report suggests that similar to female rats, endocrine responses in the reproductive system are also affected by the frequency of cocaine administration in male rats. Taken together, modulation of the reproductive system by different cocaine administration frequencies may contribute to the observed sexually dimorphic behavioral responses. The role of testosterone and progesterone induced alterations on other endocrinological responses, such as corticosterone release, remain to be elucidated.

Neurobiological mechanisms modulating sex differences in the behavioral responses to cocaine are poorly understood. However, it is well established that the reproductive hormones function in the brain to regulate neuronal activity and influence behavior through their actions on the DA, serotonin, and opioid systems (Di Paolo et al., 1979; Hull et al., 2002; Lauber et al., 1990; Pfaus and Pfaff, 1992; Romano et al., 1989; Xiao and Becker, 1994). It is likely that an interaction between cocaine and these endogenous systems plays an integral part in the cascade of events that is involved in the sexually dimorphic behavioral responses we observed. Further studies on possible mechanisms underlying these sex differences are currently underway.

CHAPTER 3: SEX DIFFERENCES IN COCAINE-INDUCED BEHAVIORAL RESPONSES, PHARMACOKINETICS, AND MONOAMINE LEVELS.

Cocaine, a psychoactive alkaloid, has a variety of pharmacological actions, however, its major effects in the central nervous system are mediated through the pre-synaptic inhibition of DA (DA), serotonin (5-HT), and norepinephrine (NE) re-uptake. The resulting increase in synaptic concentrations of these monoamines causes heightened locomotor behavior in rodents. It has been postulated that mesocorticolimbic DA projections from the ventral tegmental area (VTA) to the NAc (NAc) and nigrostriatal projections to the caudate/putamen (CPu) mediate cocaine-induced locomotion and reward/reinforcement (reviewed in Hyman and Malenka, 2001; Spanagel and Weiss, 1999). However, emerging evidence suggests that tonic regulation of DA neurotransmission by serotonergic innervation within this pathway is also critical in mediating certain behavioral effects of cocaine (Bubar et al., 2003; Parsons and Justice, 1993, Parsons et al., 1999).

An extensive body of literature describes sex differences in cocaine's behavioral and neuroendocrine effects in rat models. For example, female rats are more sensitive to cocaine-induced psychomotor stimulation (i.e., greater locomotor behavior and stereotypy), hypothalamic-pituitary-adrenal axis (HPA) activation, and cocaine's rewarding effects (Chin et al., 2001; Chin et al., 2002; Sell et al., 2000; Walker et al., 2001a; van Haaren and Meyer, 1991; Russo et al., 2000b). However, mechanisms

modulating sex-dependent responses to cocaine remain unclear.

Sex differences in both DA and 5-HT systems exist (reviewed in Becker, 1999; Klink et al., 2002). For example, in the NAc, female rats have lower D1 DA receptor levels while in the CPu, female rats have greater striatal DA release and re-uptake than male rats (Andersen and Teicher, 2000; Becker, 1999; Walker et al., 2000). Moreover, female rats have higher basal levels of 5-HT and its metabolite 5-hydroxyindole acetic acid (5-HIAA) in the NAc and CPu (Carlsson and Carlsson, 1988). It has recently been shown that there are differences in firing rates of 5–HT neurons in the dorsal raphe nucleus where male rats have higher spontaneous firing rates and lower GABAergic tonic inhibition of 5-HT firing than those of female rats (Klink et al., 2002). Additionally, female rats also have lower levels of 5-HT_{2A} receptor mRNA in the hypothalamus and greater levels in the hippocampus (Zhang et al., 1999). To date, few studies have addressed the DA/5-HT systems as potential mediators of sex differences in cocaine's behavioral effects. Therefore, elucidating where intrinsic/drug-induced sex differences in DA/5-HT tone reside may provide a clearer understanding of sex differences in vulnerability to using and subsequently abusing cocaine.

Alternatively, sex differences in cocaine pharmacodynamics may also impact behavioral activity. Bowman et al. (1999) reported that although there were no sex differences in brain and blood levels of cocaine, brain and blood levels of ecgonine

methyl ester (EME) were higher in female rats while male rats had higher blood BE levels. However, these observations have not been consistently reproduced. For example, van Harren et al. (Van Haaren and Meyer, 1997) reported that sex differences were not detected in serum BE levels after acute or chronic cocaine administration while our group demonstrated that female rats have higher serum BE levels than do male rats after acute cocaine administration (Chin et al., 2001). Moreover, no studies have addressed whether there are sex differences in the production of norcocaine, an active cocaine metabolite that binds to the monoamine transporters (Einhorn et al., 1988). As a result of the conflicting reports on cocaine metabolism, and the lack of norcocaine data, a more complete analysis of sex differences is necessary to determine if dissimilarities in motor activity are affected by bioactive metabolites. This study aims to determine the impact of sex on baseline and cocaine-induced monoaminergic levels as well as pharmacokinetic differences and their contribution to sex differences in cocaine-stimulated behaviors. A systematic study concerning these aspects of cocaine-mediated responses would provide a better understanding of possible mechanisms that contribute to sex differences in cocaine abuse.

Materials and Methods

Animals and Housing

Eight-week-old male and female Fischer rats purchased from Charles River (Kingston, NY) were individually housed in a noise minimized facility for one week prior

to experimental manipulations in standard plastic cages (20cm x 20cm x 41cm) layered with beta chips. Rats had free access to standard lab chow and water ad libitum. Rats were maintained on a 12-hour light/dark cycle with lights on at 9:00 a.m. On testing days, only the experimenter had access to the animal room. Three separate cohorts of animals were used for behavioral studies (n=7 in male rat groups with cocaine doses of 0 and 5 mg/kg, while all other groups contained 8 animals). For cocaine metabolism (one cohort) and neurochemistry studies (three cohorts), experimental groups contained 6-14 animals (see Tables II and III for number of animals per experimental group). In order to acclimate the rats to the experimental procedure, they were pre-handled and weighed on day 6. A recent report demonstrated that repeated vaginal lavage attenuates cocaine-induced activity, abolishes estrous cycle effects, and establishes a place preference in female rats (Walker et al., 2002). Furthermore, vaginal lavage requires the insertion of a dropper into the vagina and results in increased DA release in the striatum and may alter neurochemical measurements (Mermelstein and Becker, 1995). As suggested by Walker et al. (2002), the use of lavaged female rats could result in inaccurate behavioral responses when making a side-by-side comparison to male rats. Thus, animals were placed into groups at random without regard to estrous cycle. Animal care was in accordance with the Guide for the Care and Use of Laboratory Animals (NIH publication 865-112, Bethesda, MD) and approved by the Institutional Animal Care and Use Committee of Hunter College.

Drugs and Chemicals

Cocaine hydrochloride and chemicals for high-performance liquid chromatography (HPLC) were purchased from Sigma Chemical Co. (St. Louis, MO). Mobile phase for HPLC was purchased from ESA (Milford, MA). Internal standards for Liquid Chromatography-Tandem Mass Spectrometry (LC/MS/MS) were purchased from Cerilliant (Austin, TX). All solvents for LC/MS/MS were HPLC grade and were purchased from Burdick and Jackson (Muskegon, MI). Potassium hydroxide and concentrated hydrochloric acid were obtained from Mallinckrodt Specialty Chemicals, Inc. (Paris, KY). Ammonium hydroxide was purchased from Fisher Co. (Fair Lawn, NJ). Formic acid was purchased from J.T. Baker (Phillipsburg, NJ).

Cocaine Administration

Cocaine solutions were prepared daily by dissolution in physiological saline (0.9%) and injected intra-peritoneal (i.p.) in a volume of 1 mL/kg. All injections were performed in the home cage 30 minutes after lights were turned on. To establish an optimal dose for use in subsequent experiments, male and female rats were randomly assigned to cocaine (5, 15, 20, or 30 mg/kg) or saline groups. Rats were sacrificed three hours after a single injection of cocaine or saline. For cocaine metabolism determinations, a separate cohort of male and female rats each received a single injection of cocaine (20 mg/kg) and was subsequently sacrificed at 15, 45, or 60 minutes post-injection. To elucidate the role of sex, drug, and time on monoamine levels in the CPu

and the NAc, a third cohort of male and female rats was sacrificed at 15 or 45 minutes after a single injection of cocaine (20mg/kg) or saline.

Behavioral Measurement

Total locomotor, ambulatory, and rearing activities were monitored in the home cages with a Photobeam Activity System from San Diego Instruments (San Diego, CA) as previously described (Chin et al., 2002). There were a total of 16 frames which recorded locomotor activity in 6-minute bins. For each cohort of animals (n=24-30) behavior was recorded across 2 days (16 animals per day). Animal groups were balanced for sex and cocaine dose across all cohorts. Behavioral activity was recorded for 3 hours post-injection. Total locomotor activity represents the sum of counts in the horizontal frame. Ambulatory activity represents the number of counts produced by two consecutive photobeam interruptions in the horizontal frame. Rearing activity represents total counts of vertical motion. For stereotypic activity, rats were videotaped with a hand-held camcorder (30 cm away from the homecage) for 45 seconds 30 minutes after cocaine or saline administration. This time point was chosen based on prior results that demonstrated no statistically significant differences in stereotypy scores across time points of 15, 30, or 45 minutes post cocaine injection (Quinones-Jenab et al., 1999, Chin et al., 2002, Perrotti et al., 2000). The videotapes were later analyzed for behavioral stereotypy by three trained observers blind to each animal's treatment group. The rating for cocaine-induced stereotypic behaviors is based on a modified version of the Creese

and Iversen (1974) scale. This scale consists of 10 ranked scores: (1) asleep or inactive, (2) alert/actively grooming, (3) increased sniffing in one location, (4) intermittent rearing/sniffing, (5) increased locomotion/sniffing, (6) intense sniffing in one location, (7) continuous pivoting/sniffing, (8) continuous rearing/sniffing, (9) maintained rearing/sniffing, (10) splayed hind limbs. Throughout the study a score of 10 was never observed.

Extraction and LC/MS/MS

Following decapitation, brains were removed and trunk blood was collected in tubes containing K_2 EDTA and immediately placed on ice upon collection. Blood was then centrifuged at 3,000 RPM for 20 minutes at 4°C, and the serum separated. Rat brains were rapidly frozen in methylbutane (-40°C). Both brain tissue and serum were stored at -80°C. Cocaine and its metabolites were extracted as previously described with the following modifications (Lin et al., 2001). Briefly, rat brains were weighed and homogenized in a measured volume of 0.1M sodium phosphate buffer (pH 7.0). A solution containing cocaine-d_3, BE-d_3, EME-d_3, and norcocaine-d_3 was added to each sample of rat brain homogenates and serum to give a concentration of 25 ng/mL of each internal standard. Four mL of 0.1M acetate buffer (pH 4.0) was added to the homogenate and serum samples. After vortex mixing and centrifugation at 2400 rpm for 10 minutes, the supernatants were subjected to solid-phase extraction (SPE). Immediately following SPE, the retained analytes were eluted with 3 mL of methylene

chloride/isopropanol/NH_4OH (80:20:2) and the extracts were collected and dried. The residues were reconstituted with 100 μl of methanol/0.1% formic acid in Milli-Q water (10:90) and transferred to autosampler vials.

LC/MS/MS was performed as previously described with the following modifications (Lin et al., 2001). Briefly, analyses were performed using a ThermoQuest model TSQ 7000 triple-stage quadruple MS equipped with an Xcalibur data system (San Jose, CA). The LC system consisted of a Waters 600S controller and an inline degasser (Milford, MA). The LC was interfaced to the MS by means of an electrospray ionization source. Approximately 20 µL of each extracted sample was injected into the LC/MS/MS. The LC was operated isocratically with a flow rate of 150 μL/min, and the mobile phase consisted of 50% methanol and 50% water with 0.1% formic acid. MS conditions for the TSQ 7000 were as follows: corona current, 5 μA, corona voltage, 4.5 kV, vaporizer temperature, 375°C, heated capillary tube temperature, 150°C, and sheath gas pressure, 20 psi. The following selected reaction monitoring (SRM) transitions were used to quantitate the analytes and internal standards [cocaine: m/z 304 to 182, cocaine-d_3: m/z 307 to 185, BE and norcocaine: m/z 290 to 168, BE-d_3 and norcocaine-d_3: m/z 293 to 171, EME: m/z 200 to 182, EME-d_3 m/z 203 to 185]. Although the SRM transitions are the same for BE and norcocaine, each metabolite had a different retention time. Before each run, the instrument performance was evaluated by injecting 20 μL of check solution consisting of 10 pg/μL each of cocaine, BE, EME, norcocaine, and the corresponding

internal standards. Data were quantified using Xcaliber's LCquan software. Brain and serum levels of cocaine, BE, EME, and norcocaine are expressed as ng/g of tissue and ng/mL of serum.

HPLC

Following decapitation brains were rapidly removed and coronal slices (1 mm) were cut out in a matrix as previously described (Jenab et al., 2002). The CPu and NAc were dissected out (1.90 mm to 0.90 mm anterior to Bregma) on a cold glass plate and immediately frozen at -80°C. Total monoamine and metabolite levels were determined by HPLC analysis using a previously described method (Perrotti et al., 2000; Renner and Luine, 1984) with the following modifications. Prior to homogenization, tissue was stored for less than one month at -80°C. Briefly, brain tissue samples were homogenized in 1 ml sodium acetate buffer (pH=5.0) + internal standard (3,4-dihydroxybenzylamine) + ascorbate oxidase and preserved overnight (4°C). With this protocol, it has been shown that there is limited degradation of DA, 5-HT, and their respective metabolites if samples are stored overnight under these conditions in a sodium acetate buffer (see Renner and Luine, 1984). On the following day, samples were centrifuged at 13,000 rpm for 30 minutes and the supernantant was collected. To determine total protein content, tissue pellets were re-suspended in 0.01 M sodium hydroxide and analyzed using a Bradford protein assay kit (Bio-Rad Laboratories, CA).

All experimental samples for each brain area were analyzed simultaneously. Twenty μl of the supernantant was injected into a Waters Associates chromatographic system, containing a refrigerated 717 plus auto sampler (4°C) and 1525 binary pump. In line was a MD-150/RP C_{18} column with MD-TM mobile phase at pH 2.3 (ESA, Chelmsford, MA) pumped through the system. For electrochemical detection, an ESA Coulchem II detector with the screening electrodes set at -150 mV, the detecting electrode at +325 mV, and the guard cell set at +400 mV was used. Two and one-half μl of each standard solution [DA, homovanillic acid (HVA), and internal standard were dissolved in 25 ml of .01 M $HCLO_4$. 5-HT, 3,4 dihydroxyphenylacetic acid (DOPAC), and 5-hydroxyindole acetic acid (5-HIAA) were dissolved in 25 ml of saline] was combined with 25 ml of sodium acetate (pH 5.0). Concentrations of DA, DOPAC, HVA, 5-HT, 5-HIAA, and their respective turnover ratios were calculated with reference to standards using peak integration with Breeze software (Waters Associates, MA). Under the described conditions, the sensitivity of the system was approximately 500 fg (3:1 signal/noise) to detect the monoamines. Monoamine levels are expressed as pg/μg of protein.

Statistical Analysis

Total locomotor, ambulatory, and rearing data are presented in 6-minute bins as mean ± standard error of the mean (SEM). Stereotypic data is presented as median score ± semi-interquartile range. Three-way ANOVAs (ANOVAs) were conducted to

determine the effects of time interval (repeated measures factor), cocaine dose, and sex on total locomotor, ambulatory, or rearing activity as follows: time interval (30 6-minute time bins) x cocaine dose (saline or 5, 15, 20, & 30 mg/kg cocaine) x sex (male vs. female). Greenhouse-Geisser corrections adjustments were used for repeated measures ANOVAs. Behavioral data were then collapsed across time bins and separate ANOVAs were used to determine the effects of cocaine dose and sex on total locomotor, ambulatory, or rearing activity as follows: cocaine dose (saline or 5, 15, 20, & 30 mg/kg cocaine) x sex (male vs. female) and to assess the effects of sex (male vs. female) and time (15, 45, or 60 minutes) on brain or serum levels of cocaine, BE, EME, and norcocaine. Three-way ANOVAs were used to determine the effects of cocaine, sex, and length of treatment on levels of DA, 5-HT, and their metabolites as follows: cocaine (cocaine or saline) x sex (male vs. female) x time after treatment (15 or 45 minutes). A Kruskall-Wallis ANOVA followed by a Dunn's *post-hoc* test were used to assess the effects of sex or cocaine treatment on stereotypic behavior. For all other determinants, Newman-Keuls *post-hoc* tests were used when appropriate. Significance in all cases was considered to be $p<0.05$.

Results

Effects of Dose on Sex Differences in the Behavioral Response to Cocaine

In both male and female rats, a main effect of cocaine dose was observed where cocaine increased all behavioral activity measured [Total Counts: Males: $F(4,33)=3.12$,

p=0.0277; Females: F(4,35)=11.55, p=0.0001; Ambulatory Counts: Males: F(4,33)=3.54, p=0.0164; Females: F(4,35)=8.75, p=0.0001; Rearing Counts: Males: F(4,33)=9.26, p=0.0001, Females: F(4,35)=13.53, p=0.0001; Fig. 9, Fig. 10, and Fig. 11, respectively]. However, cocaine's dose-dependent effects varied according to the rat's sex and behavioral activity. For example, in male rats, cocaine-induced increases in total locomotor activity were seen at 20 mg/kg as compared to saline-treated rats while in female rats, 15, 20, and 30 mg/kg cocaine increased activity as compared to saline-treated rats (Males: p=0.0107; Females: p=0.0234, p=0.0004, p=0.0002, respectively; Fig. 9C). A cocaine dose by sex interaction was observed for total locomotor counts [F(4,68)=2.73, p=0.0365; Fig. 9C] where females showed higher cocaine-stimulated total locomotor counts at 15, 20, and 30 mg/kg than those of male rats (p=0.0497, p=0.0042, p=0.0001, respectively). Furthermore, there was a significant interaction of time and sex where, overall, total locomotor activity in female rats persisted over a longer time frame as compared to male rats [F(29,1972)=6.97, p=0.0001; Figs. 9A and 9B]. There were no statistically significant differences between saline-treated male and female rats for total locomotor counts.

As shown in Figure 10C, in male rats, only 30 mg/kg of cocaine increased ambulatory activity while female ambulatory activity was increased after 20 and 30 mg/kg of cocaine as compared to their respective controls (Males: p=0.0276; Females: p=0.0034, p=0.0002, respectively). A cocaine dose by sex interaction was also observed

for ambulatory activity [F(4,68)=2.63, p=0.0412]. Females displayed higher ambulatory counts after 20 and 30 mg/kg cocaine administration than those of male rats (p=0.0095, p=0.0006, respectively). As with total locomotor activity, there was a significant interaction of time and sex where, overall, ambulatory activity in female rats persisted over a longer time frame as compared to male rats [F(29,1972)=6.08, p=0.0001; Figs. 10A and 10B]. There were no sex differences in saline-treated rats for ambulatory activity.

In both male and female rats, rearing activity increased after administration of 15, 20, and 30 mg/kg cocaine as compared to controls (Males: p=0.0145, p=0.0102, p=0.0004, respectively; Females: p=0.0061, p=0.0002, p=0.0001, respectively; Fig. 11C). There was also a main effect of sex on rearing activity, female rats had higher rearing counts than male rats [F(4,68)=22.34, p=0.0001; Fig. 11C]. Moreover, there was a significant interaction of time and sex with rearing activity in female rats persisting over a longer time frame overall as compared to male rats [F(29,1972)=5.50, p=0.0001; Figs. 11A and 11B]. Additionally, saline-treated female rats had higher rearing counts as compared to saline-treated male rats [F(1,13)=11.94, p=0.0043; Fig. 11C].

In both male and female rats, cocaine increased behavioral stereotypy [H=24.00, p=0.0001, H=15.46, p=0.0038, respectively; Fig. 12]. Overall, female cocaine-induced stereotypy was higher than that of cocaine-treated males [H=48.24, p=0.0001]. In male

rats, cocaine increased stereotypy after doses of 20 and 30 mg/kg as compared to saline-treated males. However, cocaine-treated female rats had significantly higher scores at 15 and 30 mg/kg of cocaine as compared to saline-treated female rats. Based on these dose-response data, 20 mg/kg of cocaine was chosen and administered in all subsequent experiments since it consistently produced sex differences in cocaine-induced behaviors and was the only dose that increased total locomotor activity in male rats.

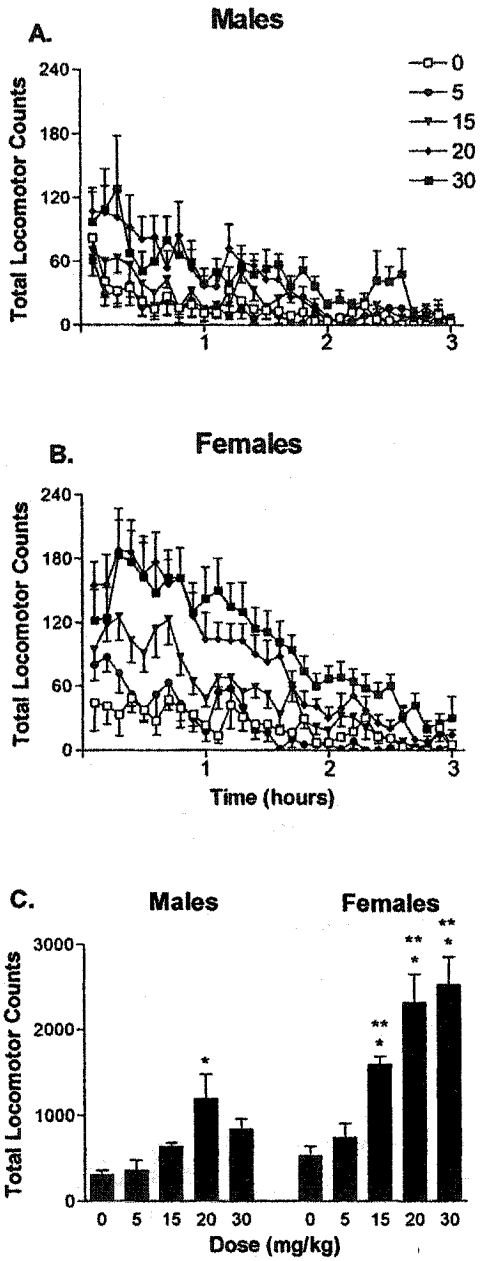


Figure 9 Sex and dose of cocaine affect total locomotor activity. Mean total locomotor counts of male (A) and female (B) rats after a single injection of cocaine (5, 15, 20, & 30 mg/kg) or saline are represented in 6-minute intervals. Figure 9C shows the sum of the total counts for male and female rats at each cocaine dose. * denotes a significant effect of cocaine as compared to respective saline-treated groups ($p<0.05$). ** denotes sex differences in total locomotor activity between doses ($p<0.05$).

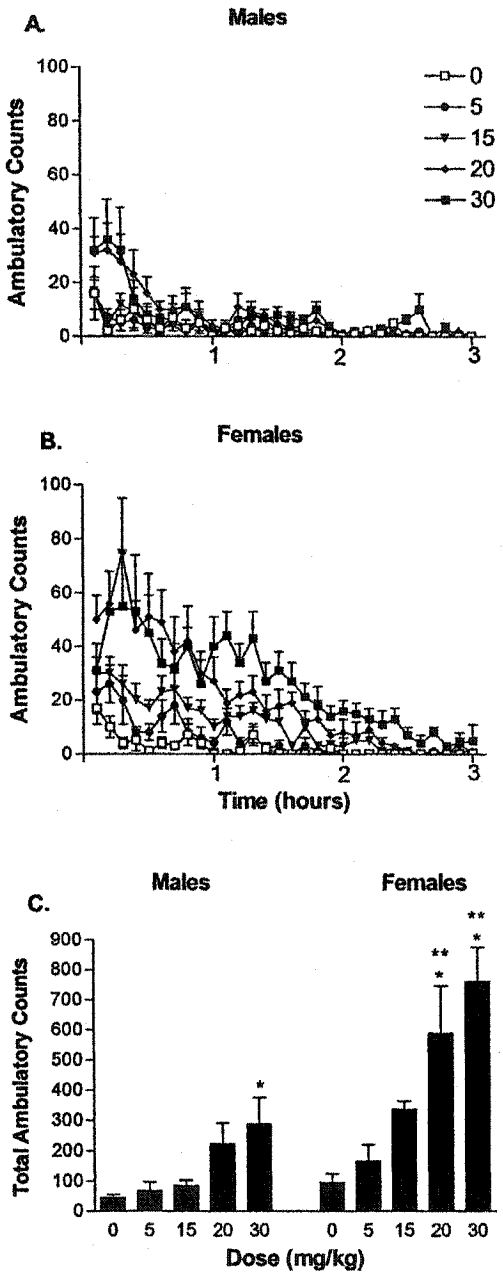


Figure 10 Sex and dose of cocaine affect ambulatory activity. Mean ambulatory counts of male (A) and female (B) rats after a single injection of cocaine (5, 15, 20, & 30 mg/kg) or saline are represented in 6-minute intervals. Figure 10C shows the mean of the summed ambulatory counts for male and female rats at each cocaine dose. * denotes a significant effect of cocaine as compared to respective saline-treated groups ($p<0.05$). ** denotes sex differences in ambulatory activity between doses ($p<0.05$).

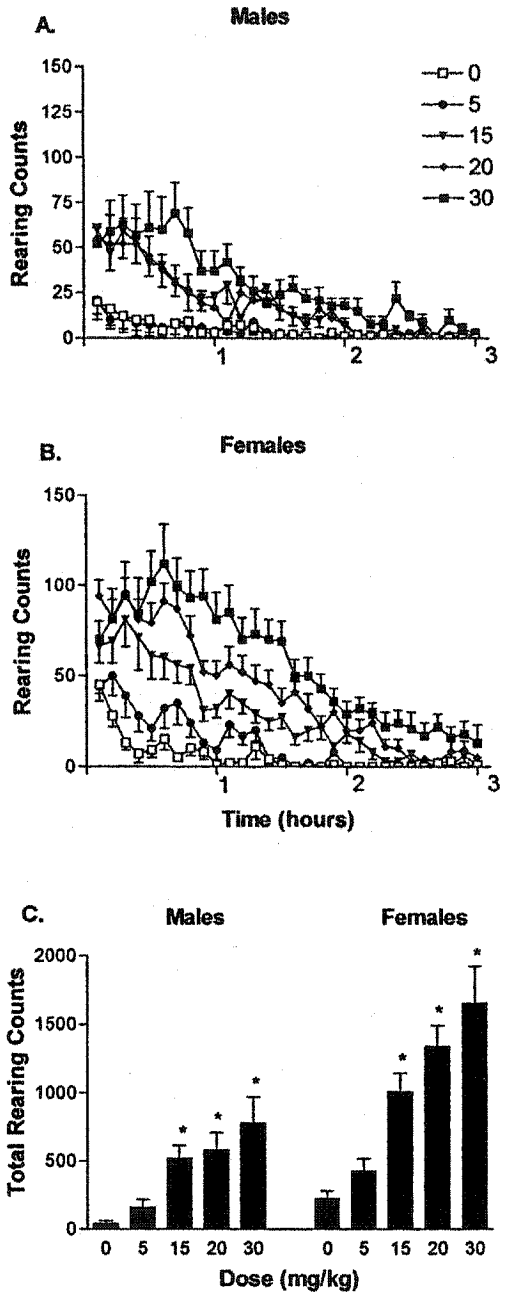


Figure 11 Sex and dose of cocaine affect rearing activity. Rearing counts of male (A) and female (B) rats after a single injection of cocaine (5, 15, 20, & 30 mg/kg) or saline are represented in 6-minute intervals. Figure 11C shows the mean of the summed rearing counts for male and female rats at each cocaine dose. * denotes a significant effect of cocaine as compared to respective saline-treated groups ($p<0.05$).

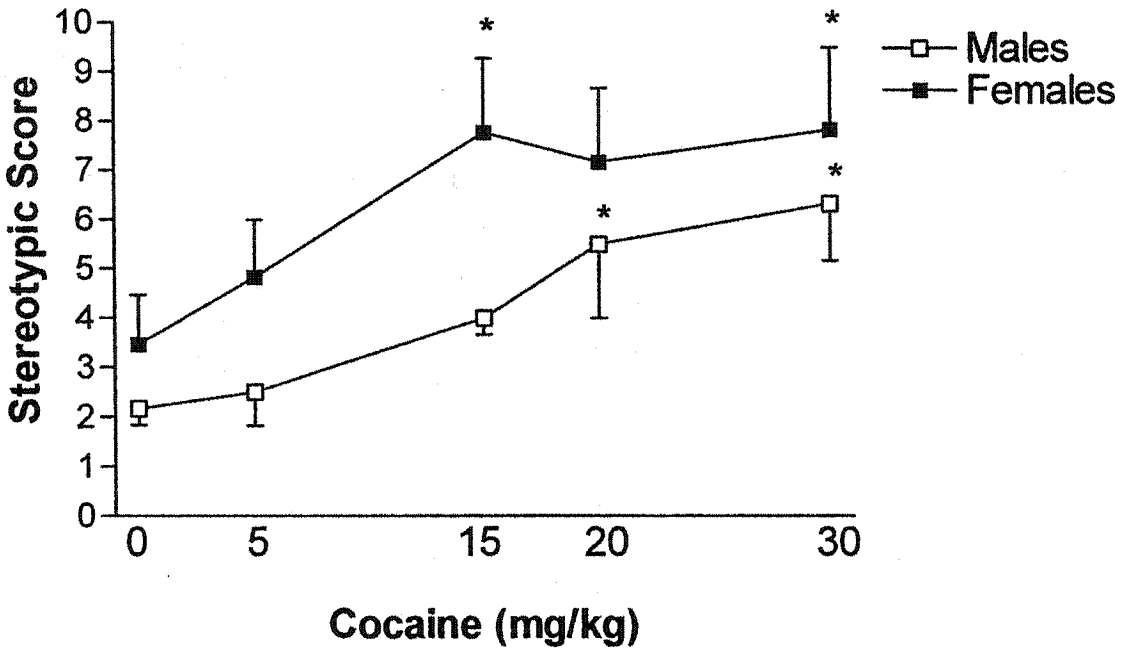


Figure 12 Sex and dose of cocaine affect stereotypic activity. Median stereotypic scores for male and female rats after a single injection of cocaine (5, 15, 20, & 30 mg/kg) or saline. 30 minutes post-injection, activity was recorded for 45 seconds. * denotes a significant effect of cocaine as compared to respective saline-treated groups ($p<0.05$).

Sex Differences in Cocaine Metabolism

Brain and serum levels of cocaine and its principal metabolites BE, EME, and norcocaine are shown in Figures 13 and 14. In both male and female rats, brain and serum levels of cocaine decreased across time [Brain: $F(2,42)=7.55$, $p=0.0016$; Serum: $F(2,39)=5.71$, $p=0.0067$], cocaine levels at 45 and 60 minutes were lower than those at 15 minutes (Figs. 13A and 14A). However, no sex differences were observed on cocaine levels in either brain or serum samples. Female rats had a non-significant peak in brain levels of BE 15 minutes following cocaine administration (Fig. 13B). Male rats had higher serum levels of BE than did female rats [$F(1,39)=8.95$, $p=0.0048$; Fig. 14B]. Brain and serum EME levels were significantly different (Figs. 13C and 14C), female rats had higher brain EME levels at 45 and 60 minutes than those of male rats and, overall, had significantly higher serum levels of EME than those of male rats [Brain: $F(1,37)=6.26$, $p=0.0046$, 45 min: $p=0.0003$ 60 min: $p=0.0001$; Serum: $F(1,39)=18.83$, $p=0.0001$]. Furthermore, EME levels in the brain were also affected by time where male and female rats had lower brain levels of EME at 15 minutes as compared to 45 and 60 minutes [Males: 15: $p=0.0357$, 60: $p=0.0236$; Females: 15: $p=0.0002$, 60: $p=0.0001$]. Norcocaine levels in both brain and serum decreased across time (Figs. 5D and 6D) [Brain: $F(1,41)=10329$, $p=0.0002$; Serum: $F(1,37)=5.36$, $p=0.0090$]. Furthermore, female rats had overall higher levels of norcocaine in the brain and serum than those of male rats [Brain: $F(1,41)=6.84$, $p=0.0124$; Serum: $F(1,37)=10.50$, $p=0.0025$].

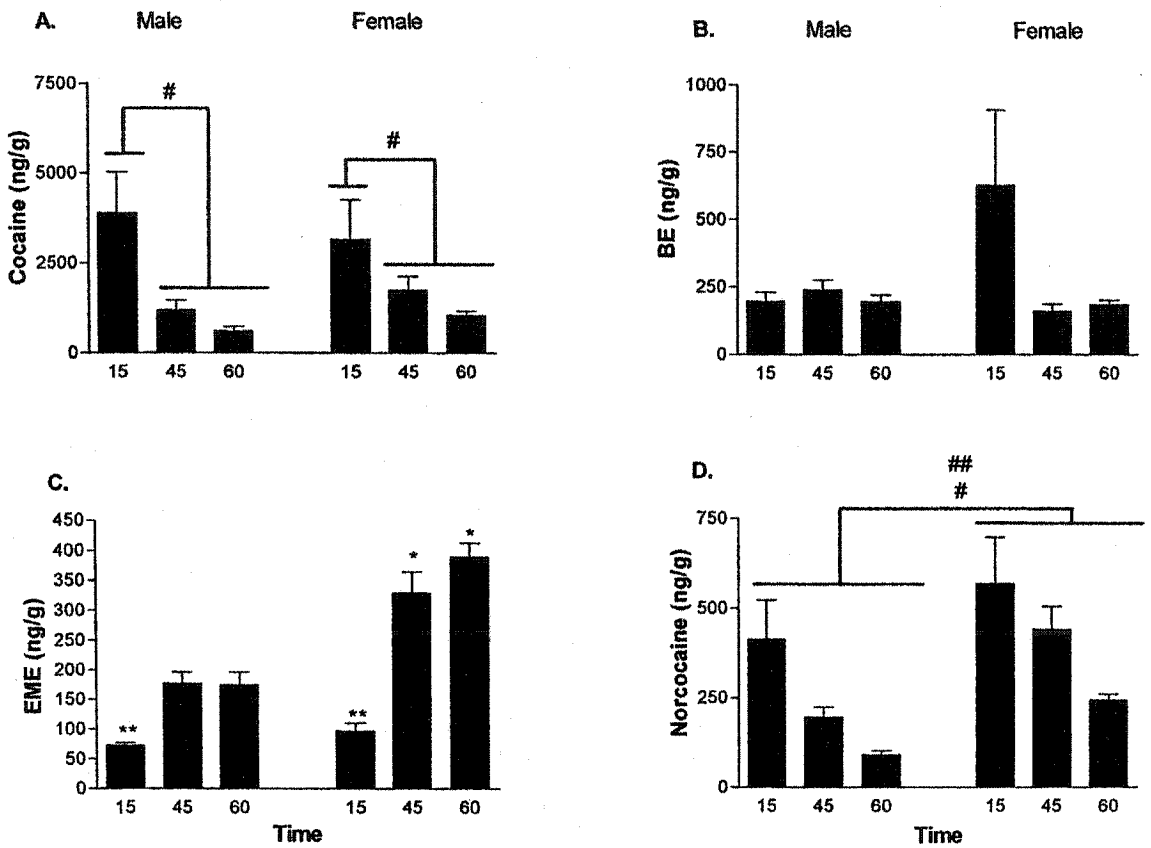


Figure 13 Sex differences in brain levels of cocaine and its metabolites. Brain tissue levels of cocaine (A), BE (B), EME (C), and norcocaine (D) (ng/g) in male and female rats 15, 45, and 60 minutes after a single injection of cocaine (20 mg/kg). * denotes significant effect of sex at 45 and 60 minutes ($p<0.05$). ** denotes a significant effect of time at 15 minutes as compared to 45 and 60 minutes ($p<0.05$). # denotes a main effect of time ($p<0.05$). ## denotes a main effect of sex ($p<0.05$).

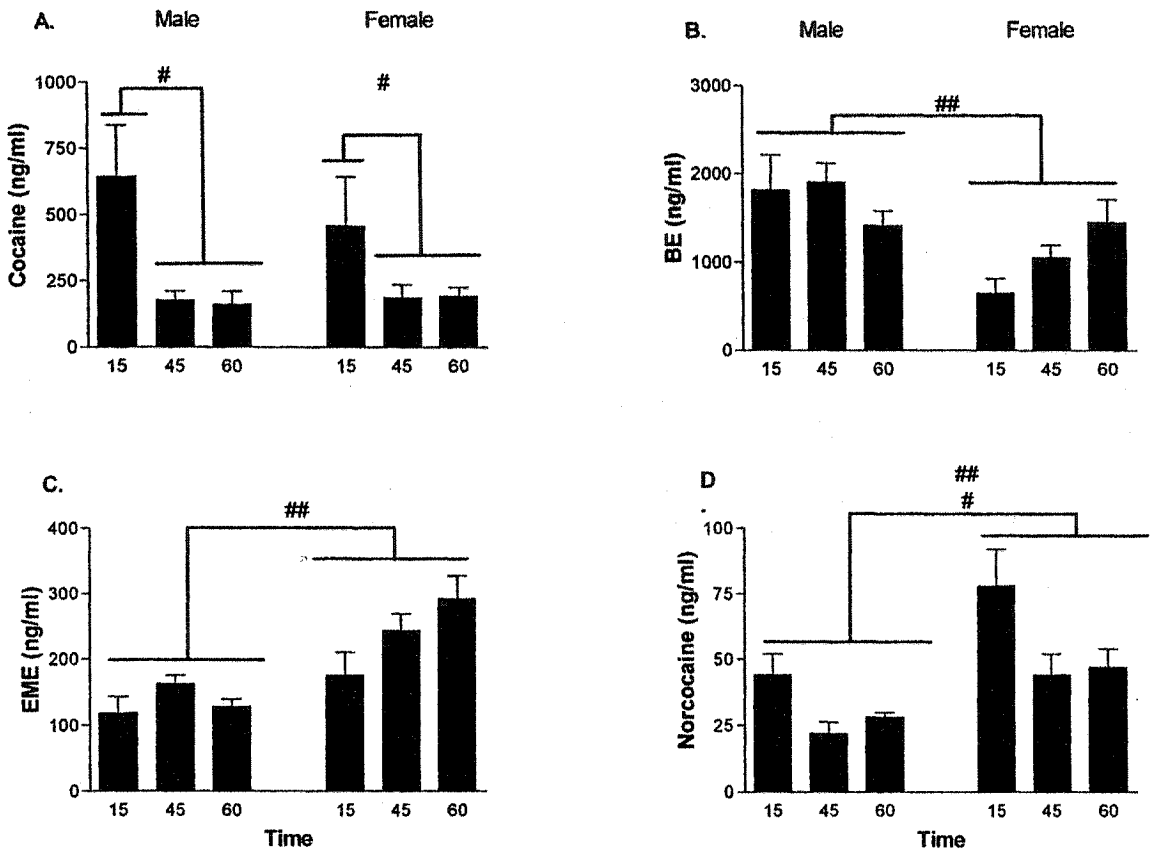


Figure 14 Sex differences in serum levels of cocaine and its metabolites. Serum levels of cocaine (A), BE (B), EME (C), and norcocaine (D) (ng/mL) in male and female rats 15, 45, and 60 minutes after a single injection of cocaine (20 mg/kg). # denotes a main effect of time ($p<0.05$). ## denotes a main effect of sex ($p<0.05$).

Sex Differences in Baseline and Cocaine-induced DA/5-HT Levels

Total levels of DA, 5-HT, and their metabolites and turnover ratios in the CPu and the NAc are summarized in Tables IV and V, respectively.

CPu:

As shown in Table IV, in both male and female rats a significant main effect of cocaine on DA levels was observed where, overall, cocaine increased DA levels [$F(1,84)=8.29$, $p=0.005$]. Furthermore, an interaction of sex and cocaine was observed on DOPAC levels [$F(1,85)=4.71$, $p=0.017$] where saline-treated males had higher levels than those of saline-treated females ($p=0.0080$). In male rats, a sex by cocaine by time interaction revealed that cocaine decreased DOPAC/DA turnover15 minutes post-injection [$F(1,85)=5.55$, $p=0.0209$]. Although 5-HT levels were not affected after cocaine administration, there was a main effect of cocaine on 5-HIAA levels where 5-HIAA decreased after cocaine administration in male and female rats [$F(1,82)=7.93$, $p=0.006$].

3.3.2. NAc:

As shown in Table V, a significant interaction of sex and drug on DA, DOPAC, and HVA levels was observed [DA: $F(1,83)=11.00$, $p=0.00131$; DOPAC: $F(1, 86)=5.541$, $p=0.0300$; HVA: $F(1,90)=7.58$, $p=0.0071$]. Overall, cocaine increased DA levels in male rats and decreased DA levels in female rats ($p=0.0207$ and $p=0.0125$,

respectively). Furthermore, cocaine decreased DOPAC and HVA levels in female rats ($p=0.0060$ and $p=0.0003$, respectively). Moreover, in both male and female rats, a main effect of drug on DOPAC/DA turnover ratios was observed where cocaine decreased DOPAC/DA turnover [$F(1,91)=13.39$, $p=0.0004$].

Overall, female rats had higher basal levels of 5-HT and 5-HIAA [5-HT: $F(1,86)=10.73$, $p=0.0015$; 5-HIAA: $F(1,63)=4.23$, $p=0.0430$]. A significant interaction between cocaine and sex was observed on levels of 5-HT [$F(1,86)=7.56$, $p=0.0072$] where cocaine decreased 5-HT in female rats ($p=0.0092$). In contrast, cocaine administration increased 5-HT levels in male rats ($p=0.0010$). Additionally, a significant interaction of cocaine and sex on levels of 5-HIAA was observed [$F(1,63)=5.98$, $p=0.0170$] where in females, cocaine decreased 5-HIAA levels ($p=0.0120$). A significant sex by cocaine interaction on levels of 5-HIAA/5-HT turnover was found [$F(1,61)=5.61$, $p=0.0210$] where, overall, females had lower basal levels of turnover as compared to male rats, and cocaine reduced 5-HIAA/5-HT turnover in male rats [$F(1,26)=10.03$, $p=0.0039$]. Further analysis in females revealed an interaction of cocaine and time where cocaine increased turnover at 15 minutes post-injection [$F(1,61)=5.61$, $p=0.0210$].

Table II. Effects of saline or cocaine (20 mg/kg) treatment on total levels of Dopamine, DOPAC, HVA their respective turnover ratios.

			Dopamine		DOPAC		HVA		Dopac/DA		HVA/DA	
Time (min)			15	45	15	45	15	45	15	45	15	45
Caudate Putamen												
Male	Saline[b]	(n=8-11)	57±5.6	68±6.2	**17±2.6**	**13±1.3**	8±1.0	8±1.0	**0.28±0.03**[c]	0.2±0.03	0.16±0.02	0.13±0.03
	Cocaine[a]	(n=10-12)	**77±7.7**	**73±4.3**	11±1.9	13±1.5	8±1.0	9±1.1	**0.13±0.02**	0.18±0.02	0.11±0.01	0.12±0.02
Female	Saline	(n=11-14)	71±7.9	68±6.2	**9±1.2**	**10±1.1**	6±0.7	8±1.0	0.16±.02	0.17±0.03	0.1±0.01	0.1±0.01
	Cocaine[a]	(n=11-14)	**81±5.8**	**89±4.6**	13±1.7	12±1.7	7±0.7	7±0.8	0.16±.02	0.14±0.02	0.09±0.01	0.08±0.01
Nucleus Accumbens												
Male	Saline	(n=8-11)	71±2.7	82±4.7	14±1.5	14±1.3	7±0.4	8±0.5	0.20±0.01	0.17±0.03	0.11±0.01	0.1±0.01
	Cocaine[d,f]	(n=10-12)	**83±5.6**	**91±5.3**	11±.9	14±1.4	7±0.6	8±0.4	**0.15±0.01**	**0.15±0.01**	0.09±0.01	0.09±0.01
Female	Saline	(n=11-14)	104±5.5	83±3.9	15±1.2	14±0.5	9±0.7	8±0.3	0.18±0.01	0.17±0.01	0.1±0.01	0.1±0.01
	Cocaine[e,f]	(n=11-14)	**77±5.6**	**77±6.7**	**9±0.8**	**10±0.9**	**6±0.6**	**6±0.5**	**0.13±0.01**	**0.14±0.01**	0.08±0.01	0.09±0.01

Levels are expressed as mean±S.E.M. in pg/ug of protein; significance is at the level of $p<0.05$. Bold typeface indicates statistical significance.
CPu: (a) Indicates a main effect of cocaine on DA levels. (b) Indicates male rats have higher baseline DOPAC levels as compared to female rats. (c) Indicates interaction of sex, drug and time on DOPAC/DA ratios. **NAc:** (d) Indicates a main effect of drug on DA levels in both sexes. (e) Indicates main effect of drug on DOPAC and HVA levels in female rats. (f) Indicates a sex by drug interaction on DOPAC/DA turnover.

Table III. Effects of saline or cocaine (20 mg/kg) treatment on total levels of 5-HT and 5-HIAA their respective turnover ratios.

			5-HT		5-HIAA		5-IAA/5-HT	
Time (min)			15	45	15	45	15	45
Caudate Putamen								
Male	Saline	(n=7-10)	10±0.8	11±1.0	13±1.3	11±0.7	1±0.1	1±0.1
	Cocaine[a]	(n=7-10)	9±0.8	11±0.9	**11±0.8**	**11±0.8**	1±0.1	1±0.1
Female	Saline	(n=11-14)	9±0.9	9±1.0	13±0.6	14±1.2	1±0.2	2±0.2
	Cocaine[a]	(n=11-14)	9±1.1	9±0.9	**11±0.5**	**11±0.6**	1±0.2	1±0.1
Nucleus Accumbens								
Male	Saline	(n=7-10)	20±1.6	20±1.4	11±0.8	12±0.1	0.5±0.02	0.58±0.05
	Cocaine[a]	(n=7-10)	**29±4.7**	**26±3.8**	12±11.0	13±1.3	**0.39±0.00[c]**	**0.44±0.02[c]**
Female	Saline[b]	(n=11-14)	**53±9.0**	**38±6.2**	**17±1.9**	**17±2.0**	0.31±0.03	0.41±0.03
	Cocaine[a,e]	(n=11-14)	**24±4.2**	**35±5.2**	**11±1.5**	**13±1.4**	**0.62±0.15[d]**	0.35±0.03

Levels are expressed as mean±S.E.M. in pg/ug of protein; significance at the level of $p<0.05$. Bold typeface indicates statistical significance. (a) Indicates a main effect of cocaine on 5-HIAA levelsin the CPu and 5-HT levels in the NAc. (b) Indicates an interaction of sex and drug on basal 5-HT and 5-HIAA levels in male and female rats. (c) Indicates a main effect of drug on 5-HIAA/5-HTI turnover in male rats. (d) Indicates drug and time interaction on 5-HIAA/5-HT turnover in female rats. (e) Indicates interaction of sex and drug on 5-HIAA levels in female rats.

Discussion

The current study demonstrates robust sex differences in cocaine-induced behavioral activities, cocaine pharmacokinetics, and monoamine content in the CPu/NAc. The general pattern of behavioral responses observed in the current study is consistent with previous results demonstrating a dose-dependent augmentation of total locomotor and ambulatory behavioral responses in female rats after acute cocaine administration when compared to male rats (Chin et al., 2001; Sell et al., 2000; Van Haaren and Meyer, 1991; Walker et al., 2001a). Additionally, regarding different aspects of the motor response to cocaine, female rats showed an enhancement of rearing and stereotypic activity as compared to male rats. This further extends published studies by demonstrating that rearing and behavioral stereotypy are also affected in a dose-dependent fashion by cocaine. It is interesting to note that cocaine dose differentially affected stereotypic behavior in male and female rats. Maximal stereotypy was reached at 15 and 30 mg/kg in female rats, but only at 30 mg/kg in male rats. Since stereotypic behavior is characterized by repetitive movements in one location (i.e., focused sniffing or repetitive pivoting/rearing), the time spent in these activities may reduce counts of other behaviors. For example, at 30 mg/kg of cocaine, male rats displayed significantly increased stereotypy, but not total locomotor counts. Other reports have found female rats to be inherently more active than male rats (Leret et al., 1994; Tropp and Markus, 2001). In the current study, we found no differences in saline-treated male and female rats for total locomotor, ambulatory, and stereotypic behaviors. However, saline-treated

female rats had significantly higher rearing counts as compared to saline-treated male rats. Since cocaine stimulated higher total locomotor, ambulatory, and stereotypic behaviors, it is unlikely that the observed sex differences are due to disparities in baseline behavior. It is more probable that sex differences in behavior are a direct effect of cocaine administration.

Although in both sexes cocaine-induced behavioral responses are dose-dependent, female rats consistently require less cocaine to achieve higher total locomotor and ambulatory activity as well as stereotypic scores than do male rats. Similar to previous reports (Walker et al., 2001a), it was observed that male rats require higher doses of cocaine to induce behavioral hyperactivity and that the greatest disparity between sexes is seen at lower doses for certain components of the behavioral response (i.e., in female rats 15 mg/kg of cocaine produces a similar level of activity to that of male rats treated with 20-30 mg/kg). Taken together, our results suggest that the potency and/or efficacy of cocaine to produce hyperactivity is lower in male rats since more drug is required to achieve responses similar to those of females. Additionally, female behavioral responses persist over a longer time frame than do male responses. This in turn may provide female rats with a longer temporal period to experience cocaine's psychomotor and/or rewarding effects. Russo et al. (2003b) reported sex differences in cocaine-induced conditioned place preference where female rats were more sensitive to the rewarding effects of cocaine through acquisition of conditioned place preference at lower doses and shorter

conditioning lengths. Thus, the prolonged cocaine-stimulated responses in female rats at lower doses may facilitate a more rapid establishment of contiguous associations between environmental context and cocaine's rewarding properties.

In concordance with Bowman et al. (Bowman et al., 1999), we showed that male and female rats had similar cocaine levels in the brain and serum. Likewise, in human studies, no sex differences in plasma cocaine levels and subjective responses after I.V. administration of cocaine were observed (Mendelson et al., 1999). Thus, based on these studies and our observations, brain and serum cocaine levels alone do not explain the observed sex differences in behavioral responses to cocaine. Cocaine is rapidly metabolized into BE, EME, and norcocaine by liver and blood enzymatic reactions. In male rats, it has been reported that some of these metabolites induce behavioral hyperactivity while others have no effect. For example, in male rats, EME administration causes no alteration of baseline behavior while BE administration causes hyperactivity (Schuelke et al., 1996). Moreover, norcocaine stimulates behavioral hyperactivity comparable to that induced by cocaine (Schuelke et al., 1996). Furthermore, studies have shown that monkeys self-administer norcocaine (Spealman and Kelleher, 1981).

Consistent with Bowman et al. (1999), we found that female rats had higher brain and serum levels of EME than those of male rats, while male rats had higher serum BE levels than those of female rats. We extend upon the report by Bowman et al. (1999) by

demonstrating that norcocaine levels in female rats were higher in the brain and serum than those of male rats. Our group has previously shown that female rats have higher serum BE levels (Chin et al., 2001). Although the strain and manner of cocaine administration are consistent across both studies, in the current study we used a higher cocaine dose and a different detection method (radioimmunoassay vs. LC/MS/MS) possibly accounting for the disparity between results. Since brain levels of BE are similar in both male and female rats, BE may have a limited impact on the observed behavioral effects. Furthermore, since EME has little effect on behavioral activity, the observed higher levels of EME in females may play a limited role in contributing to sex differences in the behavioral response. However, due to the potent effect of norcocaine on behavioral activity, sex differences in brain and serum levels of norcocaine may have the greatest potential to influence male vs. female cocaine-induced behavioral responses. Thus, contrary to Bowman et al. (1999), we postulate that, to a certain extent, the observed sex differences in behavioral responses to acute cocaine administration may in part be explained by pharmacokinetic differences.

Schuelke et al. (1996) demonstrated in male rats that equimolar concentrations of norcocaine and cocaine administered ICV resulted in similar behavioral scores. Since we observed no statistically significant differences in cocaine levels between male and female rats, one-fold higher brain norcocaine levels in female rats may in part potentiate or influence sexually disparate behavioral responses to cocaine. However, since

norcocaine displays about one-half the potency of cocaine at binding to the DA transporter (Ritz et al., 1987), and brain levels of norcocaine were 5-fold less than brain levels of cocaine in all experimental groups, the extent to which norcocaine contributes to overall sex differences in cocaine-induced behaviors remains to be elucidated. Other factors, such as the frequency of cocaine administration, may influence sex differences in cocaine pharmacokinetics since it has been previously shown following chronic cocaine administration that brain levels of cocaine increase in a temporal manner (Pettit et al., 1990). These issues require further study.

The observed sex differences in cocaine pharmacokinetics are most likely regulated by intrinsic sex differences in the enzymatic activity of proteins involved in cocaine metabolism. For example, Morishima et al. (Morishima et al., 1993) previously demonstrated higher levels of plasma cholinesterase activity in female rats, while Zhang et al. (Zhang et al., 1996) demonstrated greater decarboxylase activity in the liver of the male rat. Both of these pathways have been shown to produce EME while decarboxylase activity has been shown to produce BE (Mets et al., 1999). Thus, greater levels of decarboxylase activity in males may account for the higher serum BE levels. Meanwhile, the higher cholinesterase activity may contribute to the observed higher EME levels in females. Alternatively, endocrine effects on these enzymes may also be driving these pharmacokinetic differences. For example, P450 enzymes, which are responsible for norcocaine formation (Boelsterli et al., 1992), are regulated by both estrogen and

progesterone (Pond et al., 1992). The mechanisms which regulate sex differences in cocaine pharmacokinetics and their contribution to cocaine-stimulated behavioral responses remain to be determined.

Regarding monoamine measurements, baseline levels of DA, DOPAC, HVA, 5-HT, and 5-HIAA in male and female rats are consistent with those found in previous reports (Bisagno et al., 2002; Lindley et al., 1999). However, some discrepancies in total levels of DA and 5-HT can be attributed to the strain of rat used (Lindley et al., 1999). Consistent with Bisagno et al. (2002) and Lindley et al. (1999), total tissue levels of DA were similar in the CPu and NAc (both shell and core subareas).

Sex differences in DA and 5-HT levels following cocaine administration were observed. For the most part, there were more robust sex differences in monoaminergic levels after cocaine administration in the NAc, in female rats, cocaine reduced levels of DA, DOPAC, HVA, DOPAC/DA turnover, 5-HT, and 5-HIAA. However, in male rats, cocaine's effects on monoamines in the NAc were limited to decreases in DOPAC/DA and 5-HIAA/5-HT turnover. In the CPu of male rats DOPAC/DA turnover was decreased after acute cocaine administration, whereas this effect was not seen in female rats. Einhorn (1988) found that cocaine reduces DA release in the NAc through autoregulatory feedback by somatodendritic autoreceptors on DArgic cell bodies in the VTA. Galloway (1990) suggested that cocaine administration reduces DA cell firing and

therefore decreases DA synthesis. Due to the observed discrepancies between male and female rats in DOPAC/DA turnover ratios, our results suggest that autoreceptor-mediated regulation of DA synthesis and release in the caudate may be sexually disparate. Becker (1999) has postulated that enhanced DA release in the CPu of female rats could account for the observed sex differences in cocaine-induced activity. It is possible, as suggested by Becker (1999), that the endocrinological profile of female rats contributes to the downregulation of D_2 autoreceptors in the substantia nigra which results in enhanced DA release in the CPu. In the NAc, cocaine-induced decreases in 5-HIAA/5-HT turnover in male rats are consistent with Carey et al. (2001). In contrast, an increase in 5-HIAA/5-HT turnover in the NAc was observed in female rats 15 minutes post-injection. This finding may also reflect sex differences in autoreceptor-mediated feedback in the raphe nucleus where there are known sexual dimorphisms in neuronal activity (Klink et al., 2002).

Sex differences in basal levels of 5-HT were also observed, basal levels of 5-HT and 5-HIAA in the NAc were higher in female rats than in male rats. Carlsson and Carlsson (1987) demonstrated that sex differences in baseline levels of 5-HT were more prevalent than in the DA system which is in agreement with our findings. Thus, although the significance of these patterns in accumbal 5-HT with regard to addiction remains to be elucidated, our research is consistent with Carlsson and Carlsson (1987) in suggesting the likelihood that sex differences in basal 5-HT levels have functional consequences

(i.e., a greater potential to modulate monoaminergic function), a suggestion made by other groups as well (Broderick and Phelix, 1997).

Female patterns of cocaine-stimulated hyperactivity may be in part influenced by higher basal serotonin levels contributing to more robust DA efflux in the mesoaccumbens pathway. Indeed, Bubar et al. (2003) have recently demonstrated that serotonin re-uptake inhibitors injected into the NAc enhance cocaine's behavioral effects in male rats. Moreover, studies by Parsons and Justice (Parsons and Justice, 1993) and Parsons et al. (1999) have demonstrated that accumbal elevation of serotonin modulates cocaine-induced DA efflux. However, it has also been demonstrated that 5-HT exerts both inhibitory and excitatory effects, depending on the 5-HT concentration, on DA cell bodies in the VTA (Liu et al., 2003). Further studies are necessary to determine the interaction of 5-HT levels on DA efflux in both the VTA and NAc. One limitation of the present study is the use of whole tissue for monoamine measurement. *In vivo* microdialysis studies to measure monoamine release would further clarify the important question of serotonin's role in sex differences in the behavioral responses to cocaine.

In terms of clinical relevance, it has previously been demonstrated that chronic use of cocaine causes changes in rat serotonin function that mimics depression in humans (Baumann and Rothman, 1998; Parsons and Justice, 1993). Furthermore, women addicted to drugs have higher rates of depression which consequently decreases treatment

success (Boyd, 1993; Grant, 1995), possibly due to the modulation of serotonin function through interactions between cocaine and ovarian hormones (Klink et al., 2002; Perrotti et al., 2000). In female rats, basal differences in serotonergic tone in the NAc, which are potentiated by cocaine use, may facilitate the development of the depressive neuronal phenotype observed in the report by Baumann and Rothman (1998). Medications which specifically target the 5-HT system could be useful pharmacological adjuncts for treating female drug addicts.

Sex differences in reward and motor behavior have been postulated to be under the regulation of the hypothalamic pituitary gonadal axis. Fluctuating levels of ovarian steroids (estrogen and progesterone) affect the synthesis and secretion of several neurotransmitter systems (reviewed in Etgen, 2002; Schumacher and Robert, 2002). Our group and others have demonstrated that estrogen and progesterone influence sex differences in cocaine-induced motor and reward behaviors, possibly through alterations in serotonergic and DArgic systems (Becker et al., 1999, Perrotti et al., 2000, Russo et al., 2003b, Walker et al., 2000). Thus, it is likely that ovarian hormone effects on monoamines in female rats are contributing to their exaggerated behavioral responses.

Adolescent females are more likely than adolescent males to be dependent on cocaine as a result of self-administering the drug more frequently and in larger doses than males (Chen and Kandel, 2002). It has been shown that females experience increased

sensitivity at lower cocaine doses (Chen and Kandel, 2002). These factors may be responsible for the increased likelihood for cocaine dependence in adulthood among females as compared to males (Kandel et al., 1995). Sex differences in cocaine abuse described in the clinical literature could be based on sex differences in pharmacokinetics and/or overall neurochemical tone. Moreover, due to intrinsic sex differences in cocaine pharmacokinetics and basal/cocaine-stimulated neurochemical alterations, development of sex-appropriate treatment for cocaine dependence is needed. These important clinical issues require further study.

CHAPTER 4: SEX DIFFERENCES IN D1 AND D2 RECEPTOR ACTIVATION, mRNA, AND BINDING LEVELS FOLLOWING ACUTE COCAINE ADMINISTRATION.

Approximately 600,000 of the estimated 2 million Americans who use cocaine are woman (National Survey on Drug Use and Health, 2002). Although men have greater access to cocaine, recent evidence suggests that the onset of addiction is more rapid in women than in men (Chen and Kandel, 2002; Kosten et al., 1996; Van Etten et al., 1999). In rodents, cocaine produces robust sex differences in the behavioral and endocrine responses to cocaine (Chin et al., 2002; Sell et al., 2000; Van Haaren and Meyer, 1991; Walker et al., 2001a; Walker et al., 2001b). For example, female rats have greater motor and stereotypic activity following both acute and chronic administration and sensitize to cocaine's behavioral effects more rapidly than male rats (Chin et al., 2002; Sell et al., 2000; Walker et al., 2001b). Moreover, female rats have a more intense hypothalamic-pituitary-adrenal (HPA) axis response to acute cocaine administration than male rats (Walker et al., 2001b). Female rats also acquire cocaine conditioned place preference (CPP) at lower doses of cocaine with fewer training sessions as compared to male rats (Russo et al., 2003b). Lynch et al. (1999; 2000; 2002) has shown that female rats also acquire cocaine self-administration faster, at lower doses, and respond higher during reinstatement. However, less is known about the cellular mechanisms involved in these observed sex differences.

Cocaine exerts is psychomotor effects through blockade of the monoamine transporters. Although all three monoaminergic systems contribute to cocaine-induced responses, the mesocorticolimbic DA (DA) system has been postulated to be the primary regulator of psychomotor and rewarding effects (Hyman and Malenka, 2001; Koob, 1992). The striatum, composed of the CPu (CPu) and NAc (NAc), receives DArgic input from midbrain projection neurons and is the major neural substrate identifiable with locomotion and reward (Koob, 1992). The two classes of DA receptors, D1 and D2, are differentially distributed in the striatum. D1 receptors are located post-synaptically on striatonigral projection neurons (Gerfen et al., 1990). D2 receptors are located post-synaptically on striatopallidal projection neurons (Gerfen et al., 1990) and also function as autoreceptors pre-snynaptically on DA terminals to modulate DA release and synthesis (Surmeier et al., 1996). Both D1 and D2 receptors have been shown to modulate cocaine-induced motor activity (for review, see Hummel and Unterwald, 2002). For example, D2 activation replaces cocaine-induced motor activity with stereotyped behaviors (Ushijima et al., 1995). Blockade of D1 receptors decreases cocaine-induced activity (McCreary and Marsden, 1993), while blockade of D2 receptors decreases motor activity as well as baseline locomotion (Pierce and Kalivas, 1997; Ushijima et al., 1995).

Intrinsic sex differences in the mesocorticolimbic DA system have been documented. For example, it has been shown that male Sprague-Dawley rats have higher levels of D1 receptors in the NAc (Andersen et al., 1997). Furthermore, female rats have

a greater rate of DA release and re-uptake in the striatum (Walker et al., 2000). Becker (Becker, 1999) has suggested that D2 autoreceptors in female rats may be less sensitive to DA in the substantia nigra pars compacta, which may account for the sexual disparities seen in DA release and re-uptake (Walker et al., 2000). Although these reports have shown sex differences in DA release and re-uptake dynamics and cocaine-induced DA turnover, other components of the DA system, such as DA receptor activation and mRNA/binding levels, have not been well-studied. To this end, we examined if D1 and D2 receptor activation differentially affects the motor behavior of male and female rats following acute cocaine administration. Furthermore, a second study was preformed to determine if D1 and D2 DA receptor mRNA and protein levels are sexually disparate at baseline and/or after acute cocaine.

Materials and Methods

Subjects

Eight-week-old male (200-250 g) and female (150-200g) Fischer rats purchased from Charles River (Kingston, NY) were individually housed with free access to standard lab chow and water ad libitum. Rats were maintained on a 12-hour light/dark cycle with lights on at 9:00 a.m. To acclimate rats to experimental procedures, rats were handled and weighed for 3 days prior to experimental manipulations. Thirty minutes prior to behavioral experiments, rats were placed in behavioral activity frames to reduce the effect of novelty on motor activity. A recent report demonstrated that repeated vaginal

lavage attenuates cocaine-induced activity, abolishes estrous cycle effects, and establishes a place preference in female rats (Walker et al., 2002). Furthermore, vaginal lavage results in increased DA release in the striatum, which in turn, may alter behavioral measurements (Mermelstein and Becker, 1995). As suggested by Walker et al. (2002), the use of lavaged female rats could result in inaccurate behavioral responses when making a side-by-side comparison to male rats. Thus, animals were placed into groups at random without regard to estrous cycle. Animal care was in accordance with the Guide for the Care and Use of Laboratory Animals (NIH publication 865-112, Bethesda, MD) and approved by the Institutional Animal Care and Use Committee of Hunter College.

Chemicals

Cocaine hydrochloride, SCH-23390 hydrochloride, (-)-eticlopride hydrochloride, domperidone, S(+)-PD 128907, ketanserin, and SKF 77434 were purchased from Sigma Chemical Co. (St. Louis, MO). [^{125}I]-SCH-23982, [^{125}I]-RTI-121, and $\propto$-[^{33}P]CTP were purchased from PerkinElmer Life Sciences (Boston, MA). [^{125}I]-idiosulpride was purchased from Amersham (Arlington Heights, IL). Trizol reagent was purchased from Life Technologies (Grand Island, NY). D2 receptor plasmid was purchased from Imgenex Corporation (San Diego, CA).

Cocaine Administration and Experimental Paradigm

Cocaine, SCH-23390, and eticlopride solutions were prepared daily by dissolution

in physiological saline (0.9%) and injected intra-peritoneal (i.p.) in a volume of 1 mL/kg. All injections were performed in the home cage 30 minutes after lights were turned on. For all experiments, 20 mg/kg of cocaine was used. This cocaine dose has been previously demonstrated to produce significant increases in locomotion and robust sex differences in cocaine-stimulated motor activity without reaching a maximal effect in either sex (Festa et al., 2004).

To determine the contribution of D1 and D2 receptors to sex differences in cocaine-induced activity, four cohorts of male and female rats (n=7-9/ group) were pre-treated with SCH-23390 (0.05, 0.01, or 0.25 mg/kg), eticlopride (0.03, or 0.1 mg/kg), or vehicle (saline) 15 minutes prior to an acute injection of saline or cocaine. To establish if cocaine's regulation of D1 and D2 receptor mRNA or D1, D2, and DAT binding levels in the CPu and NAc is sexually dimorphic, two separate cohorts of rats were used (n=3-5/ group).

Behavioral Measurement

Total locomotor, ambulatory, and rearing activities were monitored in the home cages with a Photobeam Activity System from San Diego Instruments (San Diego, CA) as previously described (Chin et al., 2002). Total locomotor activity represents the sum of all counts in the horizontal frame. Ambulatory activity represents the number of counts produced by two consecutive photobeam interruptions in the horizontal frame. Rearing activity represents total counts of vertical motion. Behavioral activity was

recorded for 40 minutes post-injection. For stereotypic activity, rats were videotaped for 40 seconds 30 minutes after the onset of locomotor assessment. The videotapes were later analyzed for behavioral stereotypy by three trained observers blind to each animal's treatment group. The rating for cocaine-induced stereotypic behaviors is based on a modified version of the Creese and Iversen (1974) scale. This scale consists of 10 ranked scores: (1) asleep or inactive, (2) alert/actively grooming, (3) increased sniffing in one location, (4) intermittent rearing/sniffing, (5) increased locomotion/sniffing, (6) intense sniffing in one location, (7) continuous pivoting/sniffing, (8) continuous rearing/sniffing, (9) maintained rearing/sniffing, (10) splayed hind limbs. Throughout the study a score of 10 was never observed.

RNA Probes

D1 and D2 RNA probes were constructed from 1.5 kb *Apa*I-*Sal*I (D1) and 0.5 kb *Dsa*I-*Dsa*I (D2) cDNA fragments of the D1 and D2 receptor genes that had been previously subcloned into pGEM-T and pGEM -5Zf(-) vectors, respectively. Receptor clones were verified by sequencing (The Rockefeller University, NY). The D1 receptor clone did not contain homologous sequences for the D5 receptor, and the D2 receptor clone did not contain homologous sequences for either D3 or D4 receptors. Procedures for synthesis of RNA probes have been described in detail elsewhere (Jenab et al., 1995). Briefly, templates for sense and anti-sense transcripts of the D1 and D2 receptor probes were prepared by digestion of plasmid DNA with the appropriate restriction enzymes. The riboprobes and unlabeled sense strands were transcribed from D1 and D2 plasmids in

the presence of ∝-[^{33}P]CTP using T7 and SP6 polymerase reverse transcription systems.

Solution hybridization Rnase protection-trichloracetic (TCA) precipitation assay

Immediately after decapitation, brains were removed. Coronal slices (1 mm) were cut out in a matrix as previously described (Jenab et al., 2002). The CPu and NAc were then dissected out (1.90 mm to 0.90 mm anterior to Bregma) on a cold glass plate and frozen at -80°C. Tissues were homogenized in Trizol reagent and total RNA was extracted following the manufacturer's instructions. mRNA was stored at -80°C until assayed. D1 and D2 mRNA levels were measured by solution hybridization assay as previously described (Jenab et al., 1995; Jenab et al., 2002; Jenab and Morris, 1997). Briefly, 20 µl aliquots of total mRNA extracts were hybridized to ^{33}P-riboprobes in a hybridization buffer overnight. The mixture was then subjected to 40 µg/mL ribonuclease A and 2 µg/mL ribonuclease T1 for 1 h. Samples were precipitated with TCA and the dcms were counted by liquid scintillation. For each assay comparisons were made with respective D1 or D2 receptor standard calibration curves in each assay to quantify levels of each respective mRNA. Samples are expressed in pg of hybridized mRNA normalized to the total ug of RNA.

Quantitative Receptor Autoradiography

Rats were sacrificed 1 or 24 hours following single injection of saline or cocaine. Following decapitation, brains were rapidly removed, frozen in methylbutane (-40°C for

30 seconds), and stored at -80°C. Coronal sections (10 μm) were taken on a cryostat at the level of the rostral striatum (1.6 mm to 1.2 mm anterior to Bregma). Sections were then thaw-mounted onto Superfrost Plus Gold glass slides (Fischer, Pittsburgh, PA). The slides were stored at -80°C until the assays were preformed. Twelve hours prior to experimental analysis, slides were placed into a dessicator connected to a vacuum at 4°C. Prior to incubation, slides were warmed to room temperature for 15 minutes.

The D1 receptor binding assay was preformed as described previously (Djouma and Lawrence, 2002). Briefly, for total binding, slide-mounted sections were incubated for 30 minutes at 25°C in 220 mL of tris-HCL buffer (pH=7.4) containing 0.05 nM [^{125}I]-SCH-23982(2200 Ci/mmol). Ketanserin (50 nM) was included to block non-specific binding to 5-HT2A receptors. Nonspecific binding was determined in the presence of 10 μM SKF-77434. The D2 receptor binding assay was performed as described previously (Tella et al., 1998). For total binding, slide-mounted sections were incubated for 30 minutes at 25°C in 220 mL of tris-HCL (pH=7.4) buffer containing 0.1 nM [^{125}I]-idiosulpride (2000 Ci/mmol) and 5 nM PD128907 (to block non-specific binding to D3 receptors). Nonspecific binding was determined in the presence of 1 μM domperidone. The DAT receptor binding assay was preformed as described previously (Collins et al., 2001). For total binding, slide-mounted sections were incubated for 60 minutes at 25°C in 220 mL of NaCl (pH=7.4) buffer containing 0.07 nM [^{125}I]-RTI-121 (2200 Ci/mmol). Nonspecific binding was determined in the presence of 100 μM cocaine hydrochloride..

Following incubation with the respective radioligands, slides were subjected to two washes in fresh ice-cold buffer (5 minutes each) and then dipped in ice-cold distilled

water five times. Slides were allowed to dry overnight under a gentle stream of cool air. Labeled tissue sections were apposed to radiosensitive film (KODAK, X-OMAT AR-5, PerkinElmer Life Sciences, Boston, MA) with plastic standards (^{125}I labeled microscales) and stored in the dark for 36-48 hours. After development (KOADK X-OMAT 2000 Processor), ligand-binding was quantified using a MBA 2000 densitometer (PerkinElmer Life Sciences, Boston, MA) and ImageQuant 5.0 software by reference of the optical densities of experimental samples and ^{125}I standards. Ligand binding was quantified in both sides of the brain from 12 sections per animal and corrected for contribution of non-specific binding and background film density. All corresponding brain sections for control and experimental groups that were statistically analyzed were run in parallel and exposed on the same sheet of film. Since there were no regional differences in levels of D1 and D2 receptors in the CPu (dorsal-lateral vs. dorsal-medial) or the NAc (core vs. shell) (data not shown), the CPu and NAc were analyzed without separation into sub-regions.

Statistical Analysis

Ambulatory, and rearing data were summed for each subject and are presented as mean ± standard error of the mean (SEM). Stereotypic data is presented as median score ± semi-interquartile range. D1 and D2 receptor mRNA and binding levels are presented as the percentage change in experimental animals from saline-treated control animals ± SEM. Two-way analyses of variance (ANOVAs) were used for analysis of ambulatory and rearing activity: cocaine (cocaine or saline) x sex (male vs. female). Three-way ANOVAs were used to determine the effects of antagonist dose, cocaine, and sex on

ambulatory and rearing activity as follows: antagonist dose (vehicle, SCH-23390, or eticlopride) x cocaine (cocaine or saline) x sex (male vs. female). To test the effects of these same variables on stereotypy, Kruskall-Wallis ANOVAs followed by a Dunn's post-hoc tests were used. To determine the effects of antagonist dose on cocaine-induced behavior within sex, two-way ANOVAs were used as follows: antagonist dose (vehicle, SCH-23390, or eticlopride) x cocaine (cocaine or saline). Two-way ANOVAs were used to determine the effects of time and sex on baseline levels of D1 and D2 mRNA and D1, D2, and DAT radioligand binding as follows: time (1 or 24 hours) x sex (male vs. female). Tukey honestly significant difference tests were used for multiple comparisons when appropriate. *t*-test analyses were used to determine statistically significant differences between levels of D1 and D2 mRNA or D1, D2, and DAT radioligand binding by comparison of cocaine-treated animals to their respective saline-treated controls. Significance in all cases was considered to be $p<0.05$.

Results

D1 receptor antagonism affects cocaine-induced behavioral activity in male and female rats

As shown in Figure 15, overall, cocaine administration increased behavioral activity in both male and female rats [Ambulations: $F(1,28)=57.23$, $p<0.0001$; Rearing: [$F(1,28)=157.50$, $p<0.0001$; see Fig 15A and B; Stereotypy: $H=51.12$, $p<0.0001$; see Figs. 16C and 17C]. Female rats had overall higher ambulatory activity and cocaine-

induced rearing activity than male rats [Ambulations: $F(1,28)=4.29$, $p=0.0477$; Rearing: [$F(1,28)=15.09$, $p<0.0001$; $p=0.0002$; see Fig. 15]. As seen in Figure 16, analysis within sex revealed that 0.05 mg/kg of SCH-23390 reduced cocaine-induced ambulatory activity in female, but not male, rats as compared to their respective saline-treated control groups at the same SCH-23390 dose [Females: $F(3,86)=4.15$, $p=0.0085$; $p<0.001$]. SCH-23390 doses of 0.1 and 0.25 mg/kg attenuated ambulatory counts in both male and female rats as compared to their respective saline-treated control groups at the same SCH-23390 dose ($p<0.05$).

For rearing activity, a significant interaction of antagonist dose and cocaine was observed for both male and female rats [Males: $F(3,81)=8.96$, $p<0.0001$; Females: $F(3,86)=8.71$, $p<0.0001$; Fig. 17]; 0.1 and 0.25 mg/kg SCH-23390 reduced cocaine-induced activity in both sexes as compared to their respective saline-treated control groups at the same SCH-23390 dose. Similar to ambulatory activity, 0.25 mg/kg of SCH-23390 completely suppressed cocaine-induced rearing only in male rats. SCH-23390, at doses of 0.1 and 0.25 mg/kg, attenuated cocaine-induced stereotypy in both male and female rats. However, 0.05 mg/kg SCH-23390 reduced cocaine-induced stereotypic activity in female rats only as compared to saline-treated female rats at the same SCH-23390 dose ($p<0.05$). In saline-treated control groups, none of the SCH-23390 doses affected baseline activity for the three behavioral parameters.

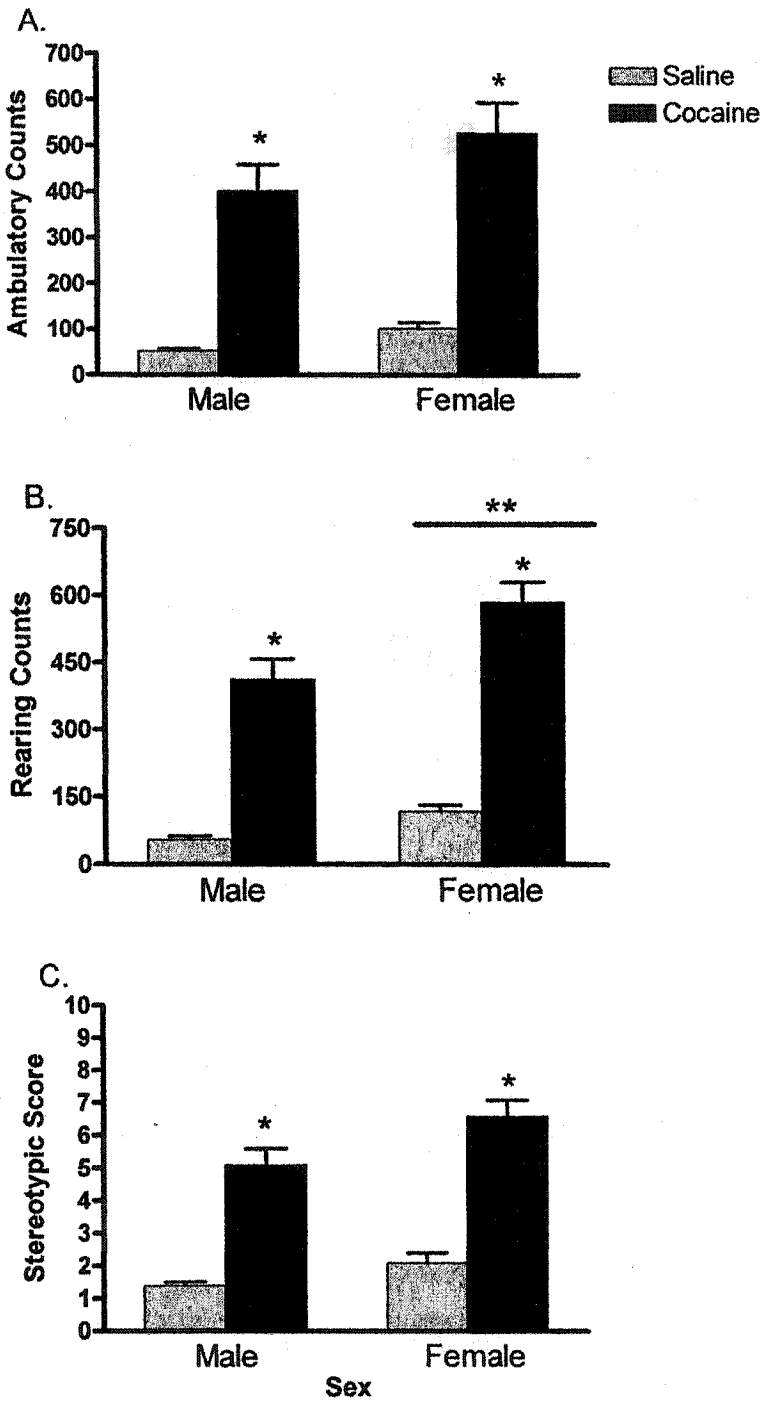


Figure 15 Ambulatory (A), rearing (B), and stereotypy (C) data are presented for male and female rats (n=8/ group) following acute cocaine (20 mg/kg) or saline administration. Behavioral activity was recorded for 60 minutes. Ambulatory and rearing activity are presented as mean number of counts ± SEM in 5 minute bins and stereotypic data is presented as median score ± semi-interquartile range . *Denotes a significant effect of cocaine. **Denotes a main effect of sex.

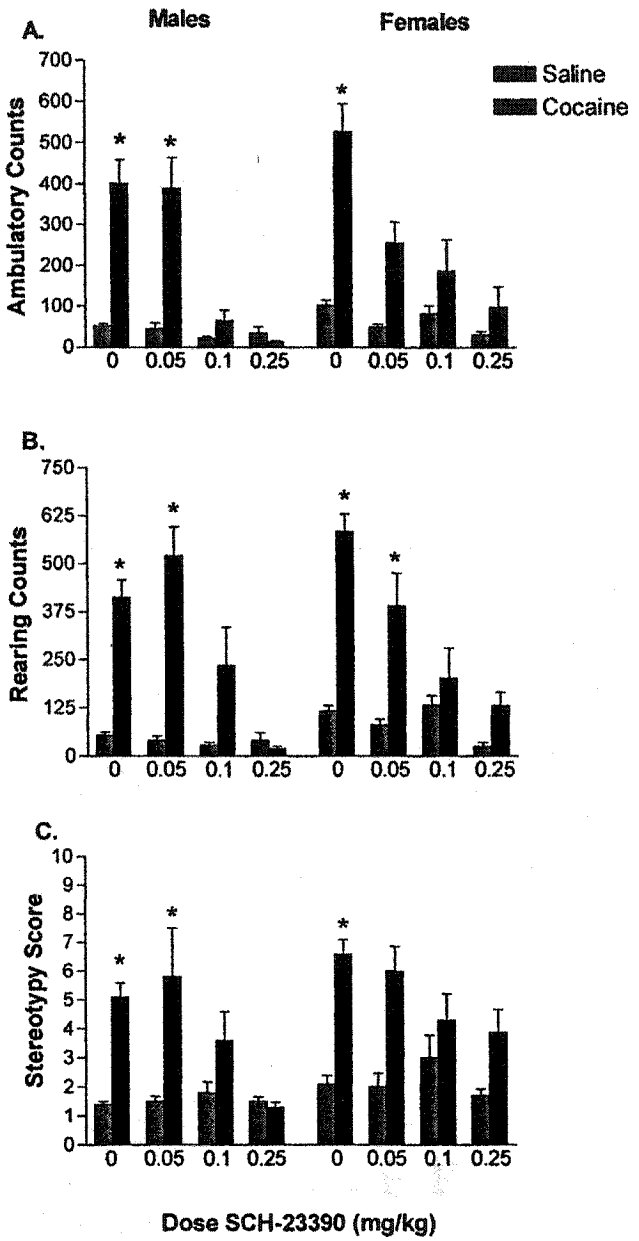


Figure 16 Ambulatory (A), rearing (B), and stereotypic (C) activity in male and female rats following acute cocaine (20 mg/kg) or saline administration. Fifteen minutes prior to cocaine or saline administration rats were pre-treated with SCH-23390 (0, 0.05, 0.1, or 0.25 mg/kg). Behavioral activity was recorded for 40 minutes. Ambulatory and rearing activity are presented as mean number of counts ± SEM in 5 minute bins stereotypic data is presented as median score ± semi-interquartile range. *Denotes a significant effect of cocaine as compared to matched saline control. Male and female rats pre-treated with vehicle contain 22-24 rats per group. All other antagonist treatment groups contain 7-9 animals per group.

D2 receptor antagonism does not affect sex differences in cocaine-induced behaviors

Overall, cocaine administration increased ambulatory and rearing activity in male and female rats [$F(2,154)=9.23$, $p=0.0028$; Figure 17A and B]. Furthermore, an interaction of sex and cocaine revealed that female rats had higher cocaine-induced ambulatory and rearing counts as compared to male rats [Ambulations: $p<0.0001$ and Rearing: $p<0.0001$ for post-hoc analyses]. As shown in Figure 17C, cocaine also produced significant increases in behavioral stereotypy in both male and female rats [$H=69.19$, $p=0.0001$]. However, none of the doses of the D2 antagonist eticlopride affected ambulatory, rearing, or stereotypic activity in both male and female rats. Furthermore, none of the doses of eticlopride affected baseline behavior in saline-treated rats.

Sex differences in cocaine's regulation of D1 mRNA and binding levels

No significant sex differences in either baseline levels of D1 and D2 mRNA, or D1, D2, and DAT binding in the CPu and NAc were observed at 1 or 24 hours post-treatment (data not shown). In the NAc of male rats, D1 mRNA levels were decreased 1 hour following cocaine administration [$t(9)=2.58$, $p=0.0299$; Fig. 4A]. This alteration was transient, since 24 hours following cocaine treatment D1 mRNA levels returned to baseline. Furthermore, in the CPu of male rats, D1 receptor binding levels were decreased 24 hours following cocaine administration [$t(16)= -3.20$, $p=0.0056$; Fig. 5A]. There were no effects of cocaine or sex on DAT binding levels in the CPu or the NAc

(Fig. 20). However, in both male and female rats, the dorsal-lateral CPu and NAc shell had greater DAT binding density as compared to dorsal-medial CPu [$F(1,18)=20.99$, $p=0.0002$] and NAc core [$F(1,15)=7.18$, $p=0.0172$], respectively. Overall, DAT binding density was lower at 24 hours as compared to one hour in male and female rats [Dorsal-medial CPu: $F(1,25)=4.81$, $p=0.03773$; NAc Core: $F(1,27)=7.18$, $p=0.0124$; NAc Shell: $F(1,28)=8.12$, $p=0.0081$].

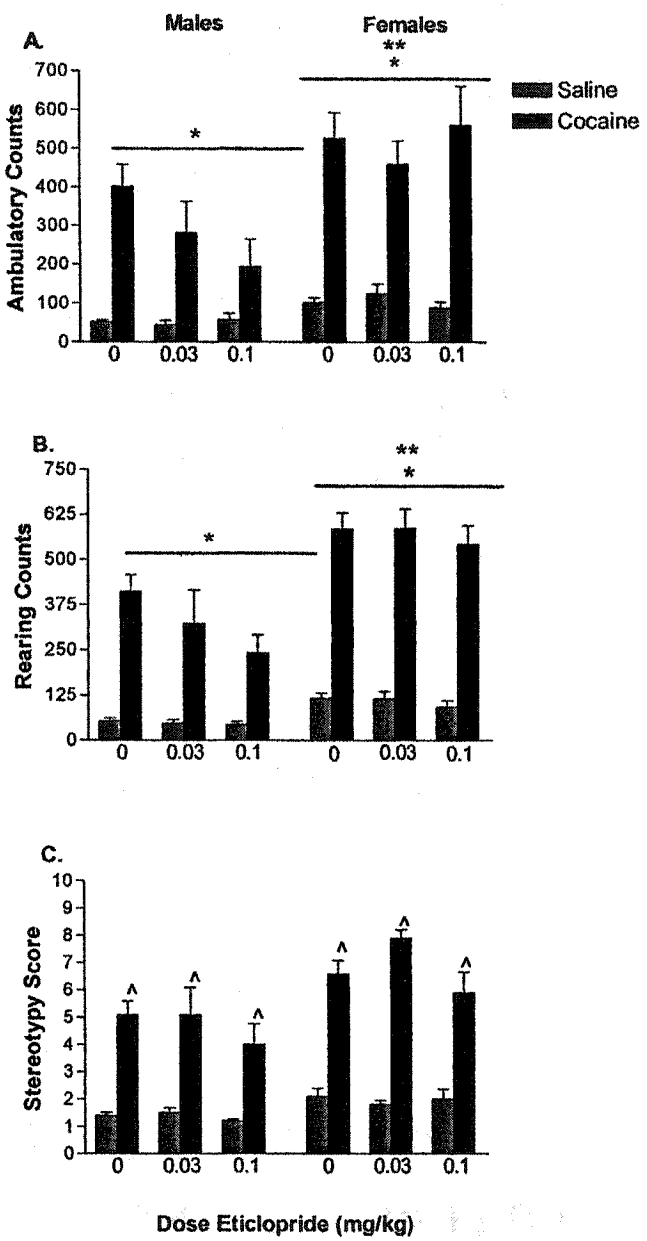


Figure 17 Ambulatory (A), rearing (B), and stereotypic (C) activity in male and female rats following acute cocaine (20 mg/kg) or saline administration. Fifteen minutes prior to cocaine or saline administration rats were pre-treated with eticlopride (0, 0.03, or 0.1 mg/kg). Behavioral activity was recorded for 40 minutes. Ambulatory and rearing activity are presented as mean number of counts ± SEM in 5 minute bins stereotypic data is presented as median score ± semi-interquartile range. *Denotes a significant effect of cocaine as compared to matched saline control. **Denotes main effect of sex. Male and female rats pre-treated with vehicle contain 22-24 rats per group. All other antagonist treatment groups contain 7-9 animals per group.

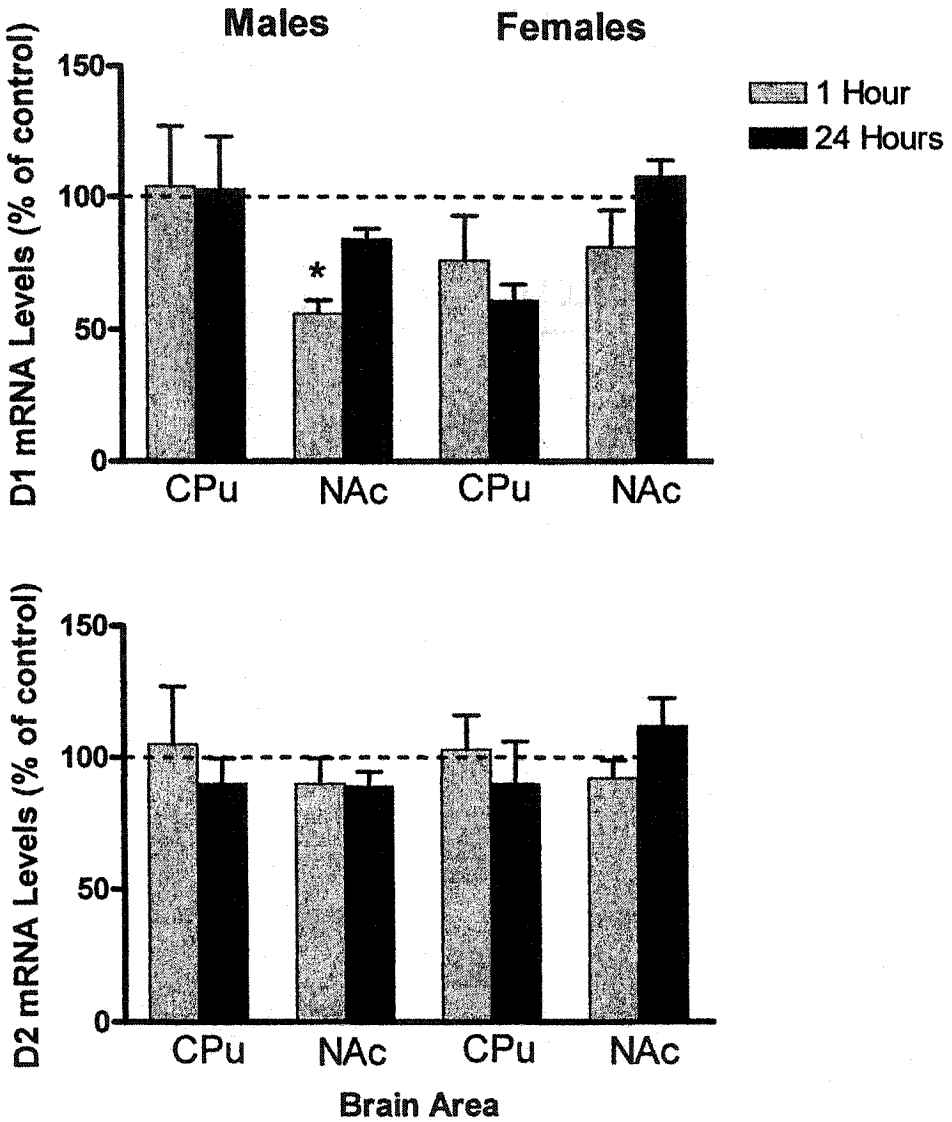


Figure 18 D1 (A) and D2 (B) mRNA levels in male and female rats 1 and 24 hours following acute cocaine (20 mg/kg) or saline administration. mRNA levels are presented as percent change from control (n=3-5/ group). *Denotes a significant effect of cocaine as compared to respective saline-treated groups ($p<0.05$).

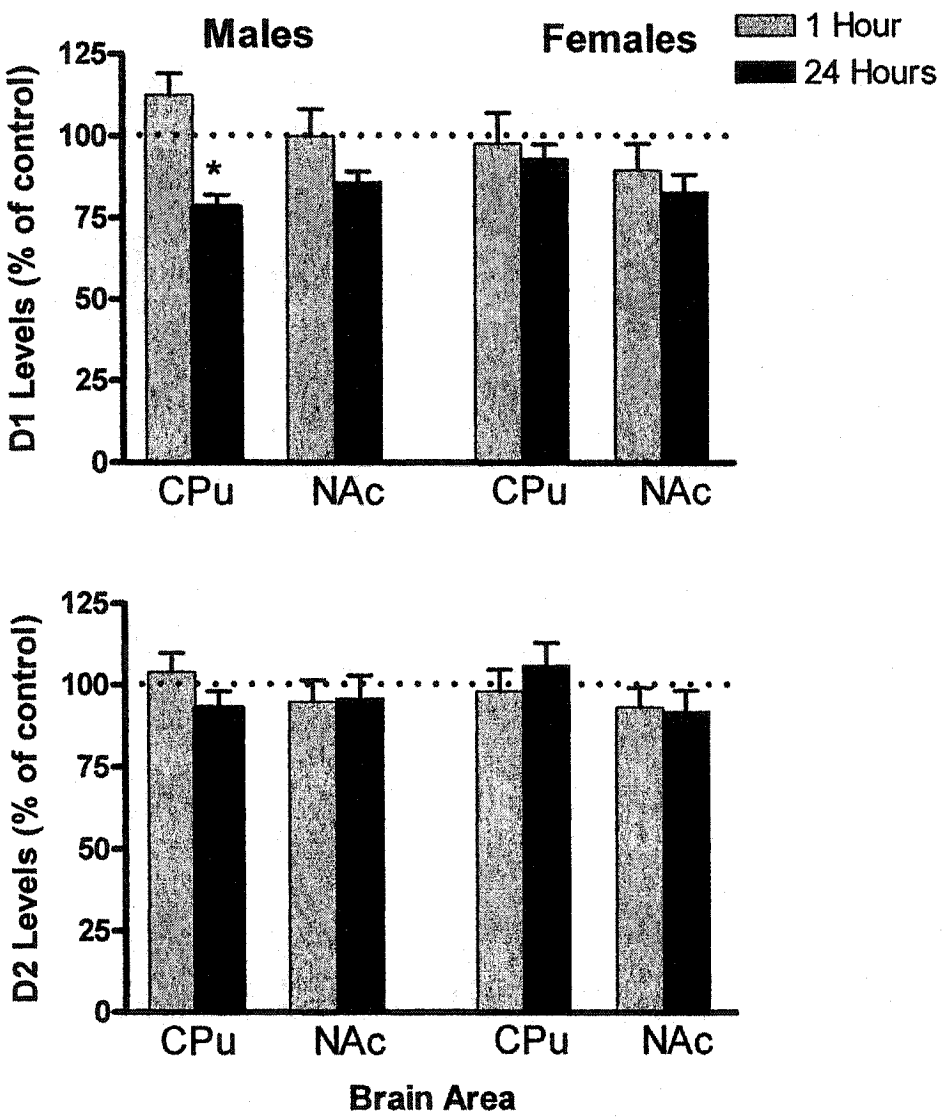


Figure 19 D1 (A) and D2 (B) binding levels in male and female rats 1 and 24 hours following acute cocaine (20 mg/kg) or saline administration. Binding levels are presented as percent change from control (n=3-5/ group). *Denotes a significant effect of cocaine as compared to respective saline-treated groups ($p<0.05$).

Male

Female

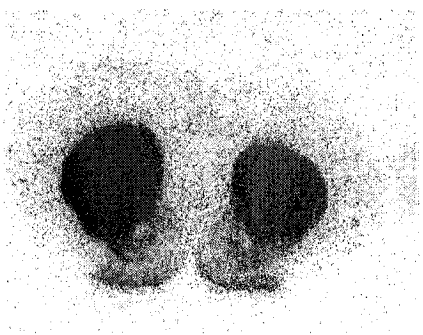


Figure 20 Dopamine transporter binding on coronal brain slices of the CPu amd NAc using the selective radioligand RTI-121 in male and female rats 24 hours following a saline injection (n=3-5/ group).

Discussion

Consistent with previous reports from our group and others, female rats had higher ambulatory and rearing activity following an acute cocaine injection (Festa et al., 2004; Walker et al., 2001a; Chin et al., 2002; Van Haaren and Meyer, 1991; Sell et al., 2000). Furthermore, in agreement with Festa et al. (2004), 20 mg/kg of cocaine did not produce significant sex differences in behavioral stereotypy. As previously reported, D1 dopamine receptor activation mediates cocaine-induced motor activity in male rats (McCreary and Marsden, 1993; Cabib et al., 1991; Xu et al., 2000). We extend upon these findings by showing that in female rats, D1 dopamine receptor activation also regulates cocaine-induced motor and stereotypic behavioral responses. However, sex differences were observed in the efficacy of SCH-23390 to inhibit of cocaine-induced activity. Cocaine-induced ambulatory activity in female rats was slightly decreased by the lower dose of SCH-23390. The highest dose tested completely abolished all three behavioral responses to cocaine in male rats, while in female rats there was an attenuation of these behavioral responses. This is in agreement with a recent study by Schindler and Carmona (2002) that demonstrated sex differences in the efficacy of SCH-23390 to inhibit cocaine-induced open field activity.

Since SCH-23390 administration did not produce alterations in behavioral activity of saline-treated rats, our results strongly suggest that a sexual dimorphism in the behavioral response to cocaine may occur via differential activation of D1 receptors.

Further supporting this postulate is the lack of sex differences in baseline levels of D1 receptor binding and/or mRNA levels in the NAc and CPu of saline-treated control rats. However, Andersen et al. (Andersen et al., 1997) demonstrated higher levels of D1 receptors in the NAc of female rats when compered to male rats. Two major methodological differences may contribute to this discrepancy. First, SCH-23390 significantly binds to 5-HT2A receptors (Bischoff et al., 1986), thus unlike Andersen et al. (1997), we used ketanserin to block 5- HT2A receptor binding. Since sex differences in the distribution of this serotonin receptor have been reported (Zhang et al., 1999), the observed sex difference in D1 receptor levels in the NAc in the Anderson et al. (1997) study may represent, in part, non-specific binding of SCH-23390 to the 5-HT2A receptor. Alternatively, the different rat strains used between these studies may contribute to the observed discrepancies. It has been shown that cocaine-induced up-regulation of cAMP in the NAc of Fischer rats is greater than in Sprague-Dawley rats (Ortiz et al., 1996).

Recent studies have consistently hypothesized that greater cocaine-stimulated striatal dopamine release in female rats may account for the prolonged and exaggerated behavioral responses as compared to male rats (Walker et al., 2000; Becker, 1999; Festa et al., 2004). Accordingly, it has been hypothesized that greater DAT levels in female rats may account for the greater release and re-uptake (Walker et al., 2000). Interestingly, we did not observe sex differences in DAT site binding densities either before or after cocaine administration (1 and 24 hours). Moreover, dopamine's affinity

for the DAT is equivalent between sexes (Walker et al., 2000). Therefore it may not be DAT site density that contributes to the higher dopamine release seen in female rats. It has also been suggested that dopamine's affinity for striatal D2 autoreceptor is lower in female rats, possibly contributing to the higher striatal dopamine levels (Becker, 1999). These observations collectively suggest that increased synaptic dopamine in female rats may contribute to the lack of complete inhibition of cocaine-induced behaviors by SCH.

The effects of acute cocaine administration on D1 binding site density in the CPu and NAc are unclear. In this study we demonstrated a 25% reduction on D1 binding site density 24 hours following acute cocaine administration only in the CPu of male rats. This down-regulation of striatal D1 binding sites may represent a compensatory mechanism in reaction to the dopaminergic surge following acute cocaine administration. However, in female rats, we did not observe significant alterations in CPu or NAc D1 binding site densities. As suggested by White and Kalivas (1998), down-regulation of dopamine receptors may reflect adaptations to maintain homeostasis where dopamine receptors acclimate to levels and frequency of ligand occupancy. Thus, we can postulate that there are sex differences in the homeostatic processes after acute cocaine administration. More specifically, this sexually dimorphic cocaine-induced alteration in dopamine receptor binding levels may represent intrinsic differences in either receptor desensitization or in components of intracellular signaling cascades such as the phosphorylation of transcription factors including CREB and/or AP-1 factors.

D1 mRNA levels in the CPu of male rats are altered after acute cocaine administration, the direction of this effect has not been consistent between studies. For example, 30 minutes after "binge" pattern cocaine administration, Yuferov et al. (2003) demonstrated a significant increase in D1 mRNA levels in the CPu of male rats. Similarly, 6 hours after a single injection of cocaine, Svensson and Hurd (1998) reported an increase in D1 receptor mRNA levels in the CPu, while Schmidt-Mutter et al. (1999) did not report any changes 6 hours after single cocaine administration. Discrepancies between these studies and ours may be attributable to either the manner of cocaine administration (binge vs. single), dose of cocaine used (Yuferov et al., (2003), a total of 45 m/kg; Svensson & Hurd (1998), 30 mg/kg; Schmidt-Mutter et al., (1999), 20 mg/kg; and in this study 20 mg/kg) or time of mRNA measurements (Yuferov et al., (2003), 3.5 hours; Svensson & Hurd (1998) and Schmidt-Mutter et al., (1999), 6 hours; and our study, 1 and 24 hours). Interestingly, in female rats, a non-significant reduction of 40% was observed in D1 receptor mRNA levels in the CPu. D1 receptor density has been shown to fluctuate with the estrous cycle in the CPu (Levesque et al., 1989). Thus, estrous cycle effects may account for the increased variability observed in D1 mRNA levels and lack of a significant effect in the current study. Moreover, our lab and others have shown that estrogen and progesterone replacement in ovariectomized females affect cocaine-induced alterations in prodynorphin mRNA and other genes in the mesocorticolimbic dopamine system (Jenab et al., 2002; Zhou et al., 2002). However, more detailed studies delineating cocaine's interaction with ovarian hormones in the

intact female rat are necessary to clarify these findings.

In the NAc, D1 receptor mRNA levels were significantly reduced in male rats one hour after cocaine administration. However, this alteration was transient because 24 hours after drug treatment transcript levels returned to that of saline-treated controls. To our knowledge, this is the first report showing sexually dimorphic molecular alterations in the striatum following acute cocaine administration. Jenab et al. (2002) demonstrated that in both male and female rats, the mRNA levels of the immediate early gene c-*fos* were increased after acute cocaine administration. On the other hand, preprodynorphin mRNA levels did not change in either male or female rats after acute cocaine administration (Jenab et al., 2002). Therefore, cocaine-induced alterations in D1 mRNA may play a more vital role in either the observed sex differences in behavior and the initial neuronal adaptations in response to acute cocaine.

Cocaine-induced alterations in D1 mRNA in male rats do not parallel changes in binding levels. However, previous studies examining chronic cocaine's effects on D1, D2, and DAT mRNA and ligand-binding have demonstrated similar discrepancies between binding and mRNA levels (Letchworth et al., 1997). Moreover, the relationship between an mRNA and it's protein cannot be completely determined by measuring two time points.

Similar to male rats (McCreary and Marsden, 1993; Ushijima et al., 1995), in female rats, cocaine-induced motor activity was not affected by D2 receptor antagonism. Moreover, no alterations in D2 receptor mRNA or binding density in either male or female rats were observed. It has been demonstrated that male rats are more sensitive to D2 agonist simulation as compared to female rats (Schindler and Carmona, 2002). As suggested by Becker (1999), D2 receptors may be less sensitive to dopamine stimulation in female rats indicating that D2 autoreceptors contribute to the sexually dimorphic motor response to cocaine. However we did not observe any sex differences in cocaine-induced motor behavior following D2 antagonism indicating that the D2 receptor may be less of a factor in sex differences in behavioral activity.

The increased DA release in the CPu {Walker et al., 2000) and the lack of any significant dopaminergic neuronal adaptations after cocaine administration in female rats may, in part, explain why their behavioral responses persist for at least 2 hours after acute cocaine treatment, while in male rats activity is rapidly decreased 45 minutes after cocaine administration (Festa et al., 2003; Festa et al., 2004). This further supports the assertion that female rats may have a dimorphic homeostatic process in response to acute cocaine administration as compared to male rats. Since pharmacological agents that target the dopamine system have long been the subject of treatment for cocaine addiction (White and Cooper, 2001), conceivably, sex should be an important variable in the development of pharmacological therapeutics for drug addiction. One confounding

factor that is not addressed in this study are the possible effects of ovarian hormones on dopamine-mediated behavioral and neuronal responses to cocaine. Mounting evidence suggests that cocaine's interaction with estrogen and progesterone contribute to the sexually dimorphic behavioral responses to cocaine (Russo et al., 2003; Sell et al., 2000; Lynch et al., 2002; Quinones-Jenab et al., 2001; Chin et al., 2002; Hu and Becker, 2003; Hu et al., 2003). Consequently, important questions remain to be answered about the impact of physiological hormonal fluctuations during the estrous cycle and how they contribute to cocaine-induced alterations in the dopaminergic system. Answers to these postulates are necessary for a more in depth understanding of the biological basis for sex differences in cocaine abuse.

CHAPTER 5: SUMMARY

The experiments presented in this thesis demonstrate sex differences in the behavioral response to acute cocaine, and possible mechanisms contributing to these disparities. Female rats have greater cocaine-induced hyperactivity as compared to male rats. These effects were dose dependent across ambulatory, rearing, and stereotypic behaviors. Furthermore, we demonstrated that cocaine is more potent in female rats than in male rats. For example, the behavioral hyperactivity demonstrated by male rats at high doses was similar to the behavior of female rats at intermediate doses of cocaine. The behavioral effects stimulated by cocaine in female rats also last for a longer time frame. Following a single injection, locomotor hyperactivity persisted for up to three hours while motor activity in male rats was attenuated by one hour. The results showing sex differences in cocaine-induced motor activity are consistent with observations of sex differences in cocaine reward. For example, female rats show a place preference for cocaine at lower doses than male rats (Russo et al., 2003b). Furthermore, female rats acquire cocaine self-administration and express a place preference for cocaine more rapidly than male rats (Lynch et al., 2002; Russo et al., 2003b). Moreover, cocaine injection frequency also affects sex differences in behavioral and endocrine alterations. In the "binge" pattern paradigm, following each of three hourly cocaine injections, behavioral activity in male rats returned to baseline prior to the next injection. In contrast, motor activity of female rats did not return to baseline levels between repeated cocaine injections. Motor activity in male rats remained stable in male rats, characterized

by a brief increase followed by a decrease in behavior. However, female rats had a more complex behavioral profile during multiple cocaine injections. Following the third cocaine injection, rearing activity significantly increased, while ambulatory behavior decreased, in female rats. This suggests the rapid development of behavioral sensitization or tolerance to specific components of the behavioral response to repeated cocaine administration in females. Taken together, these studies collectively suggest that the female CNS system is more sensitive to cocaine's psychomotor and rewarding effects.

At the endocrinological level, cocaine-induced progesterone secretion was also affected by sex and cocaine injection frequency. Cocaine-induced progesterone release in male and female rats following 3 hourly cocaine injections. However, progesterone release stimulated by cocaine was only observed female rats following a single cocaine injection. The effect of acute cocaine on progesterone secretion in females was transient because 3 hours following a single injection, progesterone levels had returned to baseline. In male rats, a similar effect of acute cocaine was observed on serum levels of testosterone. Acute and binge cocaine administration decreased testosterone levels in male rats, however, the acute effects of cocaine were temporary because 3 hours following a single cocaine injection testosterone levels returned to baseline. Thus, gonadal hormones play an important role in sex differences to the behavioral effects of acute cocaine. Testosterone may exert an inhibitory effect on cocaine-induced behaviors

in male rats, and this hypothesis is supported by the observation that DA release decreases plasma levels of testosterone (Baumann and Rothman, 1998). The role of progesterone in cocaine-induced responses is less clear. Although progesterone levels and behavioral activity increase after acute cocaine administration, numerous clinical and pre-clinical reports have shown that progesterone may play an inhibitory role in the behavioral and subjective effects to acute cocaine (Russo et al., 2003a; Sell et al., 2000; Sofuoglu et al., 2002). For example, when progesterone levels are high during the menstrual cycle, or when progesterone is exogenously administered, the subjective effects of cocaine are attenuated in women (Sofuoglu et al., 2002; Sofuoglu et al., 2003). However, progesterone's role in the addiction process remains to be elucidated.

There have been few reports regarding sex differences in cocaine metabolism. We provide evidence that cocaine metabolism may influence the hyperactivity seen in female rats following acute cocaine administration. It was observed that female rats have higher brain and blood levels of the cocaine metabolite norcocaine than male rats. Norcocaine binds to the monoamine transporters at half the potency of cocaine (Ritz et al., 1987), induces hyperactivity in rats (Schuelke et al., 1996), and is self-administered in monkeys (Spealman and Kelleher, 1981). The data also suggest that the half-life of cocaine in the brain is shorter in male rats. Thus, higher brain cocaine levels in female rats may contribute to the more robust behavioral response to acute cocaine. In the clinical literature, cocaine pharamcokinetics have not been well studied. There is

evidence that cocaine levels fluctuate with the menstrual cycle following intranasal cocaine administration (Lukas et al., 1996). However, there are no sex or menstrual cycle effects on blood levels of cocaine after an i.v. dose (Mendelson et al., 1999). To date, there have been no studies addressing norcocaine's contribution to sex differences in the subjective and physiological responses to cocaine in human males and females. We postulate that cocaine's transformation to norcocaine and it's potent CNS effects may contribute to the observed sex differences in behavior.

Monoaminergic pathways, the mesocorticolimbic DA system in particular, have been postulated to play a pivotal role in cocaine addiction. These pathways have known sexual dimorphisms, however, little is know about their role in sex differences in cocaine use. We have demonstrated sex differences in both the DA and serotonin systems that can potentially contribute to the observed sex effects following acute cocaine adminitration. We observed that male, but not female, rats show a decreased DA turnover in the CPu following acute cocaine administration. Thus, it can be suggested that autoregulatory feedback mechanisms modulating DA release are sexually dimorphic. Our data is supported by Becker's (1999) suggestion that enhanced DA release in the CPu of female rats could account for the observed sex differences in cocaine-induced activity. It is possible, as suggested by Becker (1999), that the endocrinological profile of female rats contributes to the downregulation of D_2 autoreceptors in the substantia nigra may result in enhanced DA release in the CPu. In the NAc the DArgic response to

acute cocaine was more robust in female rats (i.e., decreases in DOPAC, HVA, and DA in females only). Similar to the mesocortico DA pathway, modulation of mesolimbic dopaminergic function at the autoreceptor level can be sexually disparate in two ways, at the level of the synaptic terminal or at the cell body level in the ventral tegmental area. Alternatively, GABAergic nigrostriatal projections that feedback to dopaminergic cell bodies may also be dissimilar in male and female rats.

We also observed sex differences in the serotonin system. Basal levels of serotonin and 5-HIAA in the NAc were higher in female rats than in male rats suggesting the likelihood that sex differences in basal serotonin levels have functional consequences (i.e., a greater potential to modulate monoaminergic function). Moreover, female rats have an increased serotonin turnover ratio following acute cocaine administration and in males the opposite effect was seen. This finding may also reflect sex differences in autoreceptor-mediated feedback in the raphe nucleus where there are known sexual dimorphisms in neuronal activity (Klink et al., 2002). Additionally, our results have clinical relevance for the female cocaine addict. Women addicted to cocaine have higher rates of depression and less treatment success as compared to men (Boyd, 1993; Grant, 1995). In a rat model of cocaine addiction, chronic administration of cocaine induces changes in rat serotonin function that model the neural phenotype of clinical depression in humans (Baumann and Rothman, 1998). Thus, it can be speculated that in females, elevated basal serotonin and chronic cocaine use may facilitate the depressive neural

phenotype observed in the study by Baumann and Rothman (1998), resulting in a higher incidence of clinical depression. This speculation suggests the women cocaine addicts may need more aggressive pharamcological intervention for the treatment of depression or that the serotonin system could function as a target for medications to treat cocaine addiction.

DA D1- and D2-like receptors regulate motor behavior and play a key role in the behavioral response to cocaine. We found sex differences in D1, but not D2, receptor function. Our findings suggest that sex differences in motor behavior in response to an acute cocaine injection may be mediated, in part, by differential activation of the D1 receptor. There were no differences in D1, D2, or DAT receptor binding or in D1/D2 mRNA levels in the striatum, suggesting that differences in receptor or transporter number are not responsible for sex differences in cocaine-stimulated motor behavior. Furthermore, cocaine-induced alterations in D1 receptor binding and mRNA levels were observed in male rats only. Taken together, sex differences in the D1 receptor-signaling pathway could play a pivotal role in the dimorphic behavioral response to acute cocaine.

Figure 21 is a hypothetical model suggesting that the DA system in the striatum may be an important mediator of sex differences in the behavioral response to cocaine. We postulate that although male and female rats have equal DA levels at baseline, cocaine-induced DA turnover is a sexually disparate process where male, but not female

rats, show a robust decrease in DA turnover in the CPu. This suggests intrinsic differences in DA autoreceptor feedback systems modulating DA release and synthesis. As a result of a lack of decrease in DA turnover in the female CPu, we suggest that female rats may achieve higher synaptic DA levels following acute cocaine administration. This may contribute to the higher cocaine-induced behavioral activity seen in female rats. Also, the observed sensitivity to D1 receptor antagonism in female rats may in part be due higher snyaptic DA levels rather than intrinsic sex differences in DA receptor function. We also showed that male rats have alterations in D1 receptor mRNA and binding following acute cocaine administration, whereas these effects are absent in females. Thus, homeostatic mechanisms in response to the DA surge after cocaine administration appear to be sexually dimorphic.

The findings presented in this thesis potentially represent important advances in the understanding of cocaine abuse in humans. In the clinical literature, mechanisms underlying sex differences in cocaine use patterns are not well delineated. We postulate sex differences in cocaine metabolism patterns, dopaminergic and sertonergic autoregulatory mechanisms, and DA D1 receptor activation may contribute to the disparities observed in human cocaine addicts. Since pharamcological agents that target the DA system have long been the subject of treatment options for cocaine addiction, conceivably, sex should be an important variable in the development of pharmacological therapeutics for drug addiction.

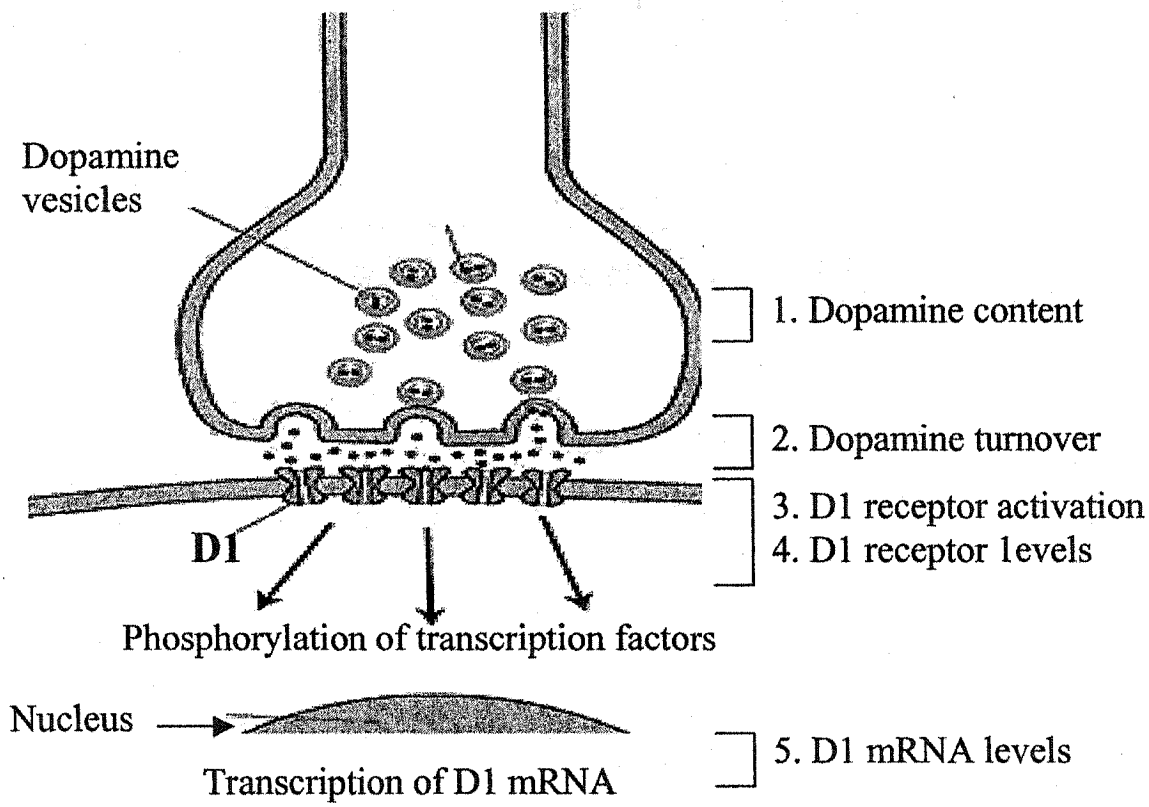


Figure 201 1. There are no sex differences in baseline dopamine levels in the CPu and NAc. **2.** In the CPu: Male rats have higher basline DOPAC/DA turnover. Male, but not female, rats show cocaine-induced decreases in DOPAC/DA turnover suggesting a more robust dopaminergic response in female rats due to a decreased sensitivity in D2 autoreceptor regulation of dopamine release. **3.** D1 receptor mediation of cocaine-induced motor behavior is sexually dimorphic where female rats are more sensitive to D1 antagonism, possibly due to increased dopamine tone in the female synaptic cleft. **4.** D1 receptor levels decrease in the CPu of male rats 24 hours following an acute cocaine injection. **5.** D1 receptor mRNA levels in male rats decrease 1 hour following acute cocaine in the NAc. These modifications of D1 mRNA and binding levels in male rats indicate that homeostatic processes in response to the dopamine surge following acute cocaine administration are sexually dimorphic.

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